

EPIDEMIOLOGY, DIAGNOSIS, AND PREVENTION OF BOVINE RESPIRATORY  
DISEASE COMPLEX

by

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B.S., Kansas State University, 1980  
D.V.M., Mississippi State University, 1991

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology  
College of Veterinary Medicine

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

2010

## Abstract

The objective of my research was to generate novel information concerning the epidemiology, diagnosis and prevention of bovine respiratory disease complex (BRDC), a common pre-weaning and post-weaning beef calf disease. To reach my objective, I conducted three prospective field trials within post-weaned calf populations, and one retrospective study of pre-weaned calves utilizing survey data.

I evaluated differences in behavior, health and performance in calves receiving multiple component health programs. Calves in a minimally invasive program, which included primarily non-injectable products, displayed less aversion to initial product administration but experienced higher BRDC morbidity ( $P = 0.02$ ) and poorer performance ( $P = 0.04$ ) compared to calves in a more invasive (all injectable products) program.

Secondly, in a study of *Mannheimia haemolytica* inoculated calves, I found that no parameter included in physical examinations, or common blood component evaluations could discern health from disease. However, disease recognition was aided by the measurement of the number of steps taken by a calf in a 24 hour period. None of the parameters that were evaluated predicted the severity of lung pathology.

Thirdly, I conducted a study in post-weaned feeder calves that determined prevalence estimates for Mollicutes in general, and *Mycoplasma bovis* specifically, and their respective associations with health and performance. Nasal Mollicutes prevalence was high on arrival, and differences in calf performance were associated with ( $P < 0.01$ ) nasal prevalence. More than half of the calves seroconverted to *M. bovis*; calves not seroconverting gained more weight (0.49 kg/head/day) during the study than those calves that did seroconvert (0.35 kg/head/day).

Finally, I conducted a retrospective analysis of national U. S. cow-calf survey data to identify herd level management practices associated with pre-weaned calf BRDC. I found feeding antibiotics to pre-weaned calves, importing cattle, the number of outside visitors, economic purpose of the cow-calf operation, and breeding management of the herd were associated with herd-level pre-weaning BRDC rates.

My research projects generated unique information concerning the epidemiology of important pathogens, differences among preventive health programs, objective BRDC diagnostic parameters, and pre-weaning BRDC risk factors. These research studies reinforce the complexity of BRDC and demonstrate the pathogen, animal and management factors affecting BRDC risk in pre- and post-weaned beef calves.

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Approved by:

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David G. Renter

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## Acknowledgements

I would like to acknowledge my advisors Drs. Brad White and David Renter. I thank them for convincing me to pursue my long-standing dream of studying epidemiology. As importantly I thank them for sharing their expertise, for enduring my numerous mistakes, and for being patient. Thanks to Dr. David Anderson for offering valuable input in several of my projects and for having the time just to talk. I would like to thank Dr. Robert Larson for sharing his knowledge and encouraging me to think “outside the box”. Thanks to Dr. Morgan Scott for sharing his knowledge, and teaching me in matters of great statistical complexity that I don’t need to sing the song, just hum the tune. Finally, thanks to Dr. Duane Davis for serving as the outside chairperson.

I would also like to acknowledge my fellow graduate students Dr. Jason Nickell, Dr. Abram Babcock, Mr. Brad Robért, Dr. Ben Wileman, and Dr. Charles Dodd. Thanks men for the help with my projects and the serious and not so serious conversations.

Without funding, my graduate program would not have been possible. Thanks to Intervet/Schering Plough and CEVA-BIOMUNE for funding my research projects. I would also like to thank the United States Department of Agriculture-Animal Health Inspection Service-Centers for Epidemiology and Animal Health, in particular Drs. Bruce Wagner and David Dargatz, for making their survey data available and offering advice during the completion of the project.

Most importantly, I would like to thank my wife Leah. She has always been supportive any time I expressed the desire to pursue new endeavors; her reaction to my pursuit of

epidemiology was no different. She willingly moved to Manhattan despite having to leave behind two of the things she loves the most, her family and Sandhills ranch. Throughout our marriage she has been a sounding-board and source of moral support. Leah, I am eternally grateful.

## **Dedication**

I dedicate this to my best friend and wife, Leah

## Preface

Bovine respiratory disease complex (BRDC) commonly afflicts both pre- and post-weaned beef calves. This disease syndrome epitomizes the term multifactorial with calf, pathogen, and management practices each playing intricate and connected roles in the epidemiologic triad. Recently, new management practices (e.g. metaphylaxis) have decreased BRDC incidence in some populations. Although multiple-component preventive health programs, including biologics, antimicrobials, parasiticides, and nutraceuticals have been developed, BRDC continues to be a health issue. Due to the complexity of this disease, research investigating the success differences among preventive health programs, and identifying novel BRDC recognition tools is important. Additionally, because the pathogens associated with this disease complex are similar in both pre- and post- weaning populations, further research into the pathogen dynamics within calf populations and their association with health and performance will help us better understand this disease complex. An important component of preventive medicine is the identification of risk factors that can be modified to improve health. Identifying those risk factors that have the largest potential impact on calf health is important information for the beef industry.

Most research has focused on comparing single products of a health program, (e.g., two types of viral vaccines), and not two programs with different combinations of products (e.g., viral-bacterial-parasiticide combinations). The difficulty, and perhaps the reason this type of research is not often undertaken, is the inability to identify the individual products within these programs that may be responsible for observed differences in health or performance. However,

combinations of products are often used; therefore, comparing combinations would be an important step in comparing the success between BRDC preventive health programs. To investigate this issue, I completed a study comparing the health, performance and behavior of calves that received two different post-weaned calf preventive health programs (Chapter 2).

No BRDC preventive health program is completely effective, and similar to other diseases, the success of medical intervention is predicated by early disease recognition. Early identification of BRDC continues to be a major challenge in the control of this disease syndrome. Presently in the beef industry, subjective observation of subtle changes in calf physical appearance, activity and apparent appetite are commonly used for BRDC recognition. Discovering objective recognition tools as indicators of early BRDC would allow more timely medical intervention and increase the probability of positive outcomes. Validated ante-mortem lung disease severity assessment tools are also lacking within the beef industry. Discovering objective measurements that could predict the severity of BRDC lung lesions would allow an objective assessment of the effectiveness of treatment regimens and could prevent suffering by improving disease outcome prognostication. The objective of my research into this topic was to investigate metabolic parameters and physical behaviors as indicators of early BRDC and the severity of lung pathology (Chapter 3).

A key to understanding the pathology and epidemiology of an organism lies in understanding its frequency and distribution. Some organisms were identified many years ago as important BRDC pathogens; therefore, many prevalence studies have been completed and associations with BRDC are well documented. Other organisms have only recently been associated with BRDC; therefore, little is known about these organisms' epidemiology as it relates to BRDC. One such group of pathogens belong the bacterial class Mollicutes (bacteria

lacking a cell wall, of which *Mycoplasma* sp. are the most dominant). The objective of my third study was to measure *Mycoplasma* nasal and serological prevalence at multiple time points (Chapter 4) and to evaluate associations with cattle health and performance indices. Both upon arrival and temporal changes of *Mycoplasma* nasal and serological prevalence would be important to understanding the epidemiology of this organism and would assist veterinarians and producers when designing preventive health programs such as those described in Chapter 2.

Another important issue for better understanding and managing BRDC in beef calves is the characterization of associated risk factors. Management practices as risk factors for BRDC in post-weaned beef calves and pre-weaned dairy calves are widely published. Despite many cow-calf operators considering pre-weaning BRDC an important disease, little research has been completed on risk factors in pre-weaned U. S. beef calves. For the purpose of disease control, extrapolating risk factors from one bovine production system to another may seem logical, but given the differences in calf age (pre-weaned calves vs. post-weaned calves) and animal management (dairy-calf hutches vs. cow-calf pairs), such an extrapolation may be inappropriate. There are likely management practices which are unique to the cow-calf industry that impact post-weaning BRDC. During the post-weaning period, this disease is difficult both to prevent, and diagnose, so being able to identify risk factors at the pre-weaning level may be an important step in preventing this disease. Discovering the management practices that enhance or reduce BRDC risk will be important to veterinarians when designing BRDC prevention programs or when assessing present cow-calf management practices. To meet my objective of evaluating cow-calf management practices as risk factors for pre-weaning BRDC, I analyzed data gathered from a national survey of U.S. cow-calf operations (Chapter 5).

My objectives in the BRDC research described here are multifaceted. First I wanted to quantify differences, based on health, performance and behavior between post-weaning preventive health programs. I also wanted to identify behavioral changes and metabolic parameters as possible accurate BRDC diagnostic tools. Additionally, I desired to investigate the population dynamics, and associated effects with health and performance of an important but only recently identified BRDC associated pathogen. Lastly, I wanted to identify those management practices that are associated with pre-weaning BRDC in a unique study utilizing data from a U.S. national survey.

# **CHAPTER 1 - Literature review: epidemiology, diagnosis, and prevention of bovine respiratory disease complex**

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## **Introduction**

Beef calf life cycles can be divided into two distinct time periods: pre- and post-weaning. Weaning is the management practice where mother and offspring are physically separated and milk feeding is terminated. This stage is a critical period in beef cattle production systems. In beef production, these two time periods share some common diseases and disease risk factors, but the primary diseases, disease timing, and risk factors of importance differ between the two periods.

Regardless of the specific disease or timing, causes of calf diseases are multi-factorial. The calf, pathogen, and environment are intimately associated arms of the epidemiological disease triad. The disease triad and the synergy that exists between each arm are important concepts that form the foundation for calf disease control and prevention programs (Radostits, 2001). Incorporating the triad concept into the decision-making process for herd disease management can significantly increase efficiency, reduce cost of production, and increase



production unit profitability (Wikse et al., 1994; Griffin, 1997). The objective of this review is to discuss the BRDC disease epidemiology for the pre-weaning and post-weaning periods. Specifically, the focus of this manuscript is on BRDC and the associated risk factors, economic ramifications, and prevention and management programs for pre-weaning and post-weaning time periods.

## **Pre-weaning health and disease**

### *Disease epidemiology*

Several studies have estimated the prevalence of beef calf pre-weaning morbidity and mortality (USDA, 1998a; USDA, 2009c). Morbidity risk for pre-weaned calves was 3.8% for respiratory disease, 3.5% for diarrhea/digestive, and 2.2% for pinkeye (USDA, 1998b). Although not the most often listed, diarrhea and pneumonia are important with regard to pre-weaning morbidity and mortality. The 1997 National Animal Health Monitoring System (NAHMS) survey reported that 5.1% of herds had at least a 2% incidence of diarrhea and 14.1% of herds had at least a 2% incidence of pneumonia (USDA, 1998a). A South Dakota study reported the following prevalence of morbidity: diarrhea (21.4%) and respiratory disease (15.5%). The NAHMS survey reported that 3.6% of all calves born alive were lost or died before weaning (USDA, 2009c). This survey also reported as the percentage of total death loss of calves less than 3 weeks of age by several perceived causes: weather (25.6%), unknown (18.6%), respiratory (8.2%), digestive (14.0%), calving problems (25.7%), predators (4.7%), and poisoning (0.0%) (USDA, 2009c). These findings differ slightly from those of the 1997 NAHMS study, which showed that calving problems was the most frequent (33.0%) cause of calf death, followed by digestive and respiratory deaths at 16.4% and 8.8%, respectively (USDA, 1998b). Also in the NAHMS Beef 2007-2008 survey, the percentage of total deaths of calves

greater than three weeks of age were listed as: weather (10.0%), unknown (19.4%), respiratory (31.4%), digestive (22.6%), calving problems (2.3%), predators (4.7%), and poisoning (0.1%) (USDA, 2009c). In 73 Colorado herds, diarrhea and respiratory infections were responsible for 11.5% and 7.6%, respectively, of total pre-weaned calf mortality (Wittum et al., 1993). These studies indicate that although multiple health issues are associated with pre-weaning morbidity and mortality, BRDC plays an important role.

Although diarrhea and BRDC are both important pre-weaning health issues, the age-associated incidence risk of neonatal diarrhea and pre-weaning pneumonia differs. Gastrointestinal infections from *Escherichia coli*, rotavirus, and coronavirus occur most commonly in calves less than 3 weeks of age, and Saif *et al.* 1985 suggested that age of susceptibility varies by pathogen and estimated age ranges for several common pathogens including *E. coli* (< 7 d), rotavirus (7 to > 30 d), coronavirus (7 to > 30 d), and *Cryptosporidia* (7 to 21 d) (Saif, 1985). Because diarrhea incidence occurs over a relatively narrow time period, labor and management focus on the disease triad needs to occur soon after birth. This may allow cow-calf producers to concentrate labor but may also be challenging if the disease demands overwhelm the available labor. In contrast, pneumonic infections associated with bovine herpes virus-1 (IBR), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus (BVDV), and *Mannheimia haemolytica* may occur at any age. However, research on a single, large herd over a 20-year period illustrated that the highest BRDC incidence rates occurred between calf ages of 70 and 170 days (Snowder, 2005). Because respiratory disease onset can occur over a long range of calf age, management focus on respiratory disease control needs to occur over the entire period from birth to post-weaning. This is a challenge for cow-calf producers, because

calves usually spend most of this phase in pasture or range environments where early disease detection and intervention are difficult.

## **Pre-weaning bovine respiratory disease**

### ***Risk factors***

Bovine respiratory disease risk factors are complex and varied. A study that followed more than 10,000 beef calves from birth to harvest revealed that dam age was a risk factor for calf BRDC incidence (Muggli-Cockett, 1992). In that study, calves born to 2-year-old dams had an increased risk for pre-weaning pneumonia, but a decreased post-weaning BRDC risk compared with calves from older dams (Muggli-Cockett, 1992). If younger dams transferred lower levels of passive immunity to their calves, the calves could be at a higher risk for pneumonia. Conversely, relatively low levels of passive immunity may have allowed for development of active immunity when vaccinated, thus decreasing the disease risk in the feeding phase. Several factors could account for differences between calf health and dam age including dystocia levels, colostrum quality and mothering ability. Future research into the association between dam age and BRDC risk is warranted.

Prior disease experience also may be a risk factor for subsequent BRDC. Calves diagnosed with scours early in their lives were found to have an increased risk of subsequent pre-weaning pneumonia (Sivula, 1996). This may indicate that previous illness can negatively affect immunity directly by overwhelming the immune system or secondarily through disease-associated anorexia and malnutrition. Additionally, calves with poor immunity may be more susceptible to many diseases. Regardless of the mechanism, it appears that general management practices that decrease disease risk for early pre-weaning diarrhea could serve to decrease future disease incidence risk (e.g., for BRDC) later in the pre-weaning period. More research is needed

to investigate interactions between health history, present immune status and pre-weaning BRDC.

Population dynamics play an important role in pre-weaning pneumonia risk. A positive association between calving season length and pre-weaning pneumonia mortality risk had been found in Canadian cow-calf herds (Dutil et al., 1999). In the same study, owners of larger herds (>40 cows) reported a higher incidence of respiratory disease (Dutil et al., 1999). The increased risk for pre-weaning pneumonia associated with higher population density and longer calving season length may be partly explained by the fact that older animals serve as common carriers of many respiratory pathogens (i.e., bovine herpes virus-1) and potentially spread the virus through saliva (Akermann, 2006). This increased risk of population density and dynamics may be explained by the concept of “herd immunity”. For example, as the calving season progresses, herd population dynamics change because disease-susceptible animals (calves) continually make up a larger percentage of the population; as the percentage of susceptible animals increases, the risk of disease outbreaks also increases. Management practices that reduce the level of exposure to BRDC pathogens will continue to be important to disease control programs.

Genetics also may play a role in pre-weaning BRDC risk. Pre-weaning BRDC resistance heritability has been estimated to be 0.07 to 0.20 (Muggli-Cockett, 1992; Snowder, 2006). In a more recent study, heritability for pre-weaning BRDC resistance was estimated to be 0.11 (+/- 0.06) in a population of 1,519 calves (Schnieder et al., 2009). Another study noted significant differences in pre-weaning BRDC resistance heritability between breeds of cattle (Snowder, 2006). In this study pre-weaning BRDC incidence was 17.8% for Hereford calves and 7.8% for Angus calves. Recently a quantitative trait loci identifying resistance to BRDC has been elucidated (Casas and Snowder, 2008). Although the heritability of BRDC resistance appears to

be low, concentrating on selecting those seedstock animals possessing inherent resistance or tolerance to BRDC or substituting more resistant breeds for susceptible breeds may be useful BRDC control strategies.

### ***Prevention and management***

Disease prevention in a population is based on the ability to incorporate all components of the standard epidemiological triad (i.e., host, environment, and pathogen) into herd health management regimes (Martin et al., 1987). Incorporating the triad concept into the decision-making process for herd disease management can significantly increase efficiency, reduce cost of production, and increase herd profitability (Wikse et al., 1994).

One of the major challenges in pre-weaning BRDC management is the timing of vaccination in the presence of maternal antibodies. For each calf, there is a unique period of time after birth during which maternal IgG is waning but immunocompetence is not complete (Chase, 2008). Susceptibility to calfhood diseases is elevated during this phase, which lasts until active immunity has reached protective levels for the current disease challenge. Traditional studies investigating passive transfer interference typically measure antibody changes after vaccination (Ridpath et al., 2003). More recent studies have evaluated innate and active immunity responses in the presence or absence of maternal antibodies (Fulton and Burge, 2000). Cell-mediated and B-cell memory responses have been demonstrated by using attenuated vaccines in the presence of maternal antibodies (Ellis, 1996). These findings may provide support for the development of novel attenuated vaccination products for use in very young calves to provide a primary memory response. Potential differences between vaccine types were evaluated in a bovine viral diarrhea study in which calves with circulating maternal antibodies were vaccinated at seven weeks of age. Calves vaccinated with modified-live BVDV mounted a

T-cell response, whereas calves given killed vaccine did not develop a T-cell response (Endsley et al., 2003). This suggests that memory cells were stimulated during a primary immune response in the presence of maternal antibodies; thus, the long-held belief that maternal antibodies interfere with parenteral vaccinations when given to young calves needs to be revisited.

Different routes of vaccination administration in young calves have been investigated for their effectiveness as components of BRDC preventive health programs. Vaccines delivered intranasally have been more protective against a clinical BHV-1 challenge than parenterally administered products in BHV-1 seronegative calves and in calves possessing maternal antibodies (Castrucci, 2002). Calves that received the intranasal vaccination did not become febrile, were not dyspnic, did not have nasal discharge, and were not observed coughing, whereas some of the calves vaccinated with the parenteral product had at least one of these clinical signs. In another study, 2-week-old Holstein-Hereford cross calves with evidence of maternal antibodies were given a single intranasal BHV-1 vaccination and challenged 16 weeks later with live BHV-1 (Patel, 2005). Although no rise in virus-neutralizing antibodies to BHV-1 was observed in any calf, all intranasally vaccinated calves were protected against pyrexia and nasal viral shedding was decreased in this group. If intranasal vaccines targeting other BRDC pathogens are effective in the presences of maternal antibodies requires more research.

Very few studies have provided vaccination timing guidelines for immunizing young calves against BRDC agents in the presence of maternally derived antibodies. If maternal antibodies block a primary immune response to vaccination, one might conclude that administration of respiratory vaccines should be delayed until passive immunity to common bovine respiratory viruses has waned. In one study, the average time for passively acquired

BVDV titers to decay to negative status (<16) was 192 days of age (Fulton et al., 2004). Other studies have shown that passive immunity to *Mannheimia haemolytica* and *Pasteurella multocida* was lowest at 60 to 90 days of age (Prado et al., 2006). Using estimations of seronegativity as guidelines for vaccination program timing disregards the distribution of seronegative and seropositive calves. Some calves will reach seronegativity earlier than the population mean, and if the mean is used, this group of calves may be left unprotected. If novel attenuated vaccines can be invented for widespread use in pre-weaned calves, then the uncertainty concerning vaccine timing or vaccine method of administration would be less important. Much more research investigating the interaction between maternal antibody presence and immune response to vaccination is badly needed.

Because BRDC is complex, prevention programs in the pre-weaning period may not be effective if concentrating on a risk factor, but may require the assessment of simultaneous risk factors such as herd age, the incidence of other diseases, genetics and vaccine management.

### ***Weaning***

As mentioned earlier, weaning is the process when mother and offspring are separated by the cow-calf producer and suckling is terminated. In addition to the cessation of milk consumption, the calf also experiences a change in living environment and solid diet components (Price et al., 2003). These weaning events are believed to be stressful to the calf, as measured by behavior changes such as increased vocalization, pacing and low feed consumption (Haley, 2006). Additionally, more subjective measures of stress such as calf serum catecholamine levels have indicated that weaning is a stressful event (Lefcourt and Elsasser, 1995; Hickey et al., 2003).

Stress is important in the immediate post-weaning period because it negatively impacts the immune system (Blecha, 1984). This occurs at a time when the exposure to viral and bacterial pathogens are increased (Duff and Galyeen, 2006). The most important post-weaning disease is BRDC, and it has been shown that stress enhances the viral-bacterial synergy that occurs between BRDC pathogens (Hodgson et al., 2005). To reduce the level of stress experienced by beef calves, several weaning strategies have been developed.

The most common weaning method is an abrupt separation of the calf and cow (Haley et al., 2005). Separating cows and calves by the greatest land distance is an important component of this weaning method, but distance separation does not appear to diminish the level of stress (Haley et al., 2005). Recently a two-stage system where nursing is first prevented, by a device placed in the calf's nose, followed later by calf-dam separation was investigated as an alternative weaning method (Haley, 2006). In this study, on the first day after separation, the two-stage weaned calves walked less distance ( $5.2 \pm 0.5$  km/d) compared to abruptly weaned calves ( $16.7 \pm 3.1$  km/d). Based on the distance difference, the authors concluded the two-stage weaned calves were less stressful. Several studies have investigated the utility of fence-line contact between calf and cow at weaning (Stookey et al., 1997; Price et al., 2003). Observers recorded the amount of time spent walking and lying down for a random sample of calves within each treatment. The fence-line contact calves spent less time walking and more time lying down compared to the abruptly weaned calves. These studies suggest that weaning methods differ with respect to the amount of stress experienced by calves. From these results, future studies investigating the association between weaning method and subsequent calf health would be a logical next step.



## Post-weaning health and disease

### *Disease epidemiology*

After weaning, calves may be moved to feedlots, backgrounding or stocker facilities. Although differences in business methods and management goals may differ between these animal production entities, they are similar in that they report BRDC as the greatest disease of concern.

According to the NAHMS Feedlot 99 survey, cumulative disease incidence risks during the feedlot phase are approximately 14.4% for BRDC, 3.1% for atypical interstitial pneumonia (AIP), 1.9% for digestive disorders, 2.2% for bullers, 1.9% for lameness, and 0.4% for central nervous system (CNS) (USDA, 2000b). Several other studies have indicated that BRDC is the most common feedlot disease (Ribble et al., 1995b; Edwards, 1996; Smith, 1998a; Loneragan et al., 2001b). In North America, BRDC accounts for 70% to 80% of morbidity and 40% to 50% of mortality (Smith, 1998b). Reported values for BRDC morbidity risk range from 0 to 86% (Kelly and Janzen, 1986; Gardner et al., 1999; Snowden et al., 2007; Step et al., 2008; Babcock, 2009; Garcia et al., 2009; Schneider et al., 2009b). Additionally, NAHMS reported that a larger percentage of calves experienced BRDC in feedlots with >8,000 capacity (15.5%) than in feedlots with smaller capacities (8.7%) (USDA, 2000a). Loneragan *et al.* found that most death loss was attributed to BRDC and that mortality incidence increased by 20% to 35% for each hundredweight decrease in cattle arrival weight (Loneragan, 2004). Reported values for the mortality risk associated with BRDC range from 0% to 8% (Step et al., 2008; Holland et al., 2010). Although other diseases are reported during the post-weaning period, and BRDC morbidity and mortality incidence differences by facility capacity or animal size, BRDC continues to plague the beef industry.

Peak BRDC incidence reportedly occurs relatively early in the feeding period. A Canadian study found BRDC morbidity peaked at 7 to 14 days and then declined toward zero by day 28 (Martin and Meek, 1986). In a South African study, peak initial BRDC incidence occurred on day 18 and 87% of all cases occurred by day 35 (Thompson et al., 2006). In a large retrospective study of 31,131 BRDC morbidities, the average days on feed to initial treatment was calculated at thirty (Babcock, 2009). In a 3-year study across 10 feedyards, the average days to first treatment was 40 days and 75% of cases occurred by day 55 (Schnieder et al., 2009). These studies suggest that days to peak morbidity increased over the study years. A need for future research to investigate the possibility for predicting temporal patterns in groups of calves is needed. This would allow feedlot personnel to concentrate labor and materials at specific times to specific groups of calves.

Several researchers have reported a seasonal pattern for BRDC. For example, two studies showed that BRDC morbidity and mortality peak in early autumn and winter (Irwin et al., 1979; Ribble et al., 1995a). And in a multiple-year, single-feedlot study, the highest risk of BRDC mortality occurred in the fall (Ribble et al., 1995a). Authors of those studies noted that the coldest temperatures and greatest amounts of precipitation occurred during this time. These findings may indicate that fall weather patterns negatively affect calf health. It is also possible that non-weather-related risk factors are present (Babcock, 2010). Most calves enter the feedyard during the fall (USDA, 2000a). This sudden influx of calves may negatively affect health through an increase in commingling stress and pathogen exposure. According to the NAHMS Feedlot 99 study, in feedlots of 8,000 head or more, the average pen observer is charged with watching 2,000 head of calves (USDA, 2000b). According to the same survey, the majority of feedlots observe each pen twice daily during the first 15 days after arrival (USDA,

2000b). Additionally, only 27.6% of feedlots require formal training with written guidelines for disease diagnosis, 45.4% offer formal non-written but not written guidelines, and 18.3% require no formal training (USDA, 2000b). Therefore, it is also possible during the fall when many groups of calves are arriving that a feedlot health system may be overwhelmed by the number of new cattle and pens that require multiple daily observations by employees who may lack adequate BRDC observational training.

Atypical interstitial pneumonia is a respiratory disease with distinctly different causes and temporal distributions. The cause of AIP is unknown, but it is believed that a synergistic event occurs between toxic rumen product production, 3-methyleneindolenine, and BRSV presence (Loneragan et al., 2001a). In a 2-year study, the mean days from feedlot arrival to AIP experience was 114 days (Ayroud et al., 2000). Mortality from this disease ranges from 0.03% to 0.05% of cattle placed (Hjerpe, 1983). Even at the low mortality risk stated here, the economic impact of this disease could be substantial when multiplied over large groups of cattle. Additionally, it has been shown that calves that become morbid closer to scheduled harvest have reduced carcass weight, presumably because they had less time to completely recover (Babcock, 2010). There may be an additional economic cost due to delayed slaughter due to treatment withholds and reduced finishing weight. Because little is known about the etiology, more research is needed in this area.

Several diseases occur during the post-weaning period including lameness and digestive disorders. Bovine respiratory disease complex continues to be the most common disease during this time. The temporal distribution of this disease may have changed over time. Whether is due to calf type changes or management changes are unknown. Future research investigating temporal pattern differences between calf groups is needed.

## **Post-weaning bovine respiratory disease complex**

### *Economics*

The economic effects of BRDC are estimated at \$750 million to \$1 billion dollars and include prevention, treatment, and production costs (Griffin, 1997). Preventive health program components include vaccinations and metaphylaxis; expenses for the latter have been estimated to range from \$5.00 to \$12.92 per calf (Guichon et al., 2000; Booker et al., 2007).

Some economic loss due to BRDC is realized through a reduction in the number of marketable animals due to mortalities, and morbidity treatment costs. One researcher estimated for every one percent increase in BRDC pen morbidity, death loss increased by 0.14% (Irsik et al., 2006). Estimates for a single BRDC treatment, realized through pharmaceuticals and supplies, ranged from \$15.57 to \$16.64 per animal, and average BRDC treatment cost was \$12.59 with a range of \$11.09 to \$16.26 (Faber et al., 1999; USDA, 2000a). With newer antimicrobials, the costs of treatment may be greater than those listed above.

Production effects of BRDC in the post-weaning phase have been extensively studied. In one study, the carcass value for untreated calves was \$23.32 dollars more than calves treated once for BRDC and \$30.15 and \$54.01 more than calves treated twice or three times, respectively (Schneider et al., 2009a). In another study, calves treated once for BRDC had lower yield grades, fat thickness, and kidney/pelvic/heart fat but did not differ in marbling or hot carcass weights (HCW) compared with non-treated calves (Garcia et al., 2009). In a 15-year study, calves treated for BRDC had lower HCW and weight of retail cuts than non-treated calves, but there were no differences in average daily gain (ADG) (Snowder et al., 2007). In another study by Snowder (2006), calves diagnosed with BRDC had lower ADG (0.95 kg/day) than healthy calves (0.99 kg/day) (Snowder, 2006). Other studies have investigated feed

efficiency differences between pens of cattle at different BRDC mortality levels. For example in one study, for each percentage increase in mortality for a pen of cattle, feed conversion decreased by 0.12 kg, which resulted in an added cost of \$1.00 per head (Irsik et al., 2006). In a recent study, BRDC affected calves gained 0.6 kg less than untreated calves over the entire feeding period (Schneider et al., 2009a). In the same study, a higher percentage (71%) of calves that were never treated for BRDC graded as Choice or better compared with calves that were treated (57%) (Schneider et al., 2009a). And Gardner *et al.* (1999) reported that calves never treated (1.53 kg/day) for BRDC had higher ADG over the entire period than calves treated once (1.49 kg/day) for BRDC (Gardner et al., 1999).

Most studies on the performance effect of BRDC reported differences between healthy and sick cattle over an entire feeding period. However, one study demonstrated that the effect of BRDC on performance differed based days from arrival and slaughter (Babcock, 2009). In that study, calves that were treated closer to slaughter had higher ADG than similar calves treated earlier in the feeding period. This may indicate that production costs from BRDC episodes are not uniform across all phases of the feeding period and that BRDC prevention and interventions that occur earlier in the feeding period may have a larger positive effect.

The economic effects of BRDC appears to differ by the timing of the event in relation to day before slaughter, and are realized through prevention and treatment costs, reduced feed performance and final product value. Further research into this area is needed.

### ***Post-weaning bovine respiratory disease complex risk factors***

Important to the management of BRDC is the identification of risk factors. Risk factors for post-weaning BRDC include arrival weight, transportation stress, gender, commingling,

receiving period management such as dehorning and castration, and dietary provisions, and pathogen exposure (Duff and Galvayan, 2006; Fike and Spire, 2006).

Arrival weight often appears to be negatively associated with calf health. Lightweight calves (< 318 kg) had a higher incidence of BRDC than heavyweight calves (Alexander et al., 1989; Sanderson et al., 2008). But in a case-control study, no association between arrival weight and BRDC morbidity was found (Booker et al., 1999). The discrepancies between studies may be explained by the lack of a standard BRDC case definition in the Sanderson study, and a strict BRDC case definition in the Booker study. Additionally, the Sanderson study had a large sample size (20,136 calves), was evaluated at the pen level compared with the Booker (200 calves) and on an individual calf level, which may have allowed for more precise group estimates. If weight is a proxy for age, lighter calves may be more immunologically naïve because they have been exposed to fewer pathogens over time than heavier calves. Additionally, it is possible that lighter calves still possess maternal antibodies that interfere with some arrival respiratory vaccines and, therefore, do not become immunized. If the studies reported in the early segments of this paper suggesting MLV may not be affected by maternal antibodies this last explanation may not be valid given the majority of feedlot calves are administered MLV upon arrival (NAHMS, 2000b).

Long transport times, defined as longer than 24 hours, have been associated with increased BRDC morbidity (Cole et al., 1988). Sanderson *et al.* (2008) observed a 10% increase in morbidity risk for each 160 km increase in travel distance (Sanderson et al., 2008). In contrast, another study conducted over a four year period showed no difference in BRDC mortality in calves hauled over long distances (Ribble et al., 1995c). This difference in study outcomes may be a reflection of calf population differences, or it is possible that travel distance

affects BRDC morbidity risk without a subsequent associated increase in mortality. It is also possible that feedlot health providers are cognizant of transport times and concentrate observations of or administer treatments (metaphylaxis) to pens of long-haul cattle, therefore reducing mortality risk.

Gender and pen gender distribution have been associated with BRDC morbidity (Alexander et al., 1989; Snowden et al., 2006). One study found that heifers were at an increased risk of BRDC mortality compared with steers (Loneragan et al., 2001b). Additionally, heifer calves not selected by cow-calf producers for replacements are typically lighter weight, and visually of lesser quality, which suggests they are younger in age and, in turn, may have an increased pathogen exposure naivety, or may have other health issues. Additionally, heifers may experience additional stresses such as estrus or pregnancy (Smith et al., 2001). Pens with a mixture of steers and heifers had an increased risk of BRDC, compared to non-mixed pens (Sanderson et al., 2008). It should be noted that in this study a direct comparison between steer and heifer pens was not completed. The mechanisms to explain the difference between pen-gender-composition is unknown and needs further research.

Commingling has been associated with BRDC risk. Calves from multiple sources housed in the same pens had a higher BRDC risk than calves in single-source pens (Ribble et al., 1995a; Sanderson et al., 2008). In the Bruce County project, mixing calf groups after arrival was associated with increased BRDC morbidity (Martin et al., 1981). In another study, calves from a single source that were not commingled at the feedyard had less BRDC morbidity than calves from auction barns or calves that were commingled upon arrival (Step et al., 2008). Comingled calves presumably experience increased stress levels and pathogen exposure compared with non-commingled calves. This provides a management option for reducing BRDC risk, but for many

large feedyards, feeding only non-commingled calves will be logistically impossible. According to the NAHMS Beef 2007-2008 study, 80% of U.S. herds have 50 or fewer cows (USDA, 2009c). Because semi truckloads of cattle are typically 23,000 kg loads and transportation costs prohibit partial truckloads, commingling during transport will continue to be a risk factor. Additionally feedlot pen sizes routinely house 150-350 or more animals, which again necessitates cattle be commingled upon arrival (USDA, 2000b).

Post-arrival management procedures such as castration and dehorning are risk factors for BRDC. Mortality was significantly higher in calves castrated upon arrival than in calves that arrived at the feedlot as steers (Martin et al., 1980). In a more recent study, BRDC morbidity risk was higher for beef stocker calves that were castrated (60%) upon arrival than for those that arrived as steers (28%) (Pinchak et al., 2004). In another study, calves dehorned after arrival were at a higher risk of BRDC morbidity than those that were not dehorned (Daniels, 2000). The majority of feedlots reported placing at least some intact males and calves with horns (USDA-2000a). Additionally, 6.3% of cow-calf operations marketed calves with horns and 23% as intact males (USDA, 2000a). This suggests that efforts to reduce the effects of these two procedures may depend on additional cow-calf operator-education and research investigating newer methods for managing castrated and dehorned calves.

Concentrate levels and dietary source may be associated with BRDC. High concentrate receiving diets were associated with increased BRDC morbidity (Galyean et al., 1999). Calves fed a 75% concentrate diet experienced more morbidity (57%) than calves fed a 25% concentrate diet (47%) (Lofgreen, 1983b). In contrast, no BRDC morbidity difference was found between calves fed 70%, 75%, and 85% concentrate diets (Fluharty and Loerch, 1996). The difference between the Lofgren and Fluharty studies may be related to the concentrate levels fed. It is



possible that morbidity risk is not different between high levels of concentrate feeding but between low concentrate and extremely high concentrate arrival diets. In the Bruce County project, calves fed a diet consisting of large amounts of corn silage during the first week after arrival were five times more likely than calves fed only grass hay to die from BRDC (Martin et al., 1980). The difference in diets in this study could still be said to be a difference in concentrate levels. In contrast, feeding only grass hay for the first three 3 days after arrival decreased BRDC morbidity risk (Lofgreen, 1983b). The differences in studies mentioned above may be more related to the effects of abrupt dietary changes than in the source of nutrients. Almost one-half of cow-calf operations report selling calves immediately upon weaning (USDA-APHIS:VS, 2009b). Presumably calves sold at weaning most likely are exposed to high forage diets, and an exposure to high concentrate diets upon feedlot arrival constitutes an abrupt dietary change.

Arrival protein levels and protein sources have also been associated with BRD incidence. In one study, morbidity risk increased linearly for diets containing 12%, 14%, 16%, and 18% crude protein although source of protein was not associated with morbidity (Fluharty and Loerch, 1995). In two subsequent trials, the same researchers found no difference in morbidity between protein levels or sources and concluded that high levels of protein were necessary in receiving diets because of low dry matter intakes (Fluharty and Loerch, 1995). In a randomized trial, Galyean, *et al.* (1993) found that BRDC morbidity risk was higher in calves fed a 16% crude protein diet than in calves fed a 14% crude protein diet (Galyean et al., 1993). Martin (1982) reported that calves fed high levels of non-protein nitrogen during the early feeding period were at a higher risk of BRDC mortality compared to calves receiving natural protein (Martin, 1982).

Studies investigating the association between BRDC and dietary protein levels are conflicting. This suggests that future research into this area is warranted.

Bovine respiratory disease complex risk factors are numerous and complex. Given the different management skills and business needs that are present in the cow-calf industry, certain risk factors (light arrival weights, intact bulls, and calves with horns) will continue to be a part of post-weaning calf health. Also that a large percentage of calves are born and raised in distant areas from feeding operations, transport times will continue to affect BRDC rates (USDA, 2009b). Finally, commingling will continue to be a risk factor given the cow-calf herds sizes, transport truck sizes, and large feedlot pen capacity (USDA 2000a; USDA, 2008a). Concentrating on evaluating and discovering other more manageable risk factors such as nutrient density may be warranted. Additionally, the invention of novel interventions designed to lessen the effects of the aforementioned risk factors may become important to the feedlot industry.

## **Pathogens**

Understanding pathogens are important to BRDC management because they are intimately associated with other arms of the epidemiological triangle. Although the risk factors between pre-weaning post-weaning BRDC may differ, the pathogens associated with this disease are similar. The list of BRDC associated pathogens is extensive and includes viruses: infectious bovine rhinotracheitis virus (IBR), bovine viral diarrhea virus (BVDV), parainfluenza-3 virus (PI-3), bovine respiratory syncytial virus (BRSV), and bacteria: *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, and *Histophilus somni* (Wikse, 1985; Smith, 1996; Griffin, 1998; Radostits, 2000; Apley, 2006).

## ***Viral pathogens***

Viral pathogens are important contributors to BRDC pathology. Although much research has already been completed investigating the pathological mechanisms of each BRDC associated virus, more research is needed to understand the immunosuppressive and synergistic nature of these organisms.

### ***Infectious bovine rhinotracheitis virus***

Infectious bovine rhinotracheitis (IBR) is caused by Bovine herpes virus-1 (BHV-1) and is transmitted by aerosol or close contact with infected animals, including indirect transfer through contaminated food and water (Engels and Akerman, 1996; Fulton, 2009). Portals of entry include the nasal cavity, eyes, and oropharynx. As a result of BHV-1 infection, the mucociliary elevator is compromised, and this reduces the clearance of bacteria pathogens from the respiratory tract (Kapil and Basarab, 1997). This virus is immunosuppressive in the lower respiratory tract because it impairs macrophage, neutrophil, and lymphocyte functions. Ultimately, this impairment can lead to increased respiratory tract infections (Kapil and Basarab, 1997).

Infectious bovine rhinotracheitis is associated with BRDC in calves entering the feedlot at less than 1 year of age (Patel, 2005; van Drunen Littel-van den Hurk, 2006). Infectious bovine rhinotracheitis naïve calves are exposed to the virus by a carrier that harbors the virus either in the upper respiratory tract or within the trigeminal nerve (Kapil and Basarab, 1997). Trigeminal nerve infections are considered latent, and reactivation occurs after stresses such as transport, commingling, and environmental conditions (Kapil and Basarab, 1997).

Martin *et al.* reported that approximately 10% of calves were IBR seropositive on arrival and approximately 2% to 3% seroconverted over a 30-day period (Martin et al., 1989). In a group of stocker calves, 7.5% had positive IBR titers upon arrival (Fulton et al., 2000). In a different study, 18.3% of calves were positive for IBR upon arrival, and calves that became BRDC cases had significantly lower arrival titers compared with controls (Martin and Bohac, 1986). There were no differences between cases and controls in arrival IBR titers, but IBR arrival titers were positively associated with a higher risk of BRDC mortality (Booker et al., 1999). It appears that calves can be exposed to IBR before entry into the feedlot, and exposure may continue through the early feeding period. Additionally, this organism may not be associated with BRDC morbidity but may be associated with mortality. More research into the mechanism of disease for this organism is warranted.

### ***Bovine viral diarrhea virus***

Bovine viral diarrhea virus (BVDV) is an important BRDC pathogen (Campbell, 2004). This virus is thought to be transmitted through close contact with carrier animals, with aerosol exposure considered a secondary mode of transmission (Pellerin et al., 1994; Ridpath et al., 1994; Fulton and Burge, 2000; Fulton et al., 2005; Ridpath, 2005). Bovine viral diarrhea virus reduces the effectiveness of the mucociliary apparatus and is considered immunosuppressive (Fulton and Burge, 2000). Bovine viral diarrhea virus is believed to have a synergistic relationship with *Mannheimia haemolytica*, PI-3 virus, BRSV, and *Mycoplasma bovis* (Potgieter et al., 1984; Fulton et al., 2000; Haines et al., 2001).

One study found that 56% of calves had positive BVDV titers upon arrival and 24% of the calves seroconverted to BVDV during the first 28 days of the feeding period (Martin and

Bohac, 1986). In that same study, seroconversion was positively associated with BRDC morbidity. In a similar study, 81.7% of calves purchased through auction markets were seronegative to BVD type I and 86.7% were seronegative to type II upon arrival, and 38.5% seroconverted to type I and 27.9% seroconverted to type II during the first 5 weeks on feed (Fulton et al., 2000). Large differences in BVDV sero-prevalence at feedlot arrival suggests that clustering of this virus among certain cattle populations occurs. Another study showed BVDV seroconversion had a sparing effect on BRDC incidence (Allen et al., 1992). Similarly, Booker *et al.* showed that BVDV seroconversion was not associated with BRDC but higher arrival BVDV titers were associated with increased BRDC risk (Booker et al., 1999). In contrast, O'Connor *et al.* (2000) found that at arrival, BVDV seropositive calves were at lower risk of BRDC morbidity than seronegative calves (O'Connor, 2000). This may suggest that seroconversion across BVDV species is not similar, and conversion to exposure to one species may be more protective than conversion to another. Additionally, these results suggest that serostatus is a measurement of blood antibody levels which do not necessarily infer protection.

### ***Parainfluenza virus***

Parainfluenza virus type 3 (PI-3) is ubiquitous in cattle populations (Kapil and Basarab, 1997). Like BHV-1, PI-3 affects the upper respiratory ciliated apparatus and predisposes calves to secondary bacterial infections. Additionally, it is believed that this virus is immunosuppressive through its negative effects on macrophage and lymphocyte function (Kapil and Basarab, 1997).

In one study 16.2% of calves were serologically positive to PI-3 upon arrival with 24.2% positive by 30 days after arrival to a feedlot (Yates et al., 1983). In a 2-year study, 11% of calves

were PI-3 seropositive upon arrival and 67% had seroconverted by day 28; in the same study, seroconversion to PI-3 was associated with an increased risk of BRDC (Martin et al., 1989). In contrast, Allen *et al.* (1992) found seroconversion to PI-3 viruses to be protective against BRDC morbidity (Allen et al., 1992). These studies suggest that exposure to PI-3 is common in some populations and that the effects of seroconversion may be different across different calf populations.

### ***Bovine respiratory syncytial virus***

Bovine respiratory syncytial virus (BRSV) is considered a primary BRDC pathogen and a component of the respiratory complex (Gershwin, 2007). Infection usually occurs through contact with nasal secretions, and with aerosols over short distances (De Jong and Kimman, 1994). As with the other BRDC viral pathogens, BRSV reduces the effectiveness of the mucociliary apparatus, allowing secondary lung bacterial infections (Ellis, 2009). Unlike BVDV and BHV-1, BRSV is not considered immunosuppressive (Baker, 1985). There appears to be a synergistic relationship between BRSV and rumen-derived pneumo-toxicants such as 3-methylindole (Gershwin, 2007). From this relationship, BRSV has been associated with AIP, a less common form of pneumonia in feedlot calves (Ellis, 2009). A unique feature of BRSV, similar to that observed with respiratory syncytial virus in humans, is the enhanced pathological reaction observed in previously vaccinated calves prior to natural BRSV exposure (Gershwin et al., 1998).

Booker *et al.* (1999) reported that less than 25% of calves were seropositive for BRSV at feedlot arrival and more than 50% seroconverted to BRSV within 30 days (Booker et al., 1999). In a 2-year study, only 4% of calves were BRSV positive upon arrival and 61% had

seroconverted by day 28 (Martin et al., 1989). In that same study, seroconversion to BRSV was associated with BRDC morbidity. In another sero-epidemiological study, 72.2% of calves purchased through auction markets were seronegative to BRSV upon arrival and 77.9% seroconverted during the first 5 weeks on feed (Fulton et al., 2000). In one study, higher BRSV titers upon arrival were associated with reduced risk of BRDC (Martin et al., 1999). In another study arrival BRSV serostatus was not associated with BRDC morbidity risk (Collins et al., 1988). From these studies, exposure to BRSV differs greatly both in pre-weaning and post-weaning calf populations, and that higher titers may be protective, seronegativity is not necessarily a risk factor. Although BRSV is considered to be a primary BRDC pathogen, more epidemiologic research is needed to establish this organism role in calf pneumonia.

### ***Corona virus***

Corona virus (BRCoV) has recently been associated with BRDC, but its role in this disease is unknown. This organism was first isolated from pneumonic calves in 1993 (Storz et al., 2000). Later BRCoV was isolated from calves experiencing diarrhea and respiratory disease (Boileau and Kapil, 2010). The pneumonic viral strains are referred to as bovine respiratory corona virus (BRCoV), and it is unknown whether the pneumonic and enteric forms are antigenically different (Boileau and Kapil, 2010). Infection is usually by fecal-oral, and aerosol routes are secondary (Boileau and Kapil, 2010).

In a recent study 20% of calves from a single source were seropositive upon arrival and 41% had seroconverted by day 35 (Thomas et al., 2006). In the same study, 14% of multi-source calves purchased from sale barns were seropositive upon arrival and 89.9% seroconverted by 35. In another study, 58% of the calves seroconverted to BRCoV during the initial 28 days of feed,

and calves that seroconverted were 1.6 times more likely to require treatment for BRDC (Lathrop et al., 2000). O'Connor *et al.* (2001) reported that 90% of the calves were seropositive to BRCoV upon arrival (O'Connor et al., 2001). In a Canadian study, 83% of calves were seropositive upon arrival and BRCoV serostatus was not associated with BRDC risk (Martin et al., 1998). Calves that seroconvert to BRCoV within the first month on feed are at an increased risk of BRDC and are more likely to require multiple treatments (Lathrop et al., 2000; Plummer et al., 2004). Exposure to this organism appears to be common, but given the conflicting studies a need to define this organism's role in BRDC is very much needed.

The information discussed above suggests that virus exposure clusters within populations. Additionally, the importance of serostatus or seroconversion to BRDC control for all the mentioned viruses is conflicting. Perhaps the conflict can be explained by these parameters not measuring protection but other unassociated, unknown factors. Future research is needed to investigate the calf-pathogen reaction concentrating on identifying parameters that can be associated with protection.

## ***Bacterial pathogens***

### ***Mannheimia haemolytica***

*Mannheimia haemolytica* is considered the most important BRDC bacterial pathogen (Griffin, 2010). It is a commensal organism that resides in the upper respiratory tract and nasopharynx and becomes pathogenic after gaining access to the lower respiratory tract (Rice et al., 2007a). Although considered highly infectious, *M. haemolytica* is not considered highly contagious (Rice et al., 2007b). Griffin (2010) noted that this lack of contagiousness did not hold true when experimentally inoculated calves were mixed with non-inoculated calves (Griffin,



2010). The contagiousness Griffin noted may be more of a result of the inoculation procedure or bacterial dose, and not necessarily the contagiousness of the organism. *Mannheimia haemolytica* is a unique BRDC pathogen because of the number of virulence factors it possesses. These include adhesins, polysaccharides, leukotoxin, iron-binding proteins, and lipopolysaccharides (Confer, 2009). Leukotoxin is the most important virulence factor (Czuprynski et al., 2004). There is a synergistic relationship between BRDC viral pathogens as immunosuppressors and *M. haemolytica* (Rice et al., 2007a).

Upon arrival to a feedlot 95.4% of calves were *M. haemolytica* seropositive and all had seroconverted by day 30 (Yates et al., 1983). In another study, 50% of calves were seropositive upon arrival and 40% seroconverted over next 30 days (Booker et al., 1999). Additionally, calves that seroconverted to *M. haemolytica* were 2.83 times more likely than seronegative calves to experience BRDC (Booker et al., 1999). In a 3-year study, 70% of incoming calves were *M. haemolytica* seropositive and 45% had seroconverted by day 28 (Martin et al., 1989). In that same study, risk of BRDC was positively associated with *M. haemolytica* seroconversion. It is apparent that exposure to this organism is common in some pre-weaning calf populations and that exposure can continue in the feedyard. What is less clear is whether *M. haemolytica* is a primary or opportunistic pathogen and if primary, how infectious this organism may be.

### ***Pasteurella multocida***

*Pasteurella multocida* is an upper airway commensal and can be found in clinically normal and sick calves (Dabo et al., 2007). In an eight year (1994-2002) randomized study, utilizing the same bacterial isolation procedure, a decrease in the *M. haemolytica*: *P. multocida* ratio isolated from fatal BRDC cases was observed (Welsh et al., 2004). Co-infections with

other pathogens are common, and *P. multocida* is believed to have a synergistic relationship with *Mycoplasma* organisms (Dabo et al., 2007). Like other BRDC-associated bacterial pathogens, *P. multocida* is considered an opportunist; thought not to be able to produce disease on its own (Autio et al., 2007). Virulence factors possessed by *P. multocida* have not been well described but include adherence and colonization factors, lipopolysaccharides, iron-regulating proteins, and proteases (Dabo et al., 2007).

In a group of 62 beef calves, 36.4% were positive to *P. multocida* upon arrival and 60.4% had seroconverted by day 30 (Yates et al., 1983). In a case-control study *P. multocida* was the most prevalent organism found in the cases (Allen et al., 1991). Seroconversion to *P. multocida* was found not to be predictive of the probability of a future BRDC episode (Virtala et al., 2000).

Although *P. multocida*'s role in BRDC has been studied for many years, understanding of its role in BRDC is not well described. There is not enough research to say if this organism is becoming more or less prevalent, and future research is needed in this area, and more research into the association between prevalence, seroconversion and subsequent BRDC risk is needed.

### ***Mycoplasma bovis***

The causal role of *Mycoplasma bovis* in BRDC is not clear (Rosendal and Martin, 1986; Maunsell, 2009). However, some European researchers believe *M. bovis* is a major cause of BRDC, mastitis, and arthritis (Nicholas and Ayling, 2003). In Europe, *M. bovis* is thought to be responsible for 25% to 33% of all BRDC cases (Gevaert, 2006). *Mycoplasma bovis* infection usually occurs through the respiratory tract, and transmission can occur by direct contact with carriers or fomites (Confer, 2009). In addition, *M. bovis* is transferred in milk, a characteristic unique to potential BRDC pathogens, and nursing calves can become infected through exposure

to contaminated milk (Maunsell, 2009). A unique clinical feature of *M. bovis* is the appearance of arthritis in calves post- infection (Confer, 2009).

In a prevalence study at multiple stocker production units, 2% of calves were nasal PCR positive for *M. bovis* upon arrival. At the stocker unit level, 0% to 4% of farms were positive according to nasal PCR and 0% to 6% were positive according to nasal culture (Wiggins et al., 2007a). Another study revealed that 50% of calves were *M. bovis* seropositive at arrival and 40% of the negative calves had seroconverted by day 28 (Martin et al., 1989). This indicates that exposure to *M. bovis* continues after arrival and that stressed calves are able to mount an immune response. Calves that experienced BRDC had higher *M. bovis* titers on day 28 than calves that had not experienced BRDC (Rosendal and Martin, 1986). In the same study, there was no association between BRDC risk and *M. bovis* seroconversion status. In two other studies, there was no difference between BRDC cases and controls with respect to serostatus or seroconversion (Rosendal and Martin, 1986; Martin et al., 1989). In a more recent study, stocker calves that were nasal PCR positive were more likely (OR 10.6) to experience fever during a background period than negative calves (Wiggins et al., 2007b).

Difficulties in assessing the role of *M. bovis* in BRDC occur because this organism is more difficult to culture compared to other BRDC associated pathogens. Additionally, the frequent use of metaphylaxis products that are ineffective against *M. bovis* may have confused the true BRDC role of this organism. More research is needed to assess the role of this organism, and as newer antibiotics targeting *M. bovis* become available the role of this organism may change.

Furthering our understanding of BRDC requires additional research to determine the specific mechanisms of pathogenicity and virulence of bacteria as pathogens. Similar to issues

mentioned with BRDC viruses, conflicting conclusions of the importance of serostatus or seroconversion would be beneficial to future BRDC control methods.

### **Disease recognition**

Observation of pens of calves by humans continues to be the primary method of BRDC recognition. Observation of clinical signs such as anorexia, nasal discharge, lack of rumen fill, lethargy, and ocular discharge are subjective, and the criteria for categorizing cattle as sick vary between feedlots (Duff and Galvayan, 2006). In addition, personnel who conduct the observations have various biases and observational abilities. Because of the subjective nature of observation, and the observational skill and experience variability between observers, diagnosis is not always accurate (Duff and Galvayan, 2006). Once an animal has been observed to have one or more of the clinical signs mentioned above, it is usually taken to a treatment facility, where a rectal temperature is taken. Abnormal rectal temperature definitions vary between production units and range from temperatures  $> 103.0^{\circ}\text{F}$  to  $> 104.5^{\circ}\text{F}$ . Clinical illness scoring systems have been devised for BRDC recognition (Apley, 1997; Fajt et al., 2003). In these systems, calves are given a score of 1 to 4 (1 = normal, and 4 = moribund). Typically, calves with a score greater than 1 are removed from the pen, and a rectal temperature is taken. If the rectal temperature is above the normal defined range for that production unit, the animal is treated for BRDC (Apley, 1997).

Several studies have confirmed the lack of sensitivity of subjective observational measures. A study of 5,976 calves in a Midwestern feedlot revealed a BRDC morbidity incidence of 8.17%, but at harvest 61.9% of the animals had lung lesions suggestive of a previous BRDC episode (Schneider et al., 2009b). In an earlier study, 68% of calves that were

not observed to be ill from birth to slaughter had lung lesions suggestive of at least one BRDC episode (Wittum et al., 1996). In another study using Bayesian statistical methods, the sensitivity and specificity for clinical signs plus rectal temperature, was only 61.8% and 62.8% (White and Renter, 2009). It is clear that because visual observations are the first “diagnostic test” used in BRDC diagnosis the low sensitivity of this test is problematic. High sensitivity tests are ideal when used as disease screening tools (Dohoo et al., 2003). Because BRDC observation as a tool has low sensitivity and accuracy, disease recognition will continue to be a struggle.

Subjective measures of normal and diseased-animal behavior have been practiced, but quantifiable measures of animal behavior have been put forth only recently (Weary et al., 2009). For example, differences in watering and feeding behavior between healthy and sick calves have been investigated as an early BRDC recognition tool. One investigator, using remote activity monitors, found that number of daily feeding times predicted BRDC morbidity better than watering behavior, and that morbid calves spent less time at the feed bunk than healthy calves (Sowell et al., 1999). In another study, calves that became ill 11 to 27 days after arrival spent more time drinking and took more frequent drinks during the first 5 days after feedlot arrival than calves that remained healthy (Buhman et al., 2000). Other detailed quantitative measures have also been investigated. A *Mannheimia haemolytica* challenge study found that total steps taken in a 24-hour period decreased significantly immediately post-inoculation and remained lower than pre-inoculation levels for up to 9 days (Hanzlicek et al., 2010). Objective measures to accurately recognize BRDC can be accomplished using mechanical devices that measure behavior parameters.

Blood metabolites have been investigated as means of diagnosing BRDC or predicting BRDC survival. Of these metabolites, acute phase proteins such as haptoglobin, fibrinogen, and serum amyloid-A have been the most intensely investigated (Arthington et al., 2003; Ganheim et al., 2003; Ganheim et al., 2004; Humblet et al., 2004; Nikunen et al., 2007). Haptoglobin was a sensitive indicator of future BRDC morbidity, but another study showed that haptoglobin concentration was unassociated with the number of BRDC treatments and was a poor predictor of disease outcome (Wittum et al., 1996; Carter et al., 2002). In another study, fibrinogen was higher in calves with early BRDC than in normal calves (Berry et al., 2004). A combination of fibrinogen and haptoglobin had a sensitivity of 71% and a specificity of 83% for BRDC diagnosis based on a gold standard of calf history, clinical examination and either bronchoalveolar lavage and paired serology or necropsy (Humblet et al., 2004). In the same study, sensitivity increased to 80% when four acute phase proteins were used concurrently. Other blood metabolites have been investigated with respect to BRDC recognition. Coghe *et al.* (1999) found that oxygen saturation and blood lactate were not sensitive indicators of early BRDC (Coghe et al., 1999). In a challenge study, no blood metabolites measured by complete blood count, blood gas, or serum chemistries were accurate at recognizing early BRDC or predicting the severity of the lung lesions (Hanzlicek et al., 2010). Whether blood metabolites are usual indicators of BRDC has not been decided. Given these tests are confirmatory tests used after the insensitive test of calf observation, the utility of their use is guarded.

Infrared pictures have been investigated to determine if skin or ocular temperature are early indicators of BRDC. In two separate BVDV induction studies, cranial and ocular-thermography were found to be sensitive indicators of infection (Schaefer, 2004; Stewart et al., 2005). In another study, sensitivity and specificity were 67.6% and 86.8%, respectively, for

ocular infrared thermography, used as an early BRDC recognition tool, when compared to the gold standard of a core temperature  $\geq 104.0^{\circ}\text{F}$ , presence of nasal discharge or cough, a white cell count  $<7$  or  $>11,000 \mu\text{L}^{-1}$  and a neutrophil: lymphocyte ratio of  $<0.1$  (Schaefer et al., 2007).

This tool was compared with the already low sensitivity realized in BRDC recognition; therefore, the usefulness of this tool requires further research. The objective nature of the BRDC screening test is a difficult problem in the diagnosis of BRDC. Other recognition tools are confirmatory tools and more research is needed to assess their specificity.

## **Prevention and management**

Post-weaning disease prevention programs can be initiated during the pre-weaning or post-weaning phases of beef production. Pre-weaning programs include preconditioning and pre-sale vaccinations, dietary management, and post-weaning programs include arrival vaccinations, dietary management, biosecurity, and metaphylaxis.

In 1965, Dr. John Herrick coined the term “preconditioning” (Miksch, 1984). Since that time, no standardized definition as it applies to pre-weaned calves has been established (Lalman and Smith, 2000). Nonetheless, the term usually infers on-farm weaning, castration, dehorning, acclimation to bunk feeding, and vaccination. A study that compared preconditioned calves with control calves found that the preconditioned calves had a lower BRDC morbidity risk than the controls (Macartney et al., 2003). Morbidity risk was lower in calves vaccinated with a killed vaccine twice, 2 to 4 weeks before sale and again when commingled at the sale barn, than in calves vaccinated with a modified live respiratory vaccine either at the sale barn or upon arrival at the feedlot (Kreikemeier et al., 1996). In another study, preconditioned calves (vaccinated against viral pathogens 3 weeks prior to weaning and held at the farm for 25 to 30 days before

shipment to the feedyard) did not have fewer BRDC episodes than non-preconditioned calves (Pritchard and Mendez, 1990). Although preconditioning is believed to be an effective BRDC control practice, deficiencies in research to assess their effectiveness are present. Much of this is due to no strict definition of preconditioning. The studies listed above all were considered preconditioning studies but differed greatly in component parts, so drawing conclusions is difficult. Even the “best” preventive health program cannot be expected to be effective if not practiced. A high percentage of cow-calf producers sell calves at weaning (USDA-APHIS:VS, 2008). If preconditioning programs can be shown to be effective through proper research, then the task of communicating to producers and lenders will be an important next step.

Pre-sale vaccination programs are becoming more popular in North America (Macartney et al., 2003). The difference between pre-sale and preconditioning programs is that the former requires vaccination, castration, and dehorning prior to shipment but not source housing for 30 to 45 days. A study followed preconditioned calves, pre-sale-vaccinated calves, and auction-barn-purchased calves for 28 days after arrival to a feedlot and found that compared with auction-barn-purchased calves, preconditioned calves were 0.22 times as likely and pre-sale-vaccinated calves were 0.68 times as likely to be treated for BRDC (Macartney et al., 2003). Difficulties in assessing the effectiveness of this practice remains because of sparse published research. It is possible that pre-sale vaccination has risen because cow-calf operators have recognized the importance of preconditioning for BRDC control but they (and their lenders) many times have high risk aversion. Vaccinating prior to sale is therefore a method used to reduce risk and simultaneously “do something” to prevent BRDC.

In addition to preconditioning and pre-sale vaccination programs, feedlot arrival vaccination programs are also widely used. Although arrival vaccination programs are common,



and hundreds of BRDC vaccines are marketed, very few direct comparison BRDC multi-pathogen vaccine field studies have been published in referred journals (USDA, 2000a; Bowland, 2000). In a review of published BRDC vaccine field efficacy studies, few were found not to contain major design flaws (Perino and Hunsaker, 1997). The authors concluded that the effects of BRDC vaccination at arrival are equivocal. Despite the paucity of vaccine field studies, 98% of all feedlot-processed cattle are vaccinated against respiratory disease upon arrival or shortly after (USDA, 2000a). Many studies have been conducted to compare single pathogen vaccines or compare single and double pathogen vaccines with multi-pathogen vaccines, but few studies have included a negative vaccine control group (Jericho, 1982; Castrucci, 2002; Schunicht et al., 2003; Wildman et al., 2008). One exception is a Canadian study in which increased BRDC risk in calves vaccinated against respiratory pathogens upon arrival was noted (Martin et al., 1981). In that study, a multitude of vaccines were used and many different pathogens were targeted; the final model vaccine variable contained all vaccines and all pathogens, so direct comparison with non-vaccinated herds is difficult. To accurately assess the effectiveness of BRDC vaccines, more negative control vaccine studies are needed in the beef industry. Given the large amount of faith in vaccines, it is understandable that cattle producers or biologic companies will be reluctant to participate in these studies.

Metaphylaxis, another common practice, is the mass administration of parenteral antibiotics at arrival to groups of calves considered to be at high risk of BRDC. In the NAHMS 99 Feedlot study, the most common reason for initiating metaphylaxis was the appearance of the group of cattle upon arrival; the second most common reason was prior poor health outcomes in cattle from the same source (USDA, 2000b). According to the NAHMS 99 feedlot survey, 56.4% of the surveyed feedlots administered metaphylaxis, and a higher percentage of large

feedlots (82.1%) used metaphylaxis compared with smaller feedlots (46.2%) (USDA, 2000a). The difference in metaphylaxis use between feedlots of different sizes may indicate that larger feedlots receive more high-risk (long transported calves from multiple sources) than small feedlots, which may feed their own cattle or locally-raised cattle, or may indicate a difference in the priority of preventive health program funds.

One of the first studies that investigated the utility of metaphylaxis occurred in 1983 (Lofgreen, 1983a). In that study, groups of calves given parenteral oxytetracycline for 3 days after arrival had fewer treatment days per calf purchased (2.9 days) than non-medicated calves (3.7 days). A meta-analysis of 107 randomized field trials indicated that parenteral mass antibiotic administration to calves upon feedlot arrival reduced BRDC morbidity risk (Van Donkersgoed, 1992). In a Canadian study, calves given tilmicosin metaphylaxis had reduced BRDC morbidity compared with a control group (Schumann et al., 1990). In another study by the same authors three treatment groups were randomly assigned:(tilmicosin upon arrival, tilmicosin 72 hours after arrival, and control), metaphylaxis-given calves experienced less BRDC morbidity than the controls during the first month after arrival (Schumann et al., 1991). In a study of 57 stressed beef calves, morbidity for calves given tilmicosin upon arrival was 0% and morbidity for a control group was 46.4% (Galyean et al., 1995). In a follow-up study of 183 calves, the tilmicosin metaphylaxis calves again experienced lower (11.9%) BRDC morbidity than control calves (43.6%) (Galyean et al., 1995). In another tilmicosin metaphylaxis study, metaphylaxis calves had a lower morbidity risk (15.0%) than controls (54.0%); the mean days to morbidity was also higher for metaphylaxis calves than for controls (15.3 vs. 3.5 days, respectively)(McClary and Vogel, 1999). The results of these studies suggest that metaphylaxis is an effective practice for reducing BRDC morbidity. However, in a consumer survey study,

33% of participants said they would not purchase meat if they knew it came from an animal that had received antibiotics during the feeding phase (Brewer and Rojas, 2008). Despite the apparent effectiveness of administering antibiotics upon feedlot arrival, other methods that are more acceptable to consumers need to be developed and evaluated.

The prevention and management practices described above concentrate on improving calf immunity, reducing exposure to pathogens or controlling resident pathogen populations. Biosecurity on the other hand concentrates on reducing exposure to known and unknown pathogens. Biosecurity (i.e., reducing pathogen exposure and transmission) has been suggested as a means to control BRDC (Callan and Garry, 2002; Brandt et al., 2008). Strict biosecurity is difficult to accomplish in large feedlots because large numbers of cattle are purchased from multiple sources and subsequently housed together (Brandt et al., 2008). Additionally, many BRDC pathogens are ubiquitous and cannot be eliminated from cattle populations. However, some biosecurity measures seem appropriate and practical in the feedyard. For example, preventing healthy calves from direct exposure to pathogens harbored by sick animals through isolation is a practical biosecurity method. Brandt *et al.* (2008) surveyed Midwestern feedlot managers and reported that 36.8% allowed direct fence-line contact between sick and health calves (Brandt et al., 2008). Because BRDC is multi-factorial and there is difficulty accomplishing suggested biosecurity practices in our modern beef production systems, it may be more effective to concentrate on other control methods in conjunction with biosecurity.

## **Conclusion**

Bovine respiratory disease complex is an important disease found in both pre-weaned and post-weaned calf populations. This disease is multifactorial and differences in risk factors between the two populations do exist, although the associated pathogens are similar.

Regardless of the population, BRDC research and control will be more effective if the multifactorial nature of this disease remains within focus. Needed research in the pre-weaning phase relates to assessing proper immunization timing, identifying management practices as risk factors, and developing methods of BRDC recognition. Research results conflict into the importance of vaccine timing or type of product used; more research is needed. No published research investigating management practices as risk factors for pre-weaning BRDC has been published. This information may be an important first step in controlling this disease on cow-calf operations. In the post-weaning phase many risk factors associated with BRDC have been recognized. Some risk factors are result of management practices that occur in the pre-weaning phase. Unfortunately, some of these risk factors will continue to be difficult to manage because of demographic constraints, herd size, and long-standing traditions that exist in the U.S. beef production systems. This necessitates research to discover objective, sensitive, and practical methods for early BRDC detection. Treatment success requires early disease recognition, and only through accurate diagnosis will we be able to assess treatments successes and offer timely prognostications. Additionally, research assessing BRDC preventive health programs will continue to be important because some risk factors will be difficult to manage. Lastly, given some public aversion to antibiotic use in livestock and the possible interaction between antibiotic use and bacterial antibiotic resistance, research into other methods, such as diet modulation or

genetic manipulation will continue to be important. Finally, bovine respiratory disease complex is aptly named, because of its multifactorial complexity. Future research to reduce BRDC's effects will require interdisciplinary efforts by researchers with common goals.

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## **CHAPTER 2 - A field study evaluating health, performance, and behavior differences in crossbred beef calves administered different vaccine-parasiticide product combinations**

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(Published in *Vaccine*, 2010, 28:5998-6005)

### **Abstract**

Bovine respiratory disease complex (BRDC) is the most important health issue in beef feeder calves. Our study was a randomized, blinded field trial to evaluate potential differences in health, production and behavior in feeder calves administered two different preventive health programs. Calves in two replicates (n=305 and n=308) were allocated to pens and then pens were randomly assigned a preventive health program. One program (Prog1) consisted of 1 injectable clostridial vaccine, 1 intranasal modified live respiratory vaccine, 1 topical and 1 oral parasiticide. The other program (Prog2) consisted of 1 injectable clostridial vaccine, 1 modified live respiratory vaccine and 1 injectable parasiticide. A greater percentage of calves in Prog1 (59.7%) experienced BRDC morbidity compared to the Prog2 program (47.8%). There were no



differences between programs in mortality, case-fatality, 1<sup>st</sup> treatment success or chronicity risks. The average daily gain over the entire study period for the Prog2 calves (1.23 kg) was greater than the Prog1 calves (1.16 kg). Calves administered Prog1 on average took more steps each day during the first 28 days of the study. Additionally, Prog1 calves spent more time lying down on certain days during the last 14 days of the study. During initial program administration, fewer Prog1 calves (39.8%) vocalized compared to Prog2 calves (47.8%). In this study, calves administered a program with fewer injections indicated less aversion to program administration than those administered more injections, but experienced greater morbidity and poorer performance.

## **Introduction**

Bovine respiratory disease complex (BRDC) continues to be the most economically important disease in post-weaned calves (Kelly and Janzen, 1986; NAHMS, 2000; Loneragan et al., 2001). The negative economic effects include reduced performance, increased treatment and labor expense, and reduced carcass quality (Jim et al., 1993; Griffin, 1997; Snowder, 2006; Duff and Galyean, 2007). This disease complex is believed to be multifactorial with components that include viruses, bacteria, stressors, environment and genetics (Edwards, 1996; Snowder et al., 2005; Berghaus et al., 2006). Primarily due to the complex nature of this disease complex, BRDC has been shown to be increasing in frequency despite the creation of new immunization and metaphylaxis regimes (Loneragan, 2004). One challenge for controlling BRDC is the few objective clinical disease recognition measures that exist (Hanzlicek et al., 2009). Some studies have shown that the number of calves treated for BRDC is much lower than the number with pulmonary lesions at slaughter (Wittum et al., 1996). This suggests that inadequacies in BRDC

identification exist, and have increased the need to find effective preventive programs, and numerous studies investigating the individual components of these control programs have been completed. For example, various vaccine types including modified live and killed products, vaccine combinations and timing of vaccine administration, have been investigated as potential BRDC control program components (Martin et al., 1983; Van Donkersgoed et al., 1990; Perino and Hunsaker, 1997; Vangeel, 2007; White et al., 2008; Wildman et al., 2008). In addition, dietary components such as roughage, concentrate, protein percentage and mineral/vitamin concentration have been investigated as possible control program components (Cole and Hutcheson, 1990; Gallo and Berg, 1995; Galyean et al., 1999; Salyer et al., 2004; Rivera, 2005).

The objective of this study was to evaluate potential differences in health, performance and behavior between two feeder-calf arrival health programs differing based on products and number of injections: one program (Prog1; 1 oral, 1 intra nasal, 1 topical, 1 subcutaneous injection) and another program (Prog2; 2 subcutaneous and 1 intramuscular injection). We hypothesized that calves administered the program with fewer injections may behave differently and outperform, both in health and performance, calves administered a more invasive program. This research is unique as it compares overall preventive health programs (including products and methods of administration) with respect to health, performance and behavioral outcomes.

## **Materials and methods**

This experimental protocol was approved by the Kansas State University Animal Care and Use Committee. (Protocol #2571).

## ***Animals***

This study was completed in two replicates using crossbred beef calves. Calves were procured in the Southeast United States (Tennessee and Kentucky) through livestock order-buyers, and transported (average distance 1223 kilometers) to the Kansas State University Stocker Unit (KSBSU), Manhattan, Kansas. Each replicate consisted of three truckloads of cattle where one load of approximately 100 calves arrived each day for 3 consecutive days. Each truckload was housed in a separate string consisting of 8 adjacent dry-lot pens.

## ***Preventive health programs***

All products contained in the health programs are commercially available and were administered according to labeled instructions. The minimally invasive program, hereafter referred to as Prog1, consisted of intranasal modified live vaccine including bovine viral diarrhea virus Type I and II, parainfluenza 3 virus, bovine respiratory syncytial virus, and infectious bovine rhinotracheitis virus, (Onset 5 IN<sup>®</sup>, Intervet/Schering Plough Animal Health, DeSoto, KS); an injectable clostridium bacterin, containing *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyii*, *Clostridium sordellii*, *Clostridium perfringens B, C & D* (Vision 7<sup>®</sup> Intervet/Schering Plough Animal Health, DeSoto, KS); oral fendbendazole endo-parasiticide (Safeguard<sup>®</sup> Intervet/Schering Plough Animal Health, DeSoto, KS) and a topical avermectin endo-ectoparasiticide (Ivomec Pour-On<sup>®</sup>, Merial, Duluth, GA) (Table 1). The more invasive program, hereafter referred to as (Prog2), consisted of an injectable modified live vaccine including bovine viral diarrhea virus, parainfluenza 3 virus, bovine respiratory syncytial virus, and infectious bovine rhinotracheitis virus (Bovishield Gold 5<sup>®</sup>, Pfizer Animal Health, New York, NY); an injectable clostridium bacterin containing *Clostridium chauvoei*, *Clostridium*

*septicum*, *Clostridium novyii*, *Clostridium sordellii*, *Clostridium perfringens* C & D (Ultrabac 7<sup>®</sup> Pfizer Animal Health, New York, NY) and an injectable doramectin endo- ectoparasiticide (Dectomax Injectable<sup>®</sup> Pfizer Animal Health, New York, NY, (Table 1). In addition on study day 28, the Prog1 calves were administered the same clostridium vaccine as on arrival (study day 0), and the MOR calves were administered an injectable modified live vaccine containing bovine respiratory syncytial virus (BRSV) (Bovishield BRSV<sup>®</sup>, Pfizer Animal Health, New York, NY) and the same clostridium vaccine as they received on arrival. The clostridium vaccines for both program groups and the BRSV vaccine for the Prog2 group were re-administered as recommended by the vaccine product label.

### ***Preventive health program allocation***

Upon arrival to the study facility (study day 0), calves were individually weighed, determined to be a bull or steer, and given a unique ear-tag identifier. Calves were assigned pens using a random number generator in a commercially available software program (Excel<sup>®</sup>, Microsoft Corporation, Redmond, WA), and pens were balanced for weight and gender. All pen assignments were done within load resulting in eight pens per load (24 pens in each replicate). A coin flip at every other pen was used to allocate the preventive health program (Prog1 or Prog2); the coin indicated the program for the first pen with the subsequent pen receiving the alternate program. Thus, one half the pens within each load were assigned to each program. Four adjacent pens from a single load in each replicate (2 pens of each program) were selected to participate in the behavior monitoring portion of the study. The random number generator was used to assign calves in the behavior monitoring pens to wear both an accelerometer and pedometer (n=32 each replicate), or pedometer only (replicate 1, n= 20 and replicate 2, n= 21)

Equal numbers of accelerometer/pedometer and pedometer only calves were contained in each program group. These numbers were chosen pre-trial considering the number of accelerometers that were available.

### ***Preventive health program administration***

The day after arrival (study day 0), calves in each load were administered their assigned program, administered metaphylaxis, ear tissue sampled and bulls were surgically castrated without the use of anesthesia. Program details are listed in Table 1. Programs were administered to all calves by the same individual according to labeled directions. Modified live viral vaccines were administered either intranasally as a single dose (2ml) in the left nostril or intramuscularly (2ml) in the right cervical region for the Prog1 and Prog2 programs, respectively. Clostridial vaccines were given subcutaneously in the left cervical region, to calves in Prog1 (2ml) and Prog2 (5ml) programs. The Prog1 calves received both a topical (500 mcg/kg BW) and oral (5 mg/kg BW) parasiticide. Calves in the Prog2 program received a subcutaneous parasiticide (200 mcg/kg BW). Castrations for all calves were performed by the same experienced veterinarian using a Newberry knife (Jorgensen Lab, Loveland, Colorado) and White's Double Crush emasculator (Jorgensen Lab, Loveland, Colorado). All calves were given ceftiofur crystalline free acid (Excede<sup>®</sup>, Pfizer Animal Health, New York, New York) subcutaneously in the base of the right ear, with the dosage (6.6 mg/kg BW) calculated on average load weight. Biopsies were collected from the right ear of each calf for bovine viral diarrhea virus antigen capture ELISA analysis. Twenty-eight days after arrival, calves in both programs were re-vaccinated with clostridial vaccines, and Prog2 calves were also administered a modified live BRSV vaccine (Table 1).

### ***Feeding program***

All ingredients are reported on a dry matter basis. The arrival diet consisted of prairie hay containing 7.0% crude protein and 0.44 mcal/kg net energy gain (NEg) and ad libitum water. Beginning two days after arrival, the calves were fed a total mixed ration (TMR) consisting of mixed grass hay, alfalfa hay, dry rolled corn, wet corn gluten feed, and a commercial premix pellet (Cargill Animal Nutrition, Minneapolis, Minnesota). This ration was formulated to contain 15.2% crude protein and 1.09 mcal/kg NEg. Beginning on post-arrival day 8 and continuing through day 18, calves were fed a TMR incorporating the same ingredients as above, but containing 15.2% crude protein and 1.14 mcal/kg NEg. On day 19 and continuing through the study endpoint, calves were fed a TMR utilizing the same ingredients formulated to contain 14.4% crude protein and 1.20 mcal/kg NEg. Feed bunks were observed and scored twice daily, and the amount of feed not consumed was used as a basis for the amount delivered at the next feeding.

### ***Vocalization and chute exit score***

A single non-blinded evaluator determined whether each calf vocalized or not during program administration, including vaccine and parasiticides. This determination was completed before metaphylaxis or castration processes were initiated, and any vocalization was considered a positive. Additionally, each calf was assessed a chute exit score (1 walk, 2 trot, 3 run, 4 jump) after program administration by the same non-blinded individual. (Grandin, 1998)

### ***Health monitoring***

Calves were observed for health status twice a day by animal care givers employed by the production unit (KSBSU). The care givers were blinded to the preventive health program

allocation of each pen. Calves with clinical illness score (CIS) greater than 1 (1 normal; 2 mild depression, gaunt; 3 severe depression, labored breathing, ocular/nasal discharge; 4 moribund, near death, little response to human approach) were taken to the working facility for physical examination. Animals with a CIS greater than 1 and a rectal temperature  $\geq 40^{\circ}\text{C}$  and not presenting of signs indicating non-respiratory disease were given antibiotics according to the production unit's standard operating procedure. The regimen for BRDC treatment was: first illness, florfenicol (Nuflor®) 40mg/kg; second illness, enrofloxacin (Baytril®) 10mg/kg, and third illness oxytetracycline (Biomycin® 200) 4 mg/kg. In both replicates combined, ten total calves were treated for diseases other than respiratory disease, (n= 4 for scrotal infection, n = 2 for lameness, n = 2 for diarrhea and n =2 for keratoconjunctivitis). No hospital pen was used in this study; all calves were returned to their original pen after physical examination and medication. Calves observed to be ill, examined and administered antibiotics for the third time were designated as chronic and were not medicated again. Health outcomes of interest included morbidity, first treatment success, chronicity, case-fatality, and mortality. (Table 2)

### ***Production parameters***

Calves were individually weighed on three occasions: arrival (day -1), revaccination (day 28) and study completion (approximately day 42). Average daily gain (ADG), was calculated for three time periods: arrival to day 28, day 28 to study completion, and from arrival to study completion. These 3 time periods will be hereafter be known as arrival, revaccination, and entire study period, respectively. Total as-fed feed delivered to each pen was recorded and used as a proxy for feed intake and to calculate pen-level feed to gain ratios from arrival to study

completion. All production parameters, except feed delivered, were analyzed with mortalities removed from the data set and are defined in Table 2.

### ***Behavioral assessment***

Behavior was monitored for all calves within designated pens using pedometers and accelerometers. The pedometers and accelerometers were applied to the right distal metatarsus using a padded self-adhesive neoprene strap. The entire apparatus, including pedometer, accelerometer and batteries, weighed approximately 0.5 kg. All calves within the behavior assessment pens were taken to the working facility once a week to download accelerometer and pedometer data. Because of the time the calves spent away from their pens during the data download days, these data were removed from the data analysis. Data were analyzed for two time periods: 1) arrival processing through study-day 13 and 2) revaccination (day 28) to study completion. These 2 time periods will be referred to as ARR and REVAC, respectively, throughout the manuscript.

Postural assessment (percent time spent standing and lying down) was completed using accelerometers (GP1 Programmable Accelerometer, Sensr, Elkader, Iowa). The accelerometer recorded five variables at 100 readings per second: average acceleration in three axis (X, Y, Z), vector magnitude average, and vector magnitude maximum. A commercial software program (Sensware, Sensr, Elkader, Iowa) was used to average the readings over a 5 second time period. The total time spent each day lying down or standing was calculated using a pre-established algorithm. (Robert et al., 2009) Pedometers (NL-800 Activity Monitor, New Lifestyles, Lee's Summit, Missouri) were used to measure total steps taken in a 24 hour period.



### *Statistical analysis*

Data were analyzed with a commercial software program (SAS v. 9.1). Logistic regression (generalized linear mixed) models were used to analyze vocalization, and health outcome data. Vocalization models included chute-exit-score as a fixed effect to control for possible confounding (Dohoo et al., 2003). Random effects in the logistic models evaluating potential associations between preventative health program and health outcomes included replicate, truckload within replicate and pen within truckload to account for lack of independence among calves (Dohoo et al., 2003). General linear mixed models were used to evaluate potential associations between the preventative health programs and pen-level ADG, ratio of kilograms of feed fed to kilograms of weight gain, and kilograms of feed delivered. Random effects in these models included, replicate, truckload and truckload within replicate. For the cattle which had behavioral measures, only data from calves not becoming morbid during any portion of the trial were used in the analyses. General linear mixed models were used to evaluate potential associations between health program and the total number of steps taken in a 24 hour period (pedometer), the study day relative to arrival or revaccination, and the potential interaction between step counts and study day; castration data were evaluated in this analysis as a potential confounder. Each model accounted for repeated measurements on calf and the lack of independence between calves within pen and replicate (Dohoo et al., 2003). Mixed effects logistic regression was used to evaluate associations between health programs and the effects of the daily percent of time spent lying (determined by accelerometers), castration, study day relative to arrival or revaccination, and the potential interaction between percent of time lying and study day. The two time periods (13 days post-arrival and 14 days post-revaccination) were modeled separately and each model included adjustments for repeated measurements on calves

and random effects for pen and replicate. A *P* value of  $\leq 0.05$  was considered significant for all models.

## **Results**

### ***Study subjects***

The study population for both replicates combined consisted of 308 calves in Prog1 and 305 in Prog2. The breakdown of calves within program by replicate was Prog1 154 and 154 and Prog2 152 and 153 for replicate 1 and 2, respectively. Mean arrival weights were 207.9 kg and 208.0 kg for Prog1 and Prog2 groups, respectively. By program group within replicate 1 the arrival weights were 207.2 kg (Prog1) and 207.5 kg (Prog2) and for replicate two, 208.7 kg (Prog1) and 208.6 kg (Prog2). The Prog1 group contained 65% bulls (202/308), 100 in the first replicate and 102 in the second replicate, and the Prog2 group contained 66% bulls (202/305), 101 in the first replicate and 101 in the second replicate. One calf in each replicate, both Prog2 calves, was positive for BVDV persistent infection by antigen capture ELISA. These calves were removed from the study and study site on day 2 and 7, for replicate 1 and 2, respectively.

### ***Health***

Morbidity in replicate 1 was 65.5% (101/154) for Prog1 and 47.8% (84/152) for Prog2. In replicate 2 morbidity was 53.8% (83/154) and 40.5% (62/153) for Prog1 and Prog2, respectively. Mortality for replicate 1 was 2.6% (4/154) and 3.2% (5/152) for Prog1 and Prog2, respectively. Replicate 2 mortality was 4.5% (7/154) for Prog1 and 0.6% (1/53) for Prog2. Case fatality for replicate 1 was Prog1: 3.9% (4/101) and Prog2: 5.9% (5/84). For replicate 2 case fatality was 8.4% (7/83) and 1.6% (1/62), for Prog1 and Prog2, respectively.

There was no significant interaction between preventive health program and replicate; therefore, data from the replicates were combined for final analysis. Morbidity for Prog1 was 59.7% (184/308) and 47.8% (146/305) for Prog2. The percentage of all morbidity that occurred during the first 28 study days was 92.7% (306/330). The percentage of mortal Prog1 program calves was 3.5% (11/308) and 1.9% (6/305) for Prog2 program calves. Case fatality for the Prog1 group was 5.9% (11/184) and for the Prog2 group 4.1% (6/146). The percentage of calves defined as chronic was 16.8% (31/184) and 11.6% (17/146) for Prog1 and Prog2, respectively. First treatment success for Prog1 was 60.9% (72/184) and for Prog2 was 64.4% (52/146). The multivariable model indicated that morbidity was lower ( $p = 0.02$ ) in Prog2 (47.8%) calves compared to Prog1 (59.7%). (Table 3) There were no significant differences between groups in first treatment success, chronicity, case fatality and mortality. (Table 3)

### ***Performance***

The ADG for ARR was 1.24 kg/day and 1.33 kg/day for Prog1 and Prog2, respectively. For REVAC the ADG for Prog1 was 0.98 kg/day and Prog2 was 1.03 kg/day. From arrival to study completion (ENTIRE) was 1.16 kg/day for Prog1 and 1.23 kg/day for Prog2. Average daily gain (kg/day), for ARR period was greater ( $p = 0.05$ ) in Prog2 compared to Prog1. (Table 4) Average daily gain for the revaccination period was not different between programs. For the period from arrival to study completion, average daily gain was greater for Prog2 (1.23 kg) compared to Prog1 (1.16 kg) ( $P = 0.04$ ). No differences were found in feed to gain ratio or feed delivered between programs. Unadjusted feed to gain ratio was 3.32 kg/kg and 3.13 kg/kg for Prog1 and Prog2, respectively. Feed delivered for Prog1 was 87.60 kg and 90.57 kg for Prog2 (Table 4).

## *Behavior*

Pedometer data from 4 calves in replicate 1 for the ARR period, and 1 calf in replicate 1 and 4 calves in replicate 2, for the REVAC period, were not used for analysis due to pedometer malfunction. As previously mentioned, data from data downloading days (7, 14, 42) were removed from the analysis. No interaction between program and study day or replicate was found for either time period. Model estimated mean steps taken per 24 hour period during the ARR period, tended to be greater ( $p=0.07$ ) for the Prog1 calves (2620) compared to the Prog2 calves (2449). There was no difference between mean steps taken during the REVAC period.

For combined replicates, 64 calves wore accelerometers ( $n=32$  in each replicate). Data from calves wearing accelerometers that became morbid (Prog1:  $n=15$ , Prog2:  $n=18$ ) during any portion of the study were removed from behavior analysis. Additionally, data from 2 calves within each program for the REVAC period were removed from the analysis due to accelerometer malfunction and incomplete data capture. For the first replicate 9 Prog1 and Prog2 calves, and for second replicate 9 Prog1 and 9 Prog2 calves were included in the analysis.

For the percentage of time spent lying down, a program by study-day interaction was found for both time periods examined: ARR (Figure 1) and REVAC (Figure 2). The Prog2 program calves did spend more time lying down on days 32 and 34 compared to the Prog1 calves. (Figure 2). Figure 1 shows that the Prog2 calves spent more time lying down during the early study days within the ARR period, but lying down behavior for the programs became similar later in this period. During the REVAC period, a similar pattern with Prog2 calves lying down more was observed until day 34, when the Prog2 calves time spent lying down decreased and the Prog1 group time spent lying down increased. (Figure 2)

## ***Vocalization***

The percentage of calves vocalizing at initial program administration was less ( $P=0.05$ ) in Prog1 (39.8%) compared to Prog2 (47.8%). An association between exit score and the probability of vocalizing was found ( $P<0.01$ ). The percentage of calves vocalizing decreased as each exit score decreased from 1 to 3. The percentage of calves that vocalized were 63.0% (CI 52.8, 72.2), 52.2% (CI 42.7, 61.5) and 34.4% (CI 25.2, 44.8) for exit score 1, 2, and 3 respectively. No difference in vocalization was found between exit score 3 and 4, 27.4% (CI 17.2, 40.8).

## **Discussion**

Calves entering feeder-calf production units are normally administered a combination of preventive health products comprised primarily of vaccines and parasiticide combinations. Although combinations of products are routinely administered, most research studies concentrate on comparing the efficacy of individual products. Our study is unique because two combinations of products were compared, which we have designated “programs”. Because we compared programs, the determination of which particular product(s) contributed to the health, performance, and behavioral differences identified in the current research is impossible to determine.

The percentage of calves vocalizing during initial program administration was higher in Prog2 program calves compared to Prog1 calves. The results from our study are similar to a study investigating aversion to injections between calves that were blinded or not-blinded to the presence of humans administering the treatments (Mitchell et al., 2004). This study concluded the injection process is responsible for the aversion demonstrated by the calves and not the mere

presence of, or handling by, humans. Other research has indicated that procedures involving the head and ears, such as ear tagging and metaphylaxis administration, result in more frequent vocalization compared to procedures involving only the cervical region (Grandin, 1998). In our study, administration of the Prog1 program did require head manipulation for intranasal vaccine application, but not ear manipulation (vocalization assessment occurred before metaphylaxis was initiated), which may help explain the difference between the two studies.

Neither program appeared to effectively prevent BRDC, but we did not have a negative control group and the level of morbidity was similar to that reported in other beef feeder cattle studies (Sowell et al., 1999; Richeson et al., 2008). Other researchers have observed differences in BRDC incidence between vaccination combinations (Wildman et al., 2008). The Prog1 program calves did experience more BRDC morbidity compared to Prog2 calves. Both programs contained vaccines and parasiticides that targeted the same organisms, but differences in route, dosage, and vaccine strains were present. Bovine viral diarrhea virus (BVDV) vaccine strains for Prog1 were Type I: Singer, Type II :125A and for Prog2 were Type I: NADL, Type II : 53637 (Ridpath, 2005). Cross reactivity between vaccine and field strains is an important consideration in vaccine selection (Ridpath, 2005). In our study, a BVDV persistently infected animal was found in each of two replicates, and transiently BVDV infected animals may have also been present. Although we did not attempt to isolate and characterize strains, it is possible that any BVDV field strains that may have been present were more genomically similar to the strains contained in the Prog2 vaccine and therefore more effective vaccine protection occurred. A difference also existed in the mode of viral vaccine delivery with the Prog1 calves receiving an intranasal vaccine and the Prog2 calves an intramuscular vaccine. Shewen *et al.* suggest that antibodies to some pathogens produced by mucosal delivered vaccines are transient and do not

persist on the mucosal surface without constant antigenic stimulation (Shewen P. E. , 2009). Some calves in the Prog1 group may not have been able to maintain long-term mucosal immunity sufficiently to prevent infection.

The parasiticide agents and route of administration also differed between programs, and this may have contributed to the morbidity differences. Internal parasites may negatively impact an animal's ability to mount immune responses to bacterial infections, and studies in mice have demonstrated a negative impact on vaccine efficacy (Mulcahy et al., 2007; Urban et al., 2007). Although previously published information has shown the products used in both programs to be effective in reducing internal parasite populations, parasiticide efficacy in the present study was not evaluated (MacGregor et al., 2001; Reinhardt et al., 2006; Ives et al., 2007). Differences in efficacy of parasiticides between programs may have contributed to our findings.

Although a greater percentage of Prog1 calves became morbid from BRDC compared to Prog2 calves, treatment success outcomes (case fatality, first treatment success, chronicity) did not differ between groups. This suggests the BRDC episodes in the Prog1 group, although more common, were not more severe than those experienced by the Prog2 group.

Calves in the Prog1 program had lower ADG compared to Prog2 calves during the arrival period and over the entire study period. This is not surprising given a greater percentage of Prog1calves became clinically ill during the trial, and our results are similar to other studies showing BRDC's negative effect on ADG (Gardner et al., 1999; Waggoner et al., 2007). Although there was a difference between preventive health programs in ADG over the entire study period, there was no difference during the revaccination period. Most initial BRDC episodes, and program morbidity differences, occurred during the first 28 study-days (arrival time period), and it would be expected that ADG would be impacted more during this time

period relative to the last 14 study days when morbidity was relatively low. The difference in ADG between Prog2 and Prog1 over the entire study contrasts with no difference found for both feed intake and feed to gain ratio over the same time period. In a different study, dry matter to gain ratio was less for morbid calves compared to non-morbid calves during the first 28 day study period (Jim et al., 1993). This discrepancy may be explained by pen size and health-observation intensity. In our study, the pen population was low (11-14 calves) possibly resulting in early BRDC recognition and treatment, which may allow morbid calves' feed intakes to return to normal levels early in the course of disease. Feed conversion results from our study differ with Chirase *et al.* who showed that calves subcutaneously vaccinated with Vision 7<sup>®</sup> had lower feed to gain ratio during the first 28 days after arrival to a feedlot compared to calves subcutaneously vaccinated with Ultrabac 7<sup>®</sup> (Chiras, 2001). In their study morbidity levels were not reported, and no other health products were administered. In our study, morbidity was greater in calves in the program that contained Vision 7<sup>®</sup> which may have reduced any feed conversion differences that may have existed because of clostridial vaccines. Additionally, because our study compared total health programs and not a single vaccine, other program components may have affected the impact of the *Clostridium* vaccines in a manner differently than was observed in the Chirase study.

Calves administered the more invasive program (Prog2), tended to take fewer steps during the ARRIV period. Because we assessed behavior only in non-morbid calves, this difference is not likely due to the difference in morbidity between groups. Our vocalization results indicated that the Prog2 program was more objectionable, through greater vocalization, and this aversion may have carried over as fewer steps taken into the early portion of the study. The difference in steps may have been an indication of covert lethargy or malaise from the



multiple invasive products given on arrival. Because more Prog1 calves experienced morbidity, it is likely more human activity occurred within the Prog1 pens (additional human activity to remove morbid calves from the pen for treatment). Therefore, it is possible that the Prog2 calves did not take fewer steps than expected, but instead the Prog1 calves took more steps due to the additional human activity. This theory is supported by the evidence that no difference in step counts between programs was observed in the REVAC period, when morbidity in both groups was relatively low. Alternatively, the low number of injections (1 or 2) received by each group at revaccination did not elicit a large enough behavioral response to be demonstrated using pedometer data.

A program by study-day interaction for time spent lying down each day was observed in both time periods (ARRIV and REVAC) evaluated in our study. In the ARRIV period, there was separation between the estimated time lying on days 3 through 5 post-arrival with the Prog2 calves spending a numerically higher amount of time lying. The Prog1 group experienced more morbidity; therefore, this discrepancy may be explained by a larger number of sub-clinical BRDC cases occurring in the Prog1 group during this time period or additional human interaction as mentioned above. The subclinical theory is substantiated by the results from a study where induced Mannheimia pneumonia calves spent less time lying down per day after disease onset (Hanzlicek et al., 2009). Evaluation of 14 days following revaccination (day 28) revealed that calves in the Prog2 program spent more time lying down on days 5 and 7 post-revaccination compared to Prog1 calves. The effect appeared to be transient and no differences were identified after 8 days post-revaccination. Lying behavior in the Prog2 calves may have been due to a transient lethargic response to the higher dose clostridium vaccine and / or the MLV BRSV vaccine. Apley, *et al.* showed an increase in post-vaccination subcutaneous lesion

size when comparing 2ml and 5ml clostridium vaccines (Apley M, 1994). If lesion size is an indication of inflammatory response, it is possible the Prog2 calves were indicating, by spending more time recumbent, the greater inflammation response to the 5cc dose clostridium vaccination. The BRSV vaccine may have also played a role in this behavioral response, but previous research did not demonstrate behavioral changes (depression) post-BRSV vaccination (Patel J, 2004). However, our study used an objective measure of behavior that may have detected smaller behavioral differences than could be identified through subjective measures.

## **Conclusion**

To the authors' knowledge this is the first controlled study comparing two complete feeder-calf health programs and demonstrating differences in some health, production, and behavior outcomes between programs. Fewer calves in the Prog1 program vocalized during initial program administration suggesting programs that incorporate primarily non-injectable products into preventive health regimes are less aversive to calves. Calves in the Prog1 program experienced higher morbidity and lower average daily gain during the early portion of the study and over the entire study period. Calves administered the (Prog1) program tended to take more steps during the early portion of the study. On days after revaccination, the calves administered the more invasive program (Prog2) spent more time lying down. Future research using objective, constant postural monitoring technology, such as accelerometers and pedometers, is needed to gain understanding into calf behavior. Because we compared complete health programs, it is impossible to say which product(s) within each program were responsible for the outcome differences. Because these programs typically contain several components, our study also illustrates the challenges in designing and evaluating new feeder calf health programs, and

the value in utilizing objective measures to evaluate calf behavior as it is affected by these programs.

## **Acknowledgements**

The authors thank Marc Epp, Brandon Greenwood, and Chance Gregory, animal care providers at the Kansas State Beef Stocker Unit, for observing and feeding the calves and recording data. For their help administering the programs and recording data, we thank Kelli Allen, Abram Babcock, Dr. Jason Nickell, Craig Pauly, Brad Robert, Debra Wilcox, and Dr. Ben Wileman.

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**Table 2-1 Animal health products, by program and study period, used in a study to compare health, performance and behavior between crossbred beef calves**

<b>Arrival</b>						
<b>Program</b>	<b>Clostridial</b>		<b>Respiratory/Viral</b>		<b>Parasiticides</b>	
	Product	Dose/route	Product	Dose/route	Product	Dose/route
Prog1 <sup>a</sup>	Vision <sup>®</sup> 7 <sup>c</sup>	2ml/SQ	Onset 5IM <sup>®c</sup>	2ml/IN	IvomecPourom <sup>®d</sup> + Safeguard <sup>®c</sup>	25ml/topical 11 ml/oral
Prog2 <sup>b</sup>	Ultrabac <sup>®</sup> 7 <sup>e</sup>	5ml/SQ	Bovishield <sup>®</sup> Gold 5 <sup>e</sup>	2ml/IM	Dectomax <sup>®</sup> injectable <sup>e</sup>	25ml/SQ

<b>Revaccination</b>						
<b>Program</b>	<b>Clostridial</b>		<b>Respiratory/Viral</b>		<b>Parasiticides</b>	
	Product	Dose/route	Product	Dose/route		
Prog1 <sup>a</sup>	Vision <sup>®</sup> 7 <sup>c</sup>	2ml/SQ	None	--		None
Prog2 <sup>b</sup>	Ultrabac <sup>®</sup> 7 <sup>e</sup>	5ml/SQ	Bovishield <sup>®</sup> BRSV <sup>e</sup>	2ml/IM		None

<sup>a</sup> Prog1= minimally invasive program containing 1 injectable component at both arrival and revaccination

<sup>b</sup> Prog2= more invasive program containing 3 injectable components on arrival and 2 injectable components at revaccination

<sup>c</sup> Intervet/Schering Plough Animal Health, Omaha, NE

<sup>d</sup> Merial, Duluth, GA

<sup>e</sup> Pfizer Animal Health, New York, NY



**Table 2-2 Definition of pen-level health and performance variables used in comparing two preventive health programs for crossbred beef feeder calves**

<b>Variable</b>	<b>Definition</b>
Morbidity	number of 1st BRDC <sup>a</sup> treatments in each pen ÷ number of animals allocated to each pen
Mortality	number of BRDC mortalities in each pen ÷ number of animals allocated to each pen
Case fatality	number of BRDC mortalities that occurred in calves previously designated as BRDC morbid in each pen ÷ number of calves with first treatments occurring in each pen
Chronicity	number animals treated 3 times ÷ number of animals allocated to each pen
1 <sup>st</sup> Treatment Success	1-(number of calves treated for the 2 <sup>nd</sup> time ÷ number of calves treated 1 time) occurring in each pen
Average daily gain (arrival)	(total weight at day 28 - total arrival weight (day ) for all animals that survived to closeout) ÷ 28 (days)
Average daily gain (revaccination)	(total weight at study completion - total weight of all animals that were alive on day 28) ÷ total days post-day 28
Average daily gain (study)	(total weight at arrival for all calves that were alive at study completion - total arrival weight of all animals) ÷ total number of days
Feed intake to gain ratio (pen level)	total feed (as-fed) delivered ÷ (total closeout weight – total arrival weight of survivors)
Feed delivered (per pen/day)	total feed (as-fed) delivered ÷ number of animals allocated to each treatment
<sup>a</sup> BRDC = bovine respiratory disease complex	

**Table 2-3 Model<sup>a</sup> adjusted percentages, standard errors, and *P*-values of health outcomes in combined replicates comparing two crossbred beef feeder-calf preventive health programs**

Health Outcomes	Program <sup>b</sup>		
	Prog1	Prog2	<i>P</i> value
Morbidity	60.0% (7.0) <sup>c</sup>	47.8% (7.3)	0.02
Mortality	1.5% (1.3)	0.8% (0.7)	0.25
Case fatality	2.7% (2.2)	1.9% (1.7)	0.53
Chronic	15.0% (4.0)	10.7% (3.4)	0.24
1 <sup>st</sup> Treatment Success	60.9% (5.3)	64.4% (5.4)	0.58

<sup>a</sup>Model included replicate, load and pen as random effects

<sup>b</sup> Prog1 = 1 injectable clostridial vaccine, 1 intra nasal modified live respiratory vaccine, 1 topical parasiticide, 1 oral parasticide: Prog2 = 1 injectable clostridial vaccine, 1 injectable modified live respiratory vaccine, 1 injectable parasiticide

<sup>c</sup> number experiencing health outcome / number at risk (SE)

**Table 2-4 Model<sup>a</sup> adjusted average daily gain (ADG) and *P*-values in combined replicates comparing two crossbred beef feeder-calf preventive health programs**

Performance Outcome	Program <sup>b</sup>		
	Prog1	Prog2	<i>P</i> value
ADG: arrival to revaccination (ARR)	1.24 (0.06) <sup>c</sup>	1.33 (0.06)	0.04
ADG: revaccination to study completion (REVAC)	0.98 (0.05)	1.03 (0.05)	0.46
ADG: arrival to study completion (ENTIRE)	1.16 (0.04)	1.23 (0.04)	0.04
Feed to gain ratio (kg)	3.32 (0.62) <sup>d</sup>	3.13 (0.62)	0.72
Feed delivered (kg)	87.60 (1.54) <sup>e</sup>	90.57 (1.54)	0.17

<sup>a</sup>Model included load, replicate as random effects

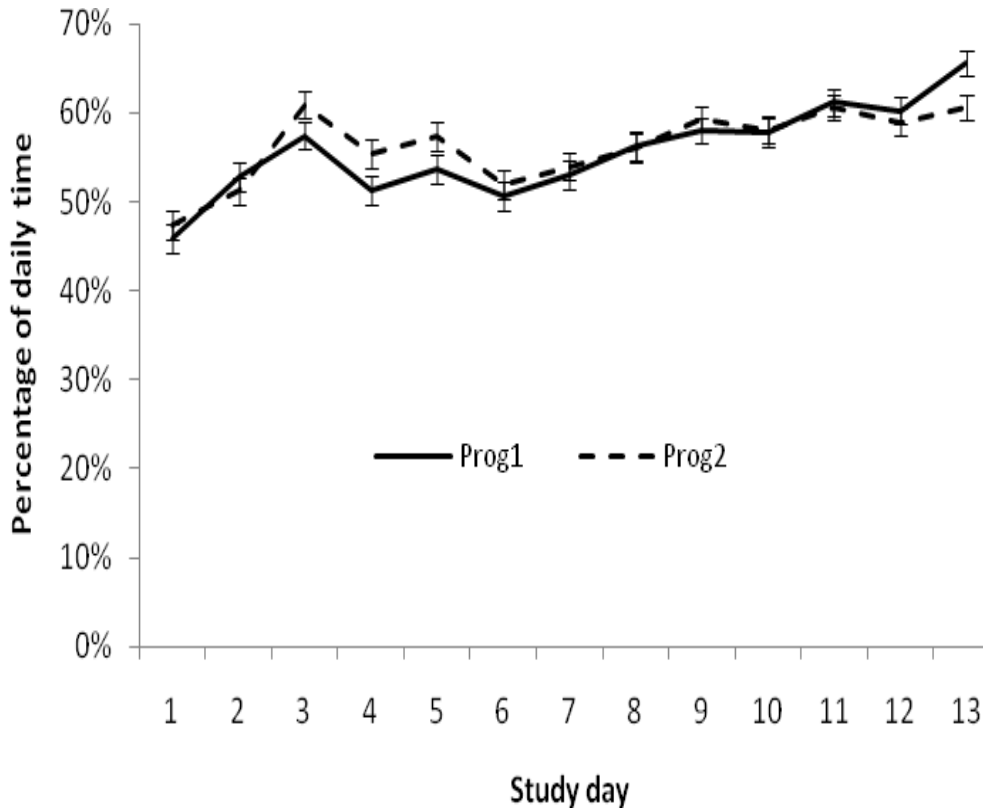
<sup>b</sup> Prog1 = 1 injectable clostridial vaccine, 1 intra nasal modified live respiratory vaccine, 1 topical parasiticide, 1 oral parasiticide: Prog2 = 1 injectable clostridial vaccine, 1 injectable modified live respiratory vaccine, 1 injectable parasiticide

<sup>c</sup> kg/day (SE)

<sup>d</sup>Feed delivered/ADG (SE)

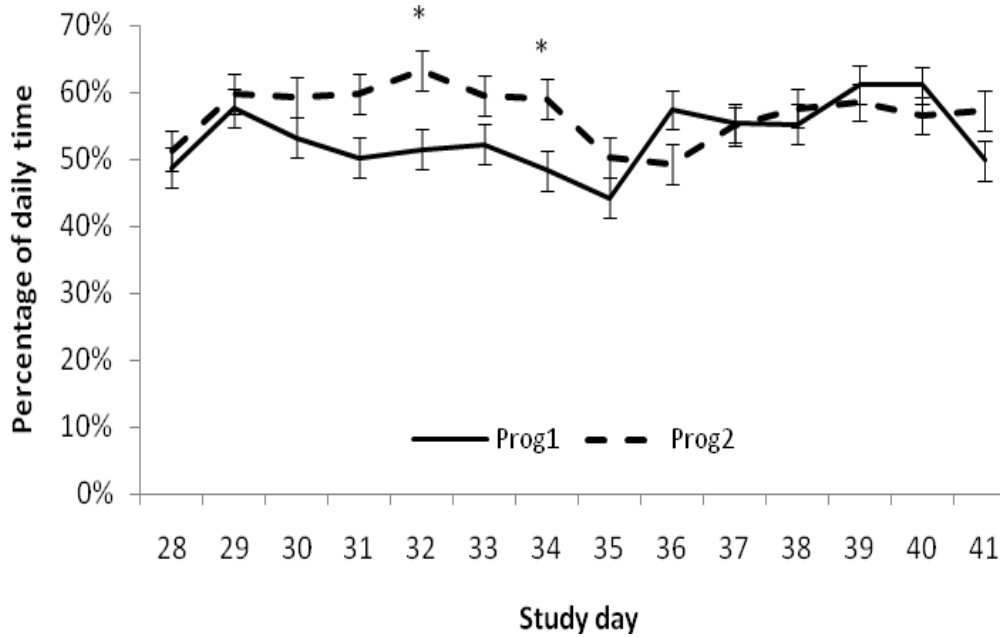
<sup>e</sup>Feed delivered per pen (SE)

**Figure 2-1 Model adjusted<sup>a</sup> mean percentage of time spent lying down for the minimally invasive program (Prog1) and more invasive program (Prog2) preventive health programs for the first 13 days of the study. Prog1 contained 1 injectable vaccination and Prog2 contained 2 injectable vaccines and 1 injectable parasiticide.**



<sup>a</sup>Model contained gender, program, studyday, and interactions terms for program by study-day and gender by study-day, and accounted for repeated measurements on calves and random effects of pen (n= ) and study replicate (n = 2).

**Figure 2-2 Model adjusted<sup>a</sup> mean percentage of time spent lying down for the minimally invasive program (Prog1) and more invasive program (Prog2) preventive health programs from study 28 to study completion. Prog1 contained 1 injectable vaccination and Prog2 contained 2 injectable vaccines and 1 injectable parasiticide.**



<sup>a</sup>Model contained gender, program, studyday, and interactions terms for program by study-day and gender by study-day, and accounted for repeated measurements on calves and random effects of pen (n= )and study replicate (n = 2).

\*Differences at the p =0.05 level for % time lying down by program and study-day.

## **CHAPTER 3 - Serial evaluation of physiological, pathological, and behavioral changes related to disease progression of experimentally induced *Mannheimia haemolytica* pneumonia in feeder calves**

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(Published in *American Journal of Veterinary Research*, 2010, 71(3):359-369)

### **Abstract**

Bovine respiratory disease complex (BRDC) continues to plague the U.S. beef industry. One of the difficulties in managing this disease is the unavailability of sensitive BRDC recognition tools. Our goal was to determine the utility of physiologic, behavioral and pathological changes as objective indicators of early respiratory disease in crossbred beef calves with *Mannheimia haemolytica* pneumonia. The disease was experimentally induced in clinically healthy calves through endoscopic pulmonary inoculation of *Mannheimia haemolytica*. Calves were necropsied on days 1, 2,3,4,7, and 9 after inoculation. Physical examination (temperature, pulse, and respiration), clinical illness score, activity level and respiratory character were assessed three times daily beginning 4 days prior to challenge and continued throughout the

study. Twice before inoculation and on days 1, 2, 3, 5, 7, and 9, arterial blood gas, blood chemistry and complete blood counts were assessed. Pedometers and accelerometers were used to monitor cattle behavior and activity throughout the trial. All calves became clinically ill after challenge and exhibited gross and histopathological signs of bronchopneumonia. No parameter was a reliable indicator of disease progression as judged by percent pulmonary involvement. However, activity as measured by total steps taken in a 24 hour period did accurately identify changes in health status. This single pathogen challenge model successfully produced clinical signs and pathology consistent with field respiratory disease. Routine laboratory parameters and subjective measures were not reliable indicators of progression of pneumonia. However activity, objectively measured with pedometers and accelerometers, appears to be a promising tool for early recognition of bovine respiratory disease.

## **Introduction**

Bovine respiratory disease complex (BRDC) is the most common and costly health problem in beef cattle today (Loneragan et al., 2001; Snowden et al., 2007). By some accounts, feedlot deaths associated with BRDC are increasing (Loneragan et al., 2001; Thompson et al., 2006; Snowden et al., 2007). Economic losses associated with BRDC primarily occur during the feeding phase and include lower average daily gain, decreased carcass quality and increased days on feed (Wittum et al., 1996; Gardner et al., 1999). If prevention and treatment costs are included, the estimated cost to the United States cattle industry is over \$3 billion annually (Griffin, 1997). Additionally, the impact on animal well-being is significant since BRDC is clearly the most common cause of morbidity and mortality in post-weaned beef cattle (Loneragan et al., 2001; Griffin, 1997).

BRDC is a multifactorial disease impacted by both pre- and post-weaning factors (Duff and Galyean, 2006). Some causative agents associated with BRDC are considered to be ubiquitous commensal organisms in cattle. The most commonly isolated organism from BRDC affected lungs, *Mannheimia haemolytica* (MH), is considered commensal in cattle.(Radostits, 2000). Successful treatment outcomes require early disease recognition, accurate prognostication and application of appropriate therapeutics. Treatment failures affect animal welfare and increase economic losses through decreased performance and increased mortality (Terhune Terry N., 2005). Therefore an accurate and timely diagnosis is critical to allow more positive economic and animal welfare decisions to be made.

Currently, subjective measures such as attitude, appetite and activity level are used to determine if calves require further examination or treatment for BRDC (Griffin, 1996). These observations are performed on individual animals; yet commercial beef production systems manage cattle in populations or herds. Since herd animals tend to mask clinical signs, overt signs of illness are often absent early in the disease process.

Objective measures to identify early clinical stages of BRDC currently do not exist. Improvements in diagnosis could be made through identification of repeatable, accurate measures associated with early stages of BRDC. We hypothesized changes in hematologic, behavioral, and physical exam parameters would be helpful for diagnosis and monitoring of BRDC. The purpose of this study was to evaluate changes in physiological, pathological and behavioral variables in beef calves at multiple time points following inoculation with MH.



## **Materials and methods**

This study was approved by the Kansas State University Institutional Animal Care and Use Committee.

### *Calves*

Calves (n=14) were selected from a population of 34 crossbred beef steers that participated in a previous 14 day observational trial during which calves were observed for clinical signs of BRD. Calves were observed for clinical illness for a total of 21 days prior to experimental MH inoculation. All calves were tested for bovine viral diarrhea persistent infection by antigen capture ELISA.<sup>a</sup> Additionally, a serum sample was collected from each calf to determine relative MH antibody levels. Nine calves of the initial 34 were not eligible for the study for the following reasons: 3 participated in another study, 1 poor temperament, 1 missing MH serology sample, 3 respiratory disease, and 1 with keratoconjunctivitis. The seven remaining calves with the highest MH optical density readings were eliminated from the potential study population to reduce challenge response variability. On day 0, four calves were removed pre-challenge and replaced by 4 other calves for the following reasons: 1 keratoconjunctivitis and 3 shoulder injuries. The average weight upon study initiation of the 14 enrolled calves was 199.2 kg. Optical MH ELISA antibody density for the study calves ranged from 0.137 to 0.571. Calves were housed in a 150 ft. by 150 ft. dry lot, fed commercial grain mix and grass hay and allowed free access to water.

## ***Inoculation***

*Mannheimia haemolytica* serotype A1 (OSU strain) isolated from a field case of BRDC was used as the challenge bacterium. The isolate was grown on brain heart infusion agar (BHI) containing 5% bovine blood in a CO<sub>2</sub> incubator for 18-22 hours. Colonies were then suspended in BHI broth and incubated at 37° C for 5.5 hours. Bacteria were pelleted by centrifugation, washed 3 times in sterile phosphate buffer saline (PBS) and re-suspended in PBS. The final concentration of approximately 1 X 10<sup>9</sup> per milliliter was based on a standard curve of colony forming units vs. optical density. Plate counts were used to confirm bacterial concentration. Four days after trial initiation, each calf was individually restrained in a portable cattle chute without sedation. The external nasal passage was cleaned and a fiberoptic endoscope<sup>b</sup> (100cm length X 6.6 mm diameter) was passed through the nares into the trachea and then tracheal bronchus into the right cranial lung lobe. Forty-milliliters of the 1 X 10<sup>9</sup> per milliliter concentration of MH inoculant in PBS was injected through a polyethylene tube (160 cm X 1.95 mm) into the bifurcation followed by a 40 ml PBS flush.

## ***Pathology***

Calves were randomly assigned to harvest day using a random number generator.<sup>c</sup> Necropsy, lung culture, and histopathology were completed on calves harvested, by captive bolt and exsanguination, on days 1, 2,3,5,7 and 9 post-inoculation (Table 1). Percent lung involvement was determined by palpation, visual observation and measurement of lesion cubic area by a board certified pathologist (DM). In each lobe the percentage of total lung consolidation was calculated using the percent of each lobe determined to be pneumonic and a reported formula: (0.53 x left cranial apical%) + (0.049 x left caudal apical lobe%)+ (0.319 x left

diaphragmatic%) + (0.043 x accessory lobe%) + (0.352 x right diaphragmatic%) + (0.061 x right middle lobe%) + (0.60 x right caudal apical lobe %) + (0.063 x right cranial apical lobe %).(Fajt et al., 2003) In addition, histopathology and bacterial lung cultures of each set of lungs were completed.

### *Data collection*

Rectal temperature, heart rate, respiratory rate, breathing type, behavior evaluation, activity assessment, and clinical illness scores (CIS) were completed three times daily beginning four days prior to inoculation until study completion. Physical examinations and all other assessments were completed by the same experienced veterinarian at each time point. Breathing type was subjectively assessed during restraint using the nominal scale; normal or rapid. Activity was recorded as normal (N) or decreased (D). Clinical illness scores were recorded while calves were being walked to the working facility using the scoring system: 1= normal, 2 = slightly ill, 3 = moderately ill, 4 = severely ill.

Arterial blood gas, blood chemistry, and complete blood counts were conducted daily, four days prior to disease induction, on inoculation day and days 1, 2, 3, 5, 7, and 9 post-challenge. Blood gas and chemistry analysis were completed using a chute side portable analyzer.<sup>d</sup> Blood gas parameters evaluated included: pH, partial carbon dioxide (PCO<sub>2</sub>), partial oxygen (PO<sub>2</sub>), base excess (BE<sub>ecf</sub>), bicarbonate (HCO<sub>3</sub>), total carbon dioxide (TCO<sub>2</sub>), oxygen saturation (SO<sub>2</sub>) and lactate. Arterial blood was collected from the medial branch of the caudal auricular artery in a non-heparinized syringe and immediately placed into 1.3 ml volume plastic lithium heparin tubes<sup>e</sup> for blood gas analysis. The time from blood collection to completed blood gas analysis was estimated at less than three minutes. Venous blood was collected from

either the jugular or coccygeal vein into 1.3 ml lithium heparin tubes. These samples were analyzed for standard serum biochemistry parameters including: sodium (Na), potassium (K), chloride (Cl), hematocrit (HCT), blood urea nitrogen (BUN), and glucose (Glu) analysis. Venous blood was collected into a 2 ml EDTA tube<sup>f</sup> and complete blood counts were completed.<sup>g</sup>

Animal behavior was continuously measured using accelerometers and pedometers. Four days before inoculation each calf was fitted with an accelerometer<sup>h</sup> and pedometer<sup>i</sup> on the right distal lateral metatarsus. These devices were housed in a plastic container and attached to the leg with two self-adhesive straps. Each calf wore the devices until harvest day. The accelerometer measured acceleration (g) in the x, y, and z axes at the rate of 100 times per second. Commercial software<sup>j</sup> was used to calculate the mean force (g), along with maximum and average vector magnitude over each 2 second period of time. Vector magnitude was calculated 100 times per second by Equation 1:

$$(1) \quad r = \sqrt{x^2 + y^2 + z^2}$$

The vector magnitude average and maximum were calculated based on all vector magnitude calculations for each 2 second period of time. Accelerometers were removed and data downloaded on days 0, 1, 2, 3, 5, 7, and 9. Data was not recorded between the time the accelerometers were removed for download and replaced on the metatarsus, (approximately 1 minute per day).

Approximately 0.5 hour of video of each calf, in a group setting within the pen, was collected over the 9 day trial period. The video was analyzed and logged by an investigator (blinded to accelerometer data) to record animal behaviors as one of three categories: lying down, standing in place, or walking. Video data was used to generate an algorithm to classify

accelerometer data in a manner previously reported.(White B. J., 2008) The combined video and accelerometer data were partitioned into training (70%) and test (30%) sets. The training set was used to generate a behavior classification algorithm using a classification tree. The test set was run through the classification algorithm and compared to actual video logged values to evaluate the accuracy of the classification algorithm by comparing Kappa values for each categorical behavior variable. The classification algorithm was used to assign a predicted behavior for accelerometer readings at each time point for each calf.

Pedometers recorded the number of steps taken in a 24 hour period (midnight to midnight). These data were collected for each day beginning 4 days before inoculation through harvest.

### *Statistical analysis*

All data were imported into a statistical program for analysis.<sup>k</sup> General linear mixed models were used to analyze continuous data for blood parameters, pedometers, temperature, weight, heart and respiratory rates while accounting for repeated measures on individual calves over time. Fixed effects evaluated included trial day in all models, and examination time (morning, noon, or evening) for temperature, heart rate and respiratory rate data. All measurements prior to pneumonia induction were grouped into a single time period (trial day -1) and mean values for pre-induction data were considered as the baseline for each calf. If significant differences ( $P < 0.05$ ) by trial day were detected, comparisons among days were made using model-generated least square mean values and corresponding standard errors (SEM).

The agreement between video recorded behavior and the algorithm used to classify accelerometer data was tested by calculating an overall Kappa value. The proportion of time

standing and lying was modeled using logistic models<sup>m</sup> with effects included to account for repeated measure on calves and trial day of the experiment. To determine potential changes in behavior patterns over time, the interaction between the percent of time spent in each behavior was also tested relative to trial day. Results from the sampling period most proximal to the necropsy were used in models as described above to compare relationship between each parameter and the percentage of lung lesions displayed in each calf.

## **Results**

All calves were negative for Bovine Viral Diarrhea Virus persistent infection by antigen capture ELISA.

### ***Pathology***

Two calves each were harvested on days 1, 2, 3, and 5 (inoculation day = 0). On trial days 7 and 9, three calves each were harvested. All lung sets exhibited gross lesions consistent with bronchopneumonia; the extent of total pulmonary involvement ranged from 1.2% to 23.4% (Table 1). Gross lesions were described as fibrinous bronchopneumonia with pleuritis on days 1 to 3. Atelectasis with resolving pneumonia described the lungs harvested on days 5, 7 and 9. Thirteen of the fourteen calves exhibited lesions on the right side. The overall mean percent lobe involvement for the target areas of the right cranial apical, right caudal apical and right middle lobes was 40%, 55.4%, and 26.1%, respectively.

Harvest day was associated with the overall percentage of pulmonary lesions ( $p = 0.03$ ). The percentage of lung lesions was significantly higher on day 2 compared to days 3 and 7 but not different from the other days (Figure 1). The percent lung involvement on day 7 was

significantly lower compared to days 2 and five. There was no apparent ascending or descending trends in percent lung involvement (Figure 1). *Mannheimia haemolytica* was cultured from 10 of 14 lung sets. Of the four sets of lungs from calves where MH was not cultured, *Arcanobacterium pyogenes* was isolated from 2 and *Histophilus somnus* from another. One lung set had no bacterial growth.

### ***Clinical illness scores***

All calves had normal CIS, during the four days prior to inoculation, but all were scored as ill post-inoculation. Statistical analysis was not completed for CIS, respiratory character or activity level because the observer was not blinded to the time relative to disease induction. All calves had CIS of 3 at the first post-inoculation observation. On day 1 post-inoculation, 32 measurements were CIS 2 and 6 were at level 3. For the rest of the study, 151 measurements were CIS 2 and 28 CIS 3. No calves received a normal CIS (1) between inoculation and harvest.

### ***Respiratory type***

Respiratory type was normal for all calves prior to inoculation, and for 4 out of 14 calves for the first reading after inoculation. From day 1 through trial completion, 17 of 179 respiratory type evaluations were classified as normal. Prior to inoculation all calves had normal activity. At the first reading post-inoculation until harvest, all calves had decreased activity.

### ***Temperature***

Least square mean rectal temperatures for all pre- and post-challenge days (range 39.8°C-40.4°C) were above published normal values (38.5°C-39.5°C).(Radostits, 2000) Only the inoculation day rectal temperature differed ( $p<0.05$ ) from all pre- and post-challenge days

(Figure 2). Rectal temperature increased throughout the day. There were differences ( $p < 0.05$ ) between measurements collected in the morning ( $39.6^{\circ}\text{C} \pm 0.049$ ), at noon ( $39.4^{\circ}\text{C} \pm 0.056$ ) and early evening ( $40.2^{\circ}\text{C} \pm 0.056$ ).

### ***Respiratory rate***

Mean respiratory rate on inoculation day (day 0) was greater ( $p=0.05$ ) compared to day 1, but not higher than any other day. Respiratory rates were greater than published values (Radostits, 2000) (30-60 breaths/minute) on all trial days and significantly greater than baseline (day -1) values on days 5 through 8 (Figure 3). Respiratory rates were less ( $p < 0.01$ ) in the morning ( $58.0 \pm 2.4$ ) relative to noon ( $75.3 \pm 2.7$ ) or evening ( $76.7 \pm 2.5$ ).

### ***Heart rate***

Heart rates were greater than reference values (60-80 per minute) on all trial days and were significantly greater on inoculation day relative to all other days in the trial. Heart rates appeared to decrease throughout the trial (Figure 4). Heart rates were greater ( $p < 0.05$ ) at noon ( $112.7 \pm 2.8$ ). Evening heart rates ( $106.0 \pm 2.7$ ) were less ( $p < .05$ ) relative to noon and greater ( $p < .05$ ) than the morning rates ( $101.1 \pm 2.6$ ).

### ***Body weight***

Body weights were only recorded daily and trial day was not significantly associated with changes in calf body weight.



### *Hematology and blood gas*

Complete blood count parameters were evaluated independently to determine if they were associated with trial day (Table 2). The effect of trial day was significant ( $p < 0.05$ ) for total leukocytes, segmented neutrophils, erythrocytes, hemoglobin, hematocrit (spun and calculated), mean cell hemoglobin concentration (MCHC), and plasma protein. The effect of trial day tended ( $p = 0.06$ ) to be associated with fibrinogen concentration. There was no overall effect ( $p > 0.10$ ) of trial day on the counts for band neutrophils, lymphocytes, monocytes, eosinophils, basophils, mean cell volume (MCV) or mean cell hemoglobin (MCH). Erythrocytes, hemoglobin, and hematocrit displayed similar trends over time with the largest values occurring prior to initiation of pneumonia and the lower values later in the study period. Plasma protein decreased on days 1 and 2 compared to pre-trial readings, and days 7 and 9 were both greater than the pre-inoculation measurements. Segmented neutrophils and total leukocyte counts displayed a different pattern with the greatest values observed on day 1. Pre-inoculation fibrinogen concentrations were less ( $p < .05$ ) compared to days 1, 3, and 9.

Serum chemistry parameters over the trial period are shown in Table 3. There was no effect ( $p > 0.10$ ) of trial day on sodium, glucose, or the anion gap, yet trial day was associated with all other chemistry parameters. All values, except pH, Hct% and hemoglobin, remained within published normal ranges from 4 days prior to inoculation through harvest. The pH value on day 5 (7.61) was outside the normal range (7.31-7.53)(Smith, 2002), and was greater compared to days 1, 2, 3, and 9. Mean hemoglobin was below the normal range (8-15 mg/dL)(Smith, 2002) on days 2 through 7, but only days 3 and 7 were less ( $p < .05$ ) than pre-inoculation values. Total carbon dioxide (TCO<sub>2</sub>) concentrations were less ( $p < .05$ ) prior to induction compared to trial days 1, 2 and five. Potassium and chloride concentrations were less

( $p < .05$ ) on trial day 1 compared to pre-challenge baseline concentrations (4.27 mmol/L, 103.12 mmol/L, respectively). On day 5, base excess was greater ( $p < 0.01$ ) compared to pre-inoculation and all post-inoculation days.

All measured blood gas parameters except oxygen saturation ( $SO_2$ ) were associated ( $p < 0.05$ ) with trial day (Table 4). Oxygen saturation tended ( $p = 0.08$ ) to be impacted by day of trial. Blood lactate concentration was greater pre-inoculation compared to all days except day 1. Arterial pH, bicarbonate ( $HCO_3$ ), total carbon dioxide ( $TCO_2$ ), and base excess (BEcf) were less ( $p < .05$ ) prior to induction compared to all days post-inoculation. Pre-inoculation oxygen ( $PO_2$ ) was less ( $p < 0.01$ ) compared to days 5, 7, and 9. Pre-inoculation  $PCO_2$  was greater ( $p < .05$ ) compared to days 1, 5 and 9.

### *Activity*

Model-adjusted mean step counts were greater ( $p < 0.01$ ) for all days post-inoculation compared to pre-inoculation (Figure 5). Mean step count pre-inoculation was 10,986 ( $\pm 692.6$ ), and on day 0 it was 6,280 ( $\pm 692.6$ ). At no time after inoculation did least square mean total steps within a 24 hour period exceed 7,137.

Video was obtained on the 14 study calves over 13 days (day -3 was not recorded) with a mean recording time of 2.2 minutes per day per calf. The overall agreement between the classification tree algorithm and the video recorded data was high (Kappa 0.91, 95% CI 0.89 to 0.92). Therefore, the classification tree was used to generate behavior estimates for each data point from the accelerometer data. Data from the first day the accelerometer was placed and each calf's harvest day were eliminated from the analysis because these were not complete 24 hour periods. Trial day was a factor ( $p < .05$ ) in the percent of time animals spent standing and

tended ( $p = 0.08$ ) to be associated with the amount of time spent lying down (Table 5). Mean time spent walking was not associated ( $p=0.86$ ) with trial day. An interaction ( $p<.05$ ) was identified in the amount of time cattle spent either standing or lying based on day of the trial (Figure 6). Prior to induction of pneumonia, cattle spent more time standing ( $53.0\% \pm 3.7\%$ ) compared to lying ( $42.3\% \pm 3.7\%$ ). After the induction of pneumonia, there were few differences in the amount of time spent in each activity, and in fact on day 4 post-inoculation, cattle spent more time lying ( $56.0\% \pm 4.6\%$ ) compared to standing ( $39.6\% \pm 4.6\%$ ).

## **Discussion**

The challenge model utilized in this study successfully produced clinical and pathological signs of respiratory disease consistent with that observed in BRDC due to MH. The distribution of pulmonary lesions in our study was similar to a bronchoscopy study where MH lesions were restricted to 15%-17% of the right lung lobes.(Reeve-Johnson, 2001b) Other researchers have reported difficulty reproducing MH pneumonia without pre-exposure to viral pathogens or stressors.(Thomson, 1974) The etiology of BRDC is multifactorial.(Wittum and Perino, 1995; Roeber et al., 2001; Fulton et al., 2002) Pre-weaning factors including colostrum intake, bovine viral diarrhea virus (BVDV) status and pre-shipment management.(Step et al., 2008) Post-weaning factors include transportation stress, castration and dehorning, gender and nutritional management.(Blecha, 1984; Wittum and Perino, 1995; Daniels, 2000; Roeber et al., 2001; Fulton et al., 2002; Rivera, 2005; Duff and Galyean, 2006) In our study, transportation and viral stress were not present but other stressors such as heat stress and frequent restraint did occur. These stressors may have contributed to the success of our challenge model.

All calves had predominately right side bronchopneumonia except one, which had lesions primarily on the left side. Presumably this was due to the calf coughing and potentially modifying the placement of the endoscope during introduction of MH. In a previous challenge study, contrast bronchography documented the dissemination of bacteria into other lung fields during coughing episodes (Vestweber et al., 1990). Gross necropsy and histopathology revealed acute bronchopneumonia on the day after inoculation. However, by day 5 post-challenge, lung lesions appeared to be resolving grossly and histopathologically. This was an unexpected finding given the extensive lung lesions observed within the first few days after inoculation. This may indicate that MH, as a single pathogen, is unable to sustain severe inflammatory responses for more than a few days (Ames et al., 1985). Other parameters such as leukocyte and neutrophil total counts indicated a rapid but transient response to infection. In addition, in our study, calves had elevated core temperatures at challenge ( $>39.7^{\circ}\text{C}$ ) which may be protective against bacterial growth.(Reeve-Johnson, 2001b) *Mannheimia haemolytica* was found in 10 of 14 lung sets, which is consistent with a previous study where 9 out of 16 lungs were found to contain *Pasteurella multocida* after inoculation.(Dowling et al., 2002)

Clinical illness score, respiratory type and activity were normal for all calves prior to disease induction, but all measures were abnormal at the first evaluation post-inoculation. This validates the success of our induction model, and indicates that these subjective measures may be used to recognize early pulmonary disease. Pyrexia and lethargy due to MH endotoxin and leukotoxin may precede measurable lung tissue damage.(Zecchinon, 2005) Clinical illness scores remained abnormal from induction to harvest, yet necropsy and histopathology results indicated a resolving bronchopneumonia starting on trial day 3. Because the observer was unblinded in our study we were unable to accurately use clinical illness score as a predictor of

lung pathology severity. Our conclusion, which agrees with others(Reeve-Johnson, 2001a), was that clinical illness scores do not give an adequate assessment of disease progression.

In our study, we found that common clinical measurements (temperature, pulse, and respiratory rate) may produce conflicting results and can vary by the time of day. Rectal temperatures were above normal throughout our study. This is likely related to the elevated environmental temperatures ( $>32.2^{\circ}\text{C}$ ) during the trial period, and restraint of the calves three times a day for physical examination. Least square mean rectal temperature was greater on inoculation day than any other study day, and this transient increase may have been a direct result of the synergistic relationship between endotoxin and leukotoxin.(Jeyaseelan, 2002) Rectal temperature varied by time of day. This is likely an effect of daily changes in ambient temperature and agrees with findings in another studies indicating maximum heat dissipation occurs overnight.(Hahn, 1999) Mean rectal temperatures for the post-inoculation morning readings were less than cut-off values ( $\geq 40^{\circ}\text{C}$ ) used to determine sickness on some production units. However, evening rectal temperature readings were above  $40^{\circ}\text{C}$ , suggesting that time of day should be taken into account when rectal temperature is used as a diagnostic tool.

Mean heart rates were above published normal values for all trial days.(Radostits, 2000) Interestingly, heart rate decreased and respiratory rate increased as the study progressed. This may be a possible source of diagnostic conflict if respiratory rate and heart rate are used together as indicators of pulmonary disease. The time of day the measurement was taken had a unique effect on heart rate relative to rectal temperature or respiratory rate with the maximum heart rate observed at the 1200 observation.

Rectal temperature, respiratory rate and heart rate immediately prior to harvest were not associated with percent lung involvement. This disagrees with a study where rectal temperature,

respiratory rate and clinical score were associated with percent lung involvement seven days post-inoculation.(Reeve-Johnson, 2001b) This difference may be a reflection of two different lung scoring systems rather than a difference in study outcomes. The difference may also be explained by differences in inoculation methods, subject age, breed, and environmental conditions.

Complete blood counts illustrated a transient increase in total leukocytes and a decrease in red blood cell count, although overall findings revealed few days when CBC parameters were beyond established normal values. Absolute neutrophil numbers were elevated above the normal range post-inoculation days 1 and 9, which conflicts with reports that neutropenia occurs with gram negative bacterial pneumonia.(Hodgson, 2006; Jones and Allison, 2007) Our observations began early after bacterial insult, and our results may indicate that neutrophilia occurs initially before an influx of neutrophils into lung tissue is precipitated by leukotoxins and endotoxins in MH infections. (Vestweber et al., 1990; Soethout, 2002; Zecchinon, 2005; Hodgson, 2006; Stockham Steven, 2008) This finding may be valuable in assessing MH disease progression if early in the course of disease neutrophilia exists. Our total leukocyte results were consistent with a similar study where leukocyte counts increased the day after inoculation and remained elevated for one day.(Ganheim, 2004) However, in another study total leukocyte and absolute neutrophil counts were highest at the first measurement (72 hours) after a blind tracheal inoculation in two week old Holsteins.(Vestweber et al., 1990) The conflicting evidence may indicate that multiple factors affect blood leukocyte numbers, and the usefulness of leukocyte counts to consistently identify early BRDC is marginal.

Mean red blood cell counts were highest prior to inoculation and above normal laboratory range pre-inoculation and on days 1, 2 and 3. Others have shown a consistent decrease in red

blood cells from inoculation throughout the rest of the trial (Corrigan et al., 2007). In contrast, polycythemia has been observed in hypoxic calves with chronic pulmonary disease. As expected, mean fibrinogen levels were elevated and statistically higher than baseline values on days 1, 3 and 9. However, fibrinogen values were not consistently elevated beyond the normal range post-inoculation. Therefore, it was not a reliable predictor of BRDC in our trial. These collective findings suggest limited utility for complete blood counts as early, objective indicators of BRDC.

Although blood chemistry values tended to vary by trial day, most indexes remained within normal ranges throughout the trial. Glucose was the only blood chemistry parameter that elevated, but this parameter was also elevated prior to disease induction. Elevated glucose is thought to be a stress indicator (Stockham, 2008). In our study, stress may have been induced from restraining and examining calves three times a day. In our trial, blood chemistry analysis illustrated few measures that could be useful for objective diagnosis of early BRDC.

Hypoxemia can be a manifestation of severe respiratory disease when both decreased ventilation and decreased tissue perfusion are occurring (Slocombe et al., 1984; Smith, 2002). Alveolar hypoxia (decreased  $SO_2$ ), as an indicator of hypoxemia, was not observed in our study after pneumonia induction. Although cranioventral lobes had large areas of pathology, the total lung involvement may not have been extensive enough to alter ventilation rates or reduce pulmonary gas exchange. Arterial pH increased after induction. This finding indicates respiratory alkalosis associated with increased respiratory rate coupled with low percent lung involvement. However, blood pH results remained normal except on day 5 when alkalosis was detected. This may be due to increased sensitivity of arterial blood to pulmonary or metabolic changes compared to venous blood (Stockham, 2008). Increased respiratory rates and gram

negative septicemia, both present in our study, can contribute to increased blood pH (Smith, 2002; Stockham, 2008). Respiratory rates were elevated throughout our trial and gram negative septicemia also likely occurred. Ambient temperatures during the trial were high, which potentially contributed to greater than normal respiratory rates in these calves with respiratory disease. Decreased arterial blood pH values (respiratory acidosis) have been reported in calves with chronic respiratory disease (Nagy, 2006). However, none of the calves in our study entered advanced stages of chronic pulmonary damage.

Increased blood lactate has been considered a reliable prognostic indicator for mortality in acute bronchopneumonia (Coghe, 2000). However, we did not find lactate to be useful in identifying early BRDC possibly because anaerobic metabolism did not occur due to the low percentage lung involvement. Changes in blood gas and chemical values are not unique to BRDC, but can be affected by many factors, and require extensive lung involvement before significant changes do occur. The use of blood gas and chemical parameters to recognize BRDC early after infection had no value in this study.

Accelerometers were not useful in determining disease progression, however these data provided evidence of a behavioral change after the disease challenge.(Figure 4) Prior to pneumonia induction, calves spent more time standing than lying indicating the behavior pattern of healthy calves in this environment. By day 4 post-inoculation, calves spent more time lying than standing. These findings are consistent with the idea that one of the primary clinical signs of BRDC is depression, but the change in the relationship between the two variables was not present until two days after induction of pneumonia. Other researchers previously suggested that animal health could be effectively monitored using accelerometer,(Schoenig et al., 2004) and these devices have been used to show calf behavior changes following a specific event such as



castration (Fajt, et al., 2003). In this study accelerometers objectively documented behavior prior to disease induction and provided evidence of behavioral pattern changes in early cases of respiratory disease.

We believe that we are the first to demonstrate the utility of pedometers as an effective indicator of respiratory disease in cattle. Previously, pedometers have been used for estrus detection, lameness and illness detection in dairy cattle (Moallem, 2002; Rorie et al., 2002; Roelofs et al., 2005; Mazrier, 2006). We found that the total number of steps taken during a 24 hour period decreased post-inoculation and remained lower than baseline levels throughout the trial. MH toxins in the inoculant may have induced lethargy and thus may explain the immediate activity decrease. Although activity was reduced following disease induction, the number of steps on the day prior to harvest was not associated with percent lung involvement at necropsy. This may be explained by a calf's ability to mask disease in a herd setting. Activity, as measured by total steps taken, may be an indicator of early respiratory disease but it does not appear to be a reliable indicator of the progression or severity of disease.

In this study we used a challenge model to replicate clinical signs and pathology of early bronchopneumonia due to MH, and investigated several parameters as potential objective indicators of early BRDC. None of the parameters predicted lung disease progression. Although physical and physiological pattern changes from pre-inoculation through harvest were noted, many times the numerical changes were too subtle to rely upon for consistent, accurate field diagnosis. Total lung involvement for most calves in our study was relatively low and that may have minimized the numerical differences; however, our focus was early respiratory disease recognition when total lung involvement would be expected to be low. Activity measures (pedometers and accelerometers) documented behavioral changes associated with disease state.

Although it is widely believed that calves suffering from BRDC are less active, our research is the first to document this behavioral change. The other disease identification tools used in this study have been used historically for many years by the livestock industry. Our research indicates these are of little value for early disease recognition. To reduce the effects of BRDC in beef calves, it is important to continue to evaluate tools for early recognition of disease.

## Footnotes

- a. Kansas State University Veterinary Diagnostic Laboratory, Manhattan, KS
- b. VetVu Flexible Endoscope, Swiss Precision Products, Spencer, MA.
- c. Excel, Microsoft Corporation, Redmond, WA.
- d. i-Stat Handheld Portable Analyzer, Heska Corporation, Loveland, CO.
- e. 1.3 ml lithium tube, Sarstedt, Numbrecht, Germany.
- f. 2 ml EDTA blood tube, BD, Franklin, TN.
- g. Clinical Pathology Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS.
- h. GP 1 Programmable Accelerometer, Sensr, Elkader, IA.
- i. NL-800 Activity Monitor, New Lifestyles, Lee's Summit, MO.
- j. Sensware, Sensr, Elkader, IA.
- k. JMP 7.0, SAS Institute, Cary NC.
- l. PEPI, Version 4.0, Sagebrush Press, Las Vegas, NV.
- m. Proc Glimmix, SAS 9.1, SAS Institute, Cary, NC.

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**Figure 3-1 Calf lung one day post-*Mannheimia haemolytica* inoculation**



**Figure 3-2 Calf lung three days post-*Mannheimia haemolytica* inoculation**





**Figure 3-3 Calf lung seven days post-*Mannheimia haemolytica* inoculation**



**Table 3-1 Percent consolidation<sup>1</sup> measured at necropsy for each calf by days post-inoculation.**

Calf ID	Harvest Day	Rt crn apical	Rt cd apical	Rt mid	Rt diaph	Acc	Lft crn apical	Lft cd apical	Lft diaph	Total Lung Score <sup>2</sup>
27	1	20	90	0	0	0	0	0	3	7.6
30	1	0	0	0	0	0	0	100	20	11.3
21	2	80	90	30	10	30	50	30	7	23.4
25	2	80	80	80	0	0	0	0	0	14.7
16	3	50	100	0	0	0	0	3	0	9.3
23	3	50	50	0	0	0	0	0	0	6.2
14	5	90	50	80	0	100	0	10	10	21.5
28	5	30	100	30	0	100	0	0	0	14.0
18	7	20	5	0	0	0	0	0	0	1.6
22	7	0	20	0	0	0	0	0	0	1.2
32	7	10	100	20	0	0	0	0	0	7.9
3	9	0	0	25	20	50	0	0	0	10.7
5	9	100	0	0	0	20	0	0	0	7.2
12	9	30	90	100	0	0	30	40	0	16.9
Average		40.0	51.8	26.1	2.1	21.4	5.7	12.9	2.9	11.8

<sup>1</sup>Abbreviations include: Rt crn apical (Right cranial apical), Rt cd apical ( Right caudal apical), Rt mid ( Right middle), Rt diaph (Right diaphragmatic), Acc( Accessory), Lft crn apical (Left cranial apical), Lft cd ( Left caudal apical), Lft diaph ( Left diaphragmatic)

<sup>2</sup> Total percentage lung consolidation = (0.053 x left cranial apical %) + (0.049 x left caudal apical %) + (0.319 x left diaphragmatic lobe %) + (0.043 x accessory lobe %) + (0.352 x right caudal lobe %) + (0.061 x right middle lobe %) + (0.060 x right caudal apical lobe %) + (0.063 x right cranial apical lobe %) (Fajt et al., 2003)

**Table 3-2 Least square mean complete blood count parameters<sup>1, 2, 3</sup> by trial day with (-1) = combine baseline and other numbers corresponding to days post-inoculation.**

Trial Day	-1	1	2	3	5	7	9	Ref Rng <sup>4</sup>	Trial d p-value
Leuk	9.58 <sup>b</sup>	12.57 <sup>a</sup>	9.82 <sup>b</sup>	8.41 <sup>b</sup>	8.67 <sup>b</sup>	10.19 <sup>ab</sup>	11.24 <sup>ab</sup>	7-14 K/ul	<0.01
Seg neut	4.20 <sup>b</sup>	7.85 <sup>a</sup>	4.98 <sup>b</sup>	3.96 <sup>b</sup>	3.73 <sup>b</sup>	4.85 <sup>ab</sup>	6.66 <sup>ab</sup>	1-5 K/ul	<0.01
Band neut	0.03	0.11	0.02	0.00	0.02	0.06	0.07	0-.2 K/ul	0.27
Lym	4.89	4.07	4.41	3.99	4.55	4.71	3.95	2.5-7.5 K/ul	0.2
Mono	0.27	0.35	0.24	0.22	0.2	0.31	0.32	0.25-.85 K/ul	0.51
Eos	0.15	0.1	0.15	0.19	0.15	0.15	0.07	0-1.6 K/ul	0.72
Bas	0.06	0.04	0.02	0.06	0.03	0.04	0	0-.2 K/ul	0.64
Erthr	9.13 <sup>a</sup>	8.63 <sup>b</sup>	8.33 <sup>bc</sup>	8.04 <sup>cd</sup>	7.71 <sup>de</sup>	7.16 <sup>e</sup>	7.53 <sup>de</sup>	5-8 M/ul	<0.01
Hemog	11.25 <sup>a</sup>	10.58 <sup>b</sup>	10.24 <sup>bc</sup>	9.87 <sup>cd</sup>	9.49 <sup>de</sup>	8.84 <sup>e</sup>	9.49 <sup>cde</sup>	8.5-14 g/dL	<0.01
Hemat (calc)	31.60 <sup>a</sup>	29.99 <sup>b</sup>	28.95 <sup>bc</sup>	28.19 <sup>cd</sup>	27.01 <sup>de</sup>	25.20 <sup>e</sup>	26.66 <sup>cde</sup>	26-42 %	<0.01
Hemat (spun)	33.41 <sup>a</sup>	32.13 <sup>a</sup>	30.23 <sup>b</sup>	29.52 <sup>bc</sup>	28.12 <sup>bc</sup>	27.23 <sup>c</sup>	28.96 <sup>bc</sup>	26-42 %	<0.01
MCV	34.94	34.91	35.08	35.2	34.88	35.16	35.08	32-51 fL	0.78
MCH	12.42	12.48	12.32	12.36	12.48	12.46	12.82	11-18 pg	0.33
MCHC	35.72 <sup>ab</sup>	35.62 <sup>ab</sup>	35.52 <sup>ab</sup>	34.98 <sup>c</sup>	35.71 <sup>ab</sup>	35.34 <sup>bc</sup>	36.16 <sup>a</sup>	33-37 g/dL	0.02
Plasma Protein	7.29 <sup>c</sup>	7.08 <sup>d</sup>	7.06 <sup>d</sup>	7.34 <sup>bc</sup>	7.22 <sup>cd</sup>	7.54 <sup>b</sup>	7.97 <sup>a</sup>	7-9 g/dL	<0.01
Fibrinogen	0.63 <sup>c</sup>	0.77 <sup>ab</sup>	0.67 <sup>bc</sup>	0.87 <sup>a</sup>	0.68 <sup>abc</sup>	0.68 <sup>abc</sup>	0.87 <sup>ab</sup>	.3-.7 g/dL	0.06

<sup>1</sup>Day -1 represents all pre-inoculation readings; all calves inoculated with *Mannheimia haemolytica* on Day 0.

<sup>2</sup>Superscripts that differ within the same column illustrate significant (p<0.05) differences in least square means.

<sup>3</sup>Abbreviations include: Seg Neut (segmented neutrophils), Band Neut (band neutrophils), Lymph (lymphocytes), Mono (monocytes), Eosin (eosinophils), Baso (basophils), Erythro (erythrocytes), Hemoglob (hemoglobin)

<sup>4</sup>Kansas State University Clinical Pathology Laboratory

**Table 3-3 Least square mean serum biochemistry parameters<sup>3</sup> by days from induction.**

	Trial Day <sup>1</sup>							Refer- ence Range <sup>4</sup>	Trial Day p-value <sup>2</sup>
	-1	1	2	3	5	7	9		
pH	7.52 <sup>bc</sup>	7.53 <sup>bc</sup>	7.53 <sup>bc</sup>	7.48 <sup>c</sup>	7.61 <sup>a</sup>	7.58 <sup>ab</sup>	7.48 <sup>c</sup>	7.3-7.5 mmol/L	<0.01
pCO2	32.81 <sup>bcd</sup>	34.21 <sup>abc</sup>	33.72 <sup>abcd</sup>	37.89 <sup>ab</sup>	30.36 <sup>cd</sup>	28.83 <sup>d</sup>	38.92 <sup>a</sup>	35-44 mmHg	0.01
HCO3	26.72 <sup>c</sup>	28.31 <sup>b</sup>	28.33 <sup>b</sup>	27.67 <sup>abc</sup>	30.07 <sup>a</sup>	26.93 <sup>bc</sup>	28.43 <sup>abc</sup>	20-30 mmol/L	<0.01
BEecf	3.83 <sup>b</sup>	5.64 <sup>b</sup>	5.71 <sup>b</sup>	3.56 <sup>b</sup>	8.74 <sup>a</sup>	4.65 <sup>b</sup>	4.99 <sup>b</sup>		<0.01
AnGap	10.7	10.5	9.48	11.05	8.24	11.14	10.48		0.14
Hb	8.31 <sup>ab</sup>	8.59 <sup>a</sup>	7.88 <sup>bc</sup>	7.41 <sup>c</sup>	7.66 <sup>bc</sup>	7.34 <sup>c</sup>	8.23 <sup>abc</sup>	8-15 g/dL	0.02
Na	136.54	134.99	135.71	136.3	135.65	136.99	136.61	132-152 mmol/L	0.39
K	4.27 <sup>b</sup>	3.70 <sup>c</sup>	4.04 <sup>bc</sup>	4.07 <sup>bc</sup>	4.13 <sup>bc</sup>	3.96 <sup>bc</sup>	5.34 <sup>a</sup>	132-152 mmol/L	<0.01
Cl	103.12 <sup>a</sup>	99.61 <sup>c</sup>	102.01 <sup>ab</sup>	101.93 <sup>abc</sup>	101.28 <sup>bc</sup>	102.58 <sup>ab</sup>	101.70 <sup>ac</sup>	132-152 mmol/L	<0.01
TCO2	27.69 <sup>c</sup>	29.32 <sup>ab</sup>	29.23 <sup>b</sup>	28.93 <sup>abc</sup>	30.99 <sup>a</sup>	28.01 <sup>bc</sup>	29.76 <sup>abc</sup>	132-152 mmol/L	<0.01
BUN	7.45 <sup>bc</sup>	8.25 <sup>ab</sup>	6.34 <sup>c</sup>	7.53 <sup>abc</sup>	8.53 <sup>ab</sup>	8.26 <sup>ab</sup>	9.78 <sup>a</sup>	6-27 mg/dL	<0.01
Glu	84.57	77.51	76.54	76.39	80.32	77.28	70.7	45-75 mg/dL	0.26
HCT	24.44 <sup>ab</sup>	25.31 <sup>ab</sup>	23.17 <sup>bc</sup>	21.72 <sup>bc</sup>	22.57 <sup>c</sup>	21.60 <sup>c</sup>	24.26 <sup>abc</sup>	24-46 %	0.02

<sup>1</sup>Day -1 represents all pre-inoculation readings; all calves inoculated with *Mannheimia haemolytica* on Day 0.

<sup>2</sup>Superscripts that differ within the same column illustrate significant (p<0.05) differences in least square means.

<sup>3</sup>Abbreviations include Na (sodium), K (potassium), Cl (chloride), TCO2 (total carbon dioxide), BUN (blood urea nitrogen), Glu (glucose), HCT%PCV (hematocrit, % packed cell volume), pCO2 (partial pressure of carbon dioxide), HCO3 (bicarbonate), BEecf (base excess), AnGap (anion gap), and Hb (hemoglobin)<sup>4</sup>Large Animal Internal Medicine, Third Edition, Mosby Publishing

**Table 3-4 Least square mean arterial blood gas parameters<sup>3</sup> by trial day.**

Trial day <sup>1</sup>	pH	PCO2	PO2	BEcf	HCO3	TCO2	SO2	Lac
-1	7.54 <sup>d</sup>	30.86 <sup>a</sup>	75.43 <sup>d</sup>	3.69 <sup>c</sup>	26.15 <sup>b</sup>	27.17 <sup>b</sup>	92.35 <sup>b</sup>	1.67 <sup>a</sup>
1	7.65 <sup>b c</sup>	26.90 <sup>b</sup>	90.28 <sup>cd</sup>	8.58 <sup>b</sup>	29.32 <sup>a</sup>	30.15 <sup>a</sup>	97.80 <sup>a</sup>	1.33 <sup>ab</sup>
2	7.64 <sup>c</sup>	27.98 <sup>ab</sup>	95.71 <sup>bcd</sup>	8.55 <sup>b</sup>	29.22 <sup>a</sup>	30.33 <sup>a</sup>	97.91 <sup>a</sup>	0.71 <sup>c</sup>
3	7.63 <sup>c</sup>	27.66 <sup>ab</sup>	97.12 <sup>bcd</sup>	8.09 <sup>b</sup>	29.01 <sup>a</sup>	29.90 <sup>a</sup>	97.72 <sup>ab</sup>	0.47 <sup>c</sup>
5	7.76 <sup>a</sup>	20.99 <sup>c</sup>	125.52 <sup>a</sup>	11.94 <sup>a</sup>	30.88 <sup>a</sup>	31.45 <sup>a</sup>	99.38 <sup>a</sup>	0.49 <sup>c</sup>
7	7.66 <sup>bc</sup>	28.07 <sup>ab</sup>	120.74 <sup>ab</sup>	9.26 <sup>ab</sup>	30.02 <sup>a</sup>	30.73 <sup>a</sup>	99.01 <sup>ab</sup>	0.57 <sup>c</sup>
9	7.79 <sup>ab</sup>	20.91 <sup>bc</sup>	153.57 <sup>a</sup>	13.10 <sup>ab</sup>	31.53 <sup>a</sup>	32.48 <sup>a</sup>	99.48 <sup>ab</sup>	0.43 <sup>bc</sup>
Reference Range <sup>4</sup>	7.31-7.53	35-44 mmHg	75-100 mmHg		20-30 mmol/L	21-32 mmol/L	94-100%	5-20 mmol/L
Trial Day p-value <sup>2</sup>	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.08	<0.01

<sup>1</sup>Day -1 represents all pre-inoculation readings; all calves inoculated with *Mannheimia haemolytica* on Day 0.

<sup>2</sup>Superscripts that differ within the same column illustrate significant (p<0.05) differences in least square means.

<sup>3</sup>Abbreviations include: PCO2 (partial pressure of carbon dioxide), PO2 (partial pressure of oxygen), BEcf (base excess), HCO3 (bicarbonate), TCO2 (total oxygen), SO2 (oxygen saturation), and Lac (lactate)

<sup>4</sup>Large Animal Internal Medicine, Third Addition, Mosby Publishing

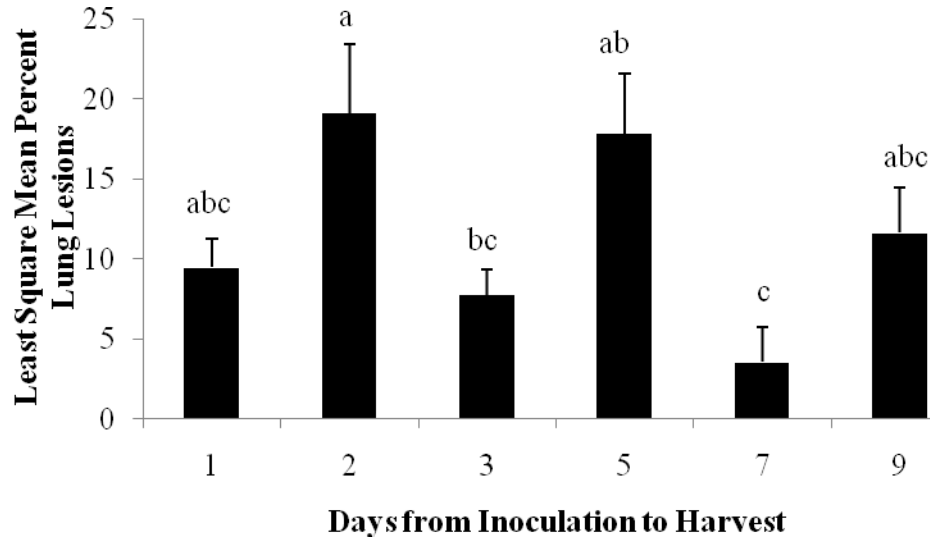
**Table 3-5 Percent of time spent in each activity as categorized by accelerometer by trial day.**

Trial Day <sup>1</sup>	% Lying	% Standing	% Walking
-1	41.1% <sup>c</sup>	54.2% <sup>a</sup>	4.7%
0	43.3% <sup>bc</sup>	52.9% <sup>a</sup>	3.8%
1	41.8% <sup>c</sup>	53.9% <sup>a</sup>	4.2%
2	45.9% <sup>bc</sup>	50.2% <sup>ab</sup>	3.8%
3	52.0% <sup>ab</sup>	43.1% <sup>bc</sup>	4.7%
4	55.9% <sup>a</sup>	39.4% <sup>c</sup>	4.6%
5	50.9% <sup>abc</sup>	45.1% <sup>abc</sup>	4.0%
6	49.0% <sup>abc</sup>	46.3% <sup>abc</sup>	4.6%
7	40.4% <sup>bc</sup>	55.9% <sup>a</sup>	3.8%
8	49.7% <sup>abc</sup>	47.2% <sup>abc</sup>	3.1%
Trial d p-value <sup>2</sup>	0.08	0.02	0.86

<sup>1</sup>Day -1 represents all pre-inoculation readings; all calves inoculated with MH on Day 0.

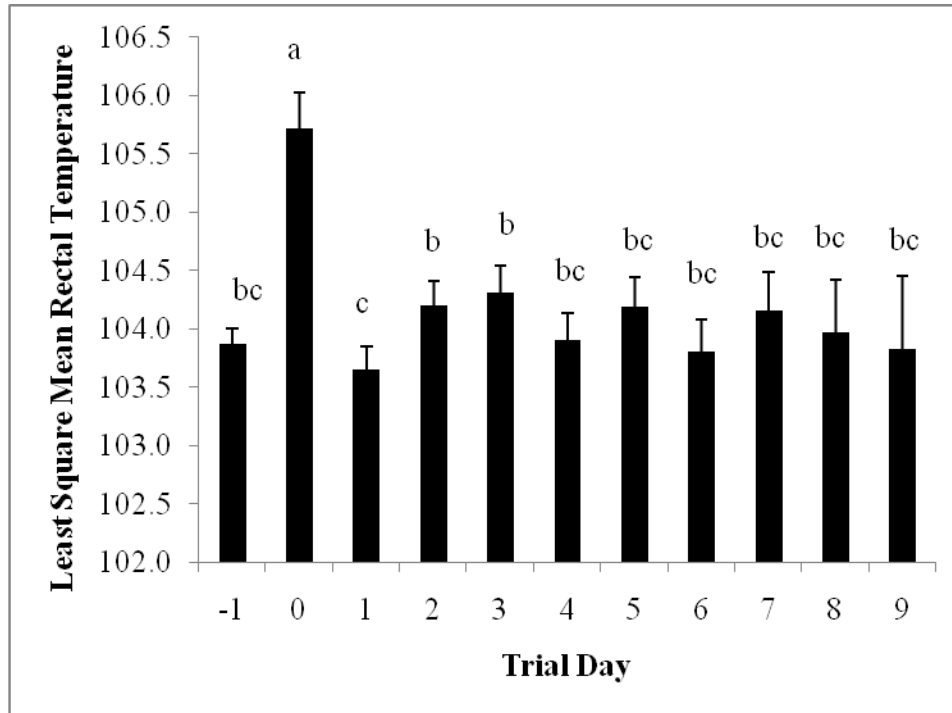
<sup>2</sup>Superscripts that differ within the same column illustrate significant ( $p < 0.05$ ) differences in least square means

**Figure 3-4 Mean<sup>1</sup> percent lung involvement by day after inoculation with *Mannheimia haemolytica*.**



<sup>1</sup>Means are model adjusted values from a generalized linear mixed model. Columns with unique superscripts statistically differ ( $p < 0.05$ ).

**Figure 3-5 Least square mean rectal temperature (°C) by trial day.<sup>1,2,3,4</sup>**



<sup>1</sup>All readings prior to inoculation are included in trial day -1.

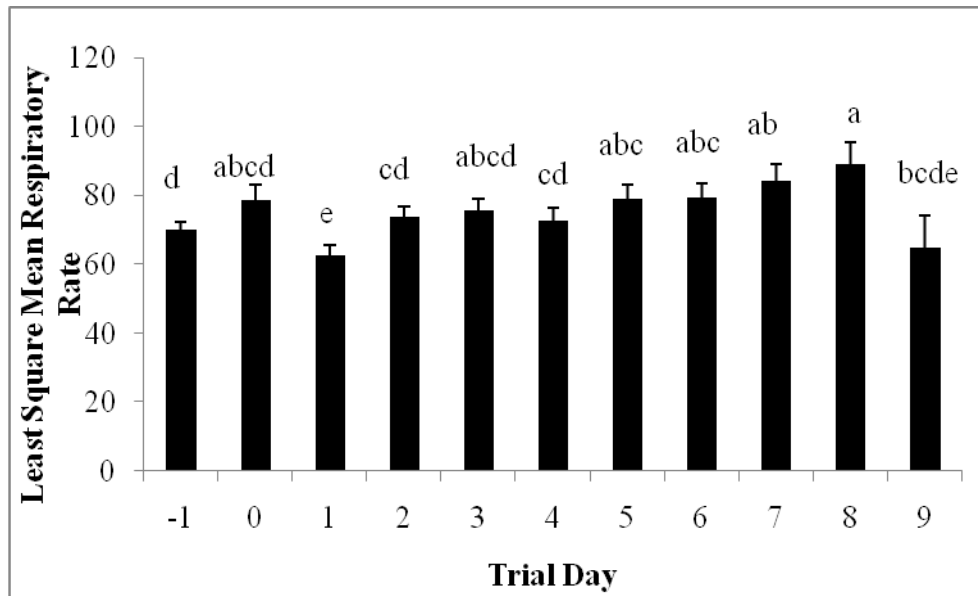
<sup>2</sup>Measurements were taken in the morning (0630), at noon, and late afternoon (1630).

<sup>3</sup>Columns with unique superscripts statistically differ ( $p < 0.05$ )

<sup>4</sup>Model accounted for the time of day measurement was taken and the repeated measures on an on individual animal.



**Figure 3-6 Least square mean respiratory rate (respirations / minute) by trial day<sup>1,2,3</sup>**

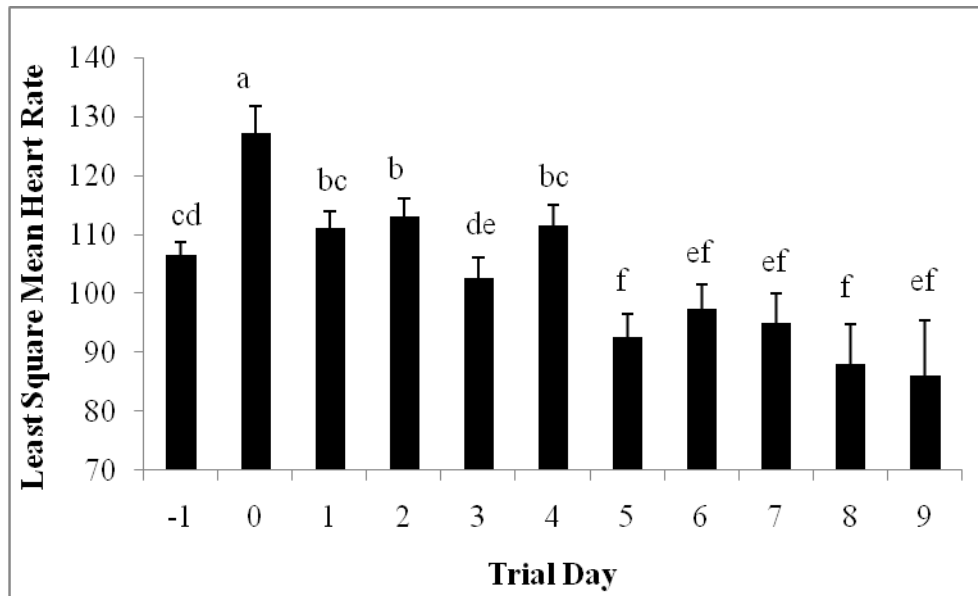


<sup>1</sup>All readings prior to inoculation are included in trial day -1.

<sup>2</sup>Columns with unique superscripts statistically differ ( $p < 0.05$ )

<sup>3</sup>Model included random effects accounting for the time of day measurement and the repeated measures on an on individual.

**Figure 3-7 Least square mean heart rate (beats per minute) by trial day<sup>1,2,3</sup>**

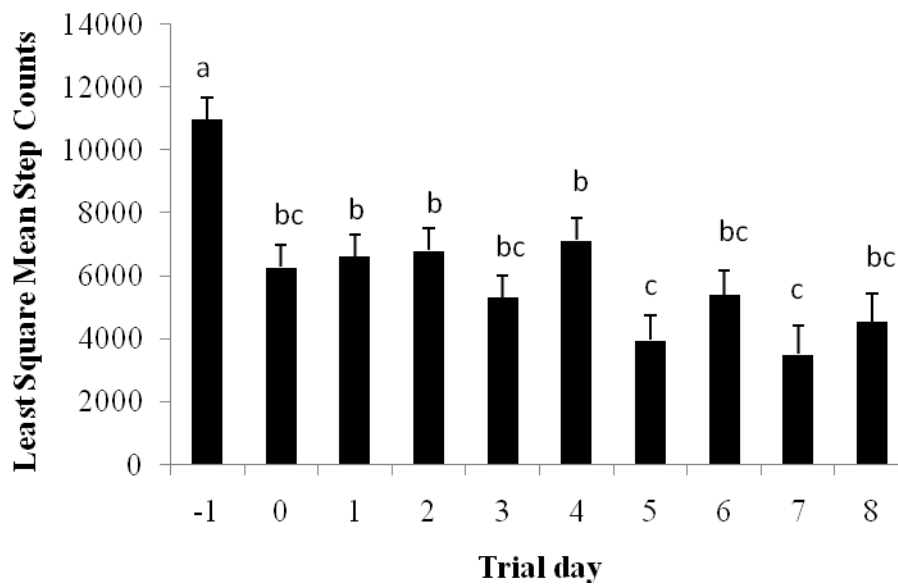


<sup>1</sup>All readings prior to inoculation are included in trial day -1

<sup>2</sup>Columns with unique superscripts statistically differ ( $p < 0.05$ )

<sup>3</sup>Model included random effects accounting for the time of day<sup>2</sup> measurement and the repeated measures on an on an individual.

**Figure 3-8 Least square means step counts by trial day<sup>1</sup> from a model including random effects.<sup>1,2,3</sup>**

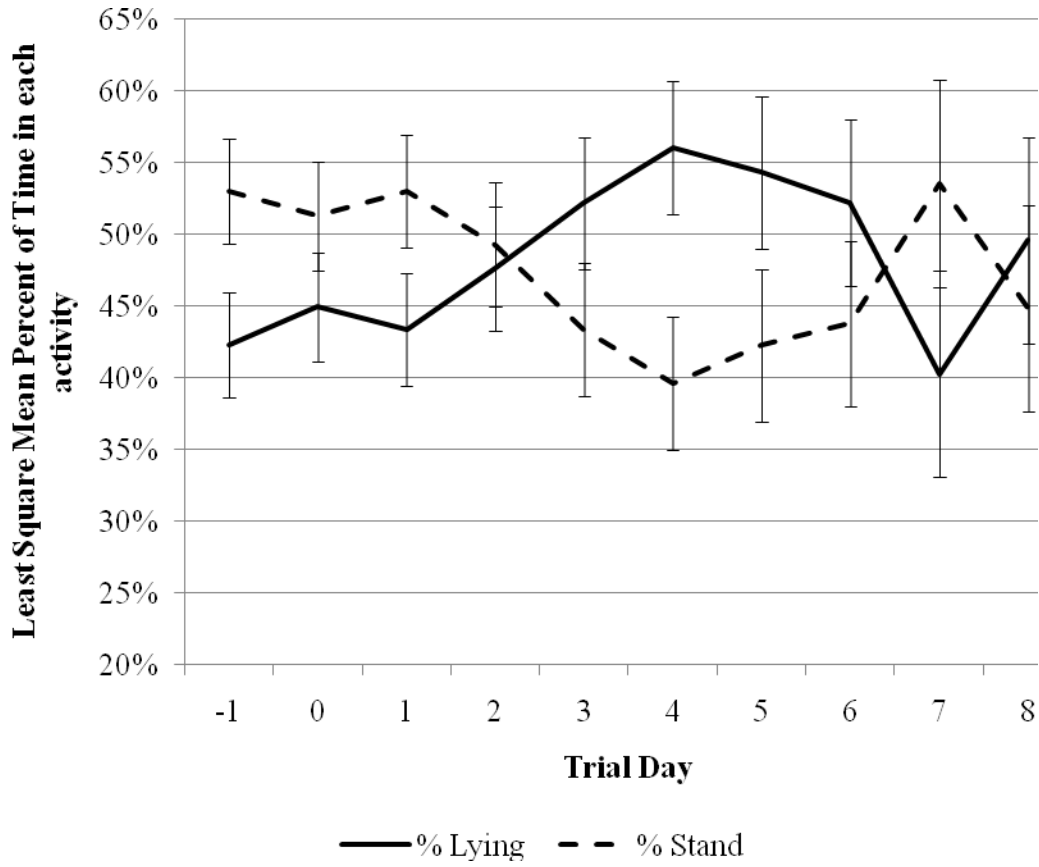


<sup>1</sup>All readings prior to inoculation are included in trial day -1.

<sup>2</sup>Columns with unique superscripts statistically differ ( $p < 0.05$ )

<sup>3</sup>Day 9 not included because it was a partial day

**Figure 3-9 Interactive model adjusted mean percent of time spent lying and standing by trial day<sup>1</sup> for calves inoculated with *Mannheimia haemolytica* Day 0.<sup>2,3</sup>**



<sup>1</sup>All readings prior to inoculation are included in trial day -1.

<sup>2</sup>Trial day 9 not included because it was a partial day

<sup>3</sup>Model was a logistic regression model accounting for repeated measures on individual calves.

## **CHAPTER 4 - Mollicutes and *Mycoplasma bovis* prevalence: associations with health and performance in stocker calves**

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(In print: *The Veterinary Record*)

### **Summary**

The purpose of this longitudinal cross-sectional times series study was to determine prevalence of nasal mycoplasma, serostatus and seroconversion and evaluate associations with health and performance in weaned beef calves during a 42 day feeding period. Nasal swabs were collected and serum was collected on days 0 (arrival), 10, 42 and at first bovine respiratory disease complex (BRDC) incidence and were evaluated for Mollicutes (culture), *Mycoplasma bovis* (PCR), and *Mycoplasma bovis* (serum antibody). On day 0, 90.4% of the calves were Mollicutes nasal culture positive. *Mycoplasma bovis* seroprevalence was 26.6% on day 0 and 98.2% by day 42 ( $P < 0.05$ ). Seroconversion to *M. bovis* between days 0 and 42 was associated ( $P = 0.04$ ) with lower weight gain. Weight gain was greater in calves that were PCR negative on

day 10 ( $P = 0.01$ ) for *M. bovis*. The percent of calves seropositive to *M. bovis* increased throughout the study indicating exposure and an immunological response to the organism. Although associations with health outcomes were not identified, seroconversion to *M. bovis* was associated with a decreased rate of weight gain during study period.

## **Introduction**

Mycoplasma organisms (class Mollicutes) are associated with some of the most economically important and severe diseases observed in cattle (Nicholas and Ayling, 2003; Gagea et al., 2006b). In the United States, mycoplasma organisms are associated with bovine respiratory disease complex (BRDC), and in other parts of the world they are associated with chronic pneumonia polyarthritis syndrome (CPPS) (Nicholas and Ayling, 2003; Clark, 2005; Gagea et al., 2006a; Caswell and Archambault, 2007).

*Mycoplasma bovis*, the most frequently identified pathogenic mycoplasma, is an upper airway commensal that is not ubiquitous, but is commonly isolated from nasal samples in diseased and non-diseased calves (Boothby et al., 1983; Allen JW, 1991; Nicholas and Ayling, 2003; Maunsell, 2009). Several studies have investigated the prevalence of Mollicutes and *M. bovis* in calf populations at single sampling periods, but few have explored the associations between prevalence and seroconversion in individual calves at multiple time points (Yates et al., 1983; Marques et al., 2007; Wiggins et al., 2007). Increased awareness of the association between the dynamics of Mollicutes and *M. bovis* and relevant health and production measures may be clinically important.

A longitudinal cross sectional time series study was designed to investigate these associations with three objectives: 1) to determine the prevalence of *M. bovis* and other mycoplasmas in nasal samples and *M. bovis* antibody concentration in weaned beef stocker

calves on three sampling days—arrival (Day 0) revaccination (Day 10), and study completion (Day 42), 2) to determine the changes in nasal Mollicutes and *M. bovis* serostatus over 3 time periods: arrival to day 10 (AR), day 10 to day 42 (RV), and arrival to day 42 (ENT), and 3) to examine associations of nasal Mollicutes status, and *M. bovis* nasal status, and *M. bovis* serostatus and seroconversion with growth performance (average daily gain) and morbidity, mortality and case fatality risk.

## **Materials and methods**

### ***Calves and preventive health program***

The study was conducted at a Kansa dry-lot, commercial stocker operation with a onetime capacity of approximately 6,000 calves. The study population consisted of male crossbred beef-calves purchased at a livestock auction market in Georgia. Before leaving the auction market, calves were administered a modified live vaccine (MLV) (Bovishield Gold 5, Pfizer) which included bovine viral diarrhea virus (BVDV) Type 1 and 2, bovine respiratory syncytial virus (BRSV), parainfluenza 3 (PI 3) and infectious bovine rhinotracheitis virus (IBR). Additionally, calves were administered a Clostridium vaccine (Ultrabac 7, Pfizer) containing *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyii*, *Clostridium sordellii*, *Clostridium perfringens* C and D.

The calves arrived in two groups one week apart. Each group was allocated to one pen and pens were fed and housed separately for the entire study period. The two study pens were isolated from other calves housed at the facility. Two days after arrival (day 0), calves were administered an intranasal MLV infectious IBR and PI3 vaccine (TSV II; Pfizer), a doramectin parasiticide (Dectomax; Pfizer) and ceftiofur, (Excede; Pfizer). An ear tissue sample was taken

at this time for real-time PCR (Enfer) BVDV status. Additionally, all calves were weighed, and intact male calves were castrated using a scalpel and Whites Emasculator (Jorgenson). Ten days after arrival, all calves were administered a MLV containing BVDV Type 1 and 2, PI3, BRSV, and IBR, (Bovishield Gold 5, Pfizer) a clostridium bacterin containing *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyii*, *Clostridium sordellii*, *Clostridium perfringens* C & D (Ultrabac 7, Pfizer) and tilmicosin phosphate (Micotil, Elanco).

### ***Health and production performance***

Experienced animal care-givers (n = 2), employed by the stocker operation, observed calves twice daily for signs of illness. The BRDC case definition was based on each calf's attitude, appetite, and clinical signs as defined by the stocker unit's standard operating procedure manual. Calves observed with clinical signs of BRDC were removed from the pen, taken to the working facility and administered an antibiotic (Draxxin; Pfizer). Rectal temperatures were recorded at this time, but the antibiotic was administered based on presumptive diagnosis of BRDC regardless of the temperature reading. After treatment, all calves were taken back to their original pen. For production performance evaluation (ADG), calves were individually weighed on three occasions: day 0, day 10, and day 42.

### ***Culture and serology sampling***

Nasal cultures were used to isolate mycoplasma and acholeplasma organisms, referred throughout the manuscript as Mollicutes. Nasal swab and blood samples were collected from each calf on three scheduled sampling days. Additionally, a nasal culture and serum sample were collected from each calf at the first BRDC episode. Nasal and lung culture samples were collected from mortalities. Nasal cultures were collected in each calf using a sterile single-



sheath guarded swab (Jorgenson) that was advanced approximately 10 cm into the ventral nasal meatus; then the swab was advanced through the sheath tip another 15 cm and a sample was collected. The swab was placed into mycoplasma broth (Hardy Diagnostics Cat. No. G212), and transported on ice to Kansas State University (KSU) for overnight shipment to a commercial microbiology laboratory (EIDE). Serum samples consisted of approximately 6 ml of blood collected and transported, on ice, to a laboratory at KSU. All mortalities were necropsied by one of the investigators (GAH) or the unit's trained personnel. Lung cultures were collected by inserting a sterile culture swab (Jorgensen Laboratory) into a stab incision made with a sterile scalpel blade, at the demarcation line between normal and diseased tissue.

### ***Mollicutes culture and identification***

Prior to inoculation, each broth tube (Hardy) containing the nasal swab was inverted several times. One hundred microliters of broth were transferred to one-half of a 15 by 100mm mycoplasma agar plate (Hardy Diagnostics); broth was then streaked across the remaining one-half of the plate using a sterile, cotton-tipped swab. The remaining broth was then placed in an incubator. The inoculum was then allowed to absorb onto the media for fifteen minutes, then the plates were inverted and incubated at 35-37° C, in 5% CO<sub>2</sub> for 14 days.

A tissue culture 40X microscope was used to search each plate for the presence of *Mollicutes* organisms. Plates were observed after 3, 7, and 14 days of incubation, and only if negative at all three observations were samples recorded as negative. For samples negative on day 3, the original sample broth was removed from incubation and used to re-inoculate new plates which were reexamined for the presence of *Mollicutes* organisms as described above.

## *Serology*

All serology tests were completed over a 3 day period using a commercial *M. bovis* antibody ELISA kit (Bio-X). Single serum dilutions were tested in random order over the testing period. Serum dilution and microwell incubation was performed as recommended by the manufacturer.

To calculate antibody levels, each OD value was subtracted from the negative control test value, and then divided by the corresponding positive control test value. As recommended by the kit manufacturer, a sample was considered to be positive if the result was greater than 13.73. Raw percentage signal values were categorized by magnitude into 6 groups (0, +, ++, +++, +++++, ++++++). The signal value numerical limits were: (  $0 < 13.73$  ); (  $13.73 < + < 50.60$  ); (  $50.60 < ++ < 87.47$  ); (  $87.47 < +++ < 124.35$  ); (  $124.35 < +++++ < 161.22$  ); (  $161.22 < ++++++$  ). Seroconversion was considered to have occurred if the signal from a serum sample increased by 2 orders (i.e. ++ initially to  $\geq$  +++++) from the previous serum sample.

## *Polymerase chain reaction (PCR)*

Polymerase chain reaction analyses were completed at the Kansas Veterinary Diagnostic Laboratory, targeting *Mycoplasma bovis* and using techniques as described by Lauerman *et al.* (Lauerman, 1998a). The primers used were forward primer: MBV-F 5' - TGATAGCAATATCATAGCGGC-3'; reverse primer: MBV-R 5' - GTAGCATCATTTCCCTATGCTAC-3' (Harasawa, 1993; Lauerman, 1998b).

### *Statistical analysis*

Data were recorded on site using a commercial spreadsheet program (Microsoft Excel). Descriptive statistics (STATA v. 10.1) and results from hypothesis testing (SAS v. 9.1) were generated using commercial statistical software packages. Individual animal was the unit of analysis. Gonadal status was forced into each model as a fixed effect to account for possible confounding. Arrival weights were categorized into quartiles: <136 kg , 136-182 kg, 183-227 kg and > 227 kg based on industry standard break points, and this variable was also forced into all models to account for potential confounding (Dohoo et al., 2003b). Pen was entered into the model as a random effect to account for the lack of independence between calves within each pen (Dohoo et al., 2003a). General linear mixed models (Proc Glimmix) were used to analyze data on health and performance outcomes potentially associated with nasal prevalence and seroprevalence at multiple sampling points accounting for repeated measures. Linear mixed models (Proc Mixed) were used to evaluate potential associations between nasal and serology results and ADG.

### **Results**

A total of 305 mixed-breed beef calves from two arrival groups (n = 134 and n = 171) comprised the study population. One calf from the second group was positive for BVDV persistent infection and removed from the study location within one day of initial processing. The remaining 304 calves had an average arrival weight of 183.5 kg; (Standard Deviation 41.29 kg) and consisted of 18.4% (n = 56) steers and 81.6% (n= 248) bulls.

### ***Mollicutes culture***

A nasal sample was not collected from one calf on arrival due to premature release from the cattle working chute. Prevalence results for all sample times and diagnostic tests are included in Table 1. The *Mollicutes* culture status of calves was associated ( $P = 0.01$ ) with sampling day. The prevalence was lower ( $P < 0.01$ ) at day 0 compared to day 42. No difference was found between the prevalence at day 10 and day 42 or day 0 and day 10 (Table 1).

### ***Mycoplasma bovis serostatus and seroconversion***

*M. bovis* seropositive calves at each sampling day were 26.6%, 62.7%, and 98.2%, for day 0, day 10, and day 42, respectively (Table 1), and serostatus was associated with sampling day ( $P < 0.01$ ). Calves seropositive at day 42 tended to have lower morbidity risk over the entire study period ( $P = 0.08$ ) compared to seronegative calves at day 42. Seroconversion was associated with sampling period ( $P < 0.01$ ); the percentage of calves seroconverting to *M. bovis* increased over each time interval (Table 1).

### ***Mycoplasma bovis nasal PCR***

The prevalence estimates for *M. bovis* are reported in Table 1. The prevalence of *M. bovis* by PCR was higher at day 10 ( $P < 0.05$ ) compared to days 0 and 42 (Table 1). *M. bovis* PCR status was associated with seroconversion over any time period. Being PCR positive for *M. bovis* at any time point was not associated with morbidity and mortality risk (Table 2).

### ***Results for samples at first BRDC treatment and necropsy***

All morbidities and mortalities were attributed to BRDC and 14.4% (44/304) of the calves were treated during the trial, with 6.5% (20/ 304 calves) dying during the period. Of the 44 BRDC morbid calves, 37 were sampled at first treatment leaving missing data for 7 calves

due to unforeseen logistical issues. All calves sampled at first BRDC treatment were *Mollicutes* nasal culture positive. At first BRDC treatment, three calves were PCR positive for *M. bovis* (8.1%). In dead calves, *Mollicutes* was cultured from 94.7% (18/19) of the nasal samples and 94.1% (16/17) of the lung samples. Three out of 19 (15.7%) calves had nasal samples positive by PCR for *M. bovis*. Only one calf (5.5%) was positive on PCR analysis of lung sample for *M. bovis*.

### ***Mollicutes, Mycoplasma species Mycoplasma bovis and average daily gain***

Average daily gain for all calves over the entire study was 0.44 kg (range of -1.57 kg to 2.14 kg). Calves *Mollicutes* culture negative on day 0 gained more weight ( $P = 0.06$ ) than positive calves (Table 3). Calves that were *Mollicutes* negative on day 42 also gained more weight ( $P = <0.01$ ) compared to positive calves (Table 3). There was no association between ADG and serostatus at any single sampling time point. However, calves that did not seroconvert during ENT period had a higher rate of weight gain ( $P = 0.04$ ) compared to calves that did seroconvert during the same period (Table 3). Calves *M. bovis* PCR negative at day 10 gained more weight per day ( $P = 0.01$ ) compared to positive calves, and calves remaining negative to *M. bovis* PCR at all sampling days tended to gain more ( $P = 0.09$ ) than calves with at least one positive PCR result (Table 3).

## **Discussion**

Most calves were *Mollicutes* nasal culture positive upon arrival and at each subsequent sampling period. Our culture results contrast with one study where only 15% of calves were found to be nasal *Mollicutes* culture positive when sampled within 10 days after arrival (Wiggins et al., 2007). This difference may be explained by different culture techniques that were used in

each study, but this could also indicate a difference in prevalence among different groups of stocker calves. Additionally, researchers have suggested that management practices such as commingling and long transport distances, both of which occurred in our study, can increase *Mollicutes* nasal prevalence (Boothby et al., 1983). The *Mollicutes* populations may also be explained by the 2 metaphylactic antibiotics, used by the stocker operation in our trial, one (Excede, Pfizer) being ineffective against all *Mollicutes* and the other (Micotil, Elanco) having limited activity against *Mollicutes* (Rosenbusch et al., 2005; Gerchman et al., 2009). It is possible other commensal bacterial populations were reduced by the antibiotics which allowed *Mollicutes* populations to flourish.

Over 25% of all calves were *M. bovis* seropositive upon arrival, and by study completion almost all calves (98.2%) were seropositive. The half-life of *M. bovis* antibodies has been estimated to be 20 days, and given the young age (based on arrival weight) of some of the calves in this study, it is plausible that maternal antibodies may have been present upon arrival (Tschopp, 2001). However, an analysis of the data revealed no association between arrival weight and arrival serostatus. If calf body weight is a proxy for age, then it is less likely that arrival seropositive status was a result of maternal antibodies and more likely that it was due to previous *M. bovis* exposure and infection. One study found measurable antibodies, in seronegative calves, within 7 days post-*M. bovis* inoculation (Vanden Bush and Rosenbusch, 2003). We found a high percentage of calves became seropositive during the first 10 days of our study, which suggests that calves were exposed to *M. bovis* immediately prior to arrival or early in the study period.

By study completion 59.7% of the calves had seroconverted to *M. bovis*. Our findings agree with a previous study where 55% of calves entering a feedlot seroconverted during the first

7 weeks after arrival (Tschopp and others 2001). The relatively low percentage of calves that seroconverted during the study period may be explained by the percentage of calves that were seropositive upon arrival. Seroconversion was defined as a 2 fold increase in serum signal between samples. It seems plausible that seropositive animals (those with higher initial values) were less likely to seroconvert than seronegative animals (those with lower initial values). Our study indicates that commingled calves, hauled long distances, and stressed by dietary and social challenges, are capable of mounting an immune response to *M. bovis* within 2 weeks after arrival. The arrival nasal *M. bovis* PCR status and seroconversion were not associated and may indicate that the PCR is not a good indicator of the presence of the organism or that the tests were performed at a time when the organism was not present. Seroconversion to *M. bovis* likely requires exposure, invasion, and infection of the lower respiratory. Risk factors associated with *M. bovis* seroconversion have been reported to include mixing calves of different age groups, metaphylactic antibiotic use and contact with a seropositive animal. (Tschopp and others 2001) The arrival weight range in our study was wide (90.9 kg to 345.5 kg) and assuming calf weight is a proxy for age, all three risk factors were present in our study population.

Morbidity risk tended ( $P = 0.08$ ) to be lower in calves that were seropositive at study completion, versus those that were seronegative. Other studies have failed to find an association between *M. bovis* serostatus and morbidity or mortality (Rosendal and Martin, 1986; Martin et al., 1989). No association was found between *M. bovis* seroconversion status and health outcomes. This contrasts with two published studies that found calves which seroconverted to *M. bovis* were more likely to experience respiratory disease compared to calves that did not seroconvert (Rosendal and Martin, 1986; Martin et al., 1990). That only tendencies were

observed in our study may be explained by the relatively low number of morbidity and mortality cases that occurred.

Several associations were identified between Mollicutes and *M. bovis* presence and ADG. The presence of Mollicutes by nasal culture at day 42 was associated with lower ADG. There also tended to be an association between ADG and arrival Mollicutes culture prevalence. This may indicate that Mollicutes negatively effects performance when present in the upper respiratory tract or Mollicutes were also present in other anatomical areas and their presence was manifested by subclinical disease. Other studies found a high proportion of apparently healthy calves with lung lesions at slaughter suggesting subclinical BRDC infections are common (Wittum et al., 1996; Gardner et al., 1998). Additionally, a Bayesian analysis of two studies found that clinical signs, as a test for BRDC diagnosis, have relatively low sensitivity and specificity (White and Renter, 2009). In the study described here, seroprevalence at any single time point was not associated with ADG, although seroconversion to *M. bovis* over the entire study period was negatively associated with performance. Previously, researchers have shown that calves that seroconverted to *M. bovis* experienced a 7.6% decrease in average daily gain over a 49 day period, compared to calves that did not seroconvert (Tschopp and others 2001). Our findings suggest interventions that reduce seroconversion after arrival, such as prior immunization, or a reduction in exposure to *M. bovis* just before or during the early feeding period, may be beneficial in reducing the negative impact of this organism in the stocker phase of production. That few BRDC morbidities occurred in our study but seroconversion was common may indicate a performance expense in response to *M. bovis* subclinical infections.

The study subjects were typical of high stressed calves that enter United States stocker units. The study described here indicates that in some populations Mollicutes organisms are



nasal ubiquitous, dynamic populations, and exposure to this class of organism may be common in newly arrived stocker calves. We found 26.5% of calves seropositive to *M. bovis* on arrival, which suggests exposure to this organism may occur sometime prior to entry into a stocker facility. Additionally, our data indicate that highly stressed beef calves are able to mount an antibody response to *M. bovis* soon after arrival, but seroconversion, or associated subclinical disease, has a cost that is expressed through a decrease in weight gain. This production cost was observed even though BRDC morbidity and mortality risks were low, suggesting Mollicutes, *Mycoplasma* species and *M. bovis* organisms have important effects on beef calves even if disease events are not clinically apparent. That seroconversion and not serostatus was associated with weight gain indicates that longitudinal studies are important in assessing the role of mycoplasma organisms in stocker unit performance. Further research is needed to define the epidemiology of these organisms in other production settings and their effects on beef calf health and production performance.

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**Table 4-1 Unadjusted data from beef stocker calves showing prevalence based on Mollicutes nasal culture, *M. bovis* serostatus, *M. bovis* nasal PCR by sampling time.**

	Sample Day		
	Day 0	Day 10	Day 42
Mollicutes nasal culture*	90.4% <sup>a</sup> (274/303)	100.0% <sup>ab</sup> (298/298)	96.4% <sup>b</sup> (271/281)
<i>M. bovis</i> seropositive	26.6% <sup>a</sup> (81/304)	62.7% <sup>b</sup> (187/298)	98.2% <sup>c</sup> (276/281)
<i>M. bovis</i> nasal PCR	6.6% <sup>a</sup> (20/303)	11.4% <sup>b</sup> (34/298)	4.2% <sup>a</sup> (12/280)
	Sampling Interval**		
	AR	RV	ENT
<i>M. bovis</i> seroconversion	10.7% <sup>a</sup> (32/298)	41.2% <sup>b</sup> (115/279)	59.7% <sup>c</sup> (168/281)

\*Data with different superscripts in the same row are different (P <0.05) based on multivariable regression model including effects of arrival group, morbidity, arrival weight and gonadal status.

\*\*Relative to arrival at a stocker facility: AR = day 0 to day 10, RV = day 10 to day 42, ENT= day 0 to day 42

**Table 4-2 Unadjusted calf respiratory disease (BRDC) morbidity by *Mollicutes* culture status, *M. bovis* nasal PCR status, *M. bovis* serostatus, seroconversion and nasal PCR status by sampling day\* or period.**

<b>BRDC Morbidity by Test Results</b>			
	<b>Test Negative</b>	<b>Test Positive</b>	<b>P-value**</b>
<b><u>Mollicutes culture</u></b>			
Day 0	3.4% (1/29)	15.3% (42/274)	0.11
Day 10	0% (0/0)	14.0% (42/298)	---***
Day 42	0% (0/10)	11.8% (32/271)	0.97
Negative at all sampling days ****	0% (0/279)	11.4% (32/279)	---
<b><u>M. bovis serostatus</u></b>			
Day 0	15.2% (34/223)	12.3% (10/81)	0.84
Day 10	13.5% (15/111)	14.4% (27/187)	0.70
Day 42	40.0% (2/5)	10.8% (30/276)	0.08
Negative at all sampling days	50.0% (1/2)	11.1% (31/279)	0.19
<b><u>M. bovis seroconversion</u></b>			
AR	12.7% (34/266)	25.0% (8/32)	0.58
RV	10.9% (18/164)	12.1% (14/115)	0.58
ENT	8.8% (10/113)	13.0% (22/168)	0.39
Negative for all sampling days	8.4% (9/107)	13.3% (23/172)	0.33
<b><u>M. bovis PCR</u></b>			
Day 0	15.1% (43/283)	0.0% (0/20)	0.96
Day 10	14.7% (39/264)	8.8% (3/34)	0.51
Day 42	10.8% (29/268)	25.0% (3/12)	0.43
Negative for all sampling days	12.0% (26/216)	9.6% (6/62)	0.66

\*Sampling day in relation to arrival to stocker facility: day 0, day 10, day 42, and sampling periods: AR = day 0 to day 10, RV = day 10 to day 42, ENT = day 0 to day 42

\*\*P-values based on comparisons of model-adjusted means from multivariable logistic regression models containing effects of group, gonadal status, and arrival weight..

\*\*\* Unable to assess significance due to 0% for one group

\*\*\*\* Represents parallel interpretation of test results; i.e. negative animals tested negative at all sample days or periods, and positive calves were positive at least 1 sample day or period.

**Table 4-3 Model-adjusted\* means for daily weight gains (kg/day) by *Mollicutes* nasal culture, *M. bovis* serostatus and seroconversion, and nasal sample polymerase chain reaction (PCR) testing for *M. bovis* .**

	Average Daily Gain by Test Result		P-value*
	Negative	Positive	
<b><u>Mollicutes nasal culture</u></b>			
Day 0**	0.59 n = 29	0.40 n = 274	0.06
Day 10	-- n = 0	0.41 n = 298	--
Day 42	0.93 n = 10	0.41 n = 271	< 0.01
Negative for all sampling days***	-- n = 0	0.41 n = 279	--
<b><u>M. bovis serostatus</u></b>			
Day 0	0.40 n = 223	0.45 n = 81	0.53
Day 10	0.38 n = 111	0.44 n = 187	0.38
Day 42	0.68 n = 5	0.40 n = 276	0.25
Negative for all sampling days	0.38 n = 2	0.41 n = 279	0.92
<b><u>M. bovis seroconversion</u></b>			
AR	0.40 n = 266	0.51 n = 32	0.34
RV	0.45 n = 164	0.35 n = 115	0.17
ENT	0.49 n = 113	0.35 n = 276	0.04
Negative for all sampling periods	0.49 n = 107	0.36 n = 279	0.06
<b><u>M. bovis nasal PCR</u></b>			
Day 0	0.40 n = 283	0.55 n = 20	0.25
Day 10	0.44 n = 264	0.20 n = 34	0.01
Day 42	0.43 n = 268	0.18 n = 12	0.12
Negative for all sampling days	0.45 n = 216	0.31 n = 62	0.09

\*From a multivariable regression model of average daily gain from arrival to day 42 (with mortalities removed), which included effects for arrival weight, group, gonadal status , morbidity

\*\*Sampling day or period in relation to arrival to stocker facility: AR = day 2 to day 10, RV = day 10 to day 42, ENT = day 0 to day 42

\*\*\*Negative = negative test result for all testing days or periods: Positive = 1 or more positive test

## **CHAPTER 5 - Management practices associated with the rate of pre-weaning disease from a national survey of U.S. cow-calf operations**

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### **Abstract**

National survey data representative of 79.6% of U.S. cow-calf operations and 87.8% of U.S. beef cows were used to investigate associations between herd management practices and bovine respiratory disease complex (BRDC) rates in pre-weaned beef calves. Data from surveys on management practices and health outcomes were combined for this assessment.

Multivariable negative binomial regression models and a platform specifically designed for assessing weighted survey data were used to evaluate factors associated with herd BRDC rates, as defined by counts of reported BRDC treatments and total calf-days at risk from birth to weaning. The final multivariable model contained six variables associated with pre-weaning

BRDC rates. Higher BRDC rates were found in operations that fed antibiotics to calves to prevent pre-weaning BRDC (incidence rate ratio (IRR) 3.46; 95% confidence interval (CI) 1.39, 8.60) compared to herds that did not feed antibiotics. Importing bred heifers was associated with lower BRDC rates (IRR 0.40; CI 0.19, 0.82), but operations that imported weaned steers (IRR 2.62; CI 1.15, 5.97) had higher rates than operations that did not import this class of animal. The number of reported visits by outsiders also was associated with herd-level BRDC rates; compared to 0 visits, IRRs were 2.06 (CI 0.59, 7.13), 0.57 (CI 0.19, 1.70), 0.46 (CI 0.16, 1.31), 1.26 (CI 0.45, 3.51) for 1-2, 3-5, 6-30, and >30 visits per month, respectively. Operations whose calves were from composite genetics (IRR 2.27; CI 1.00, 5.16), two breed crosses (IRR 2.36; CI 1.30, 4.29) or three breed crosses (IRR 4.00; CI 1.93, 8.31) had higher BRDC rates than single breed herds. Compared to respondents that considered the cow-calf operation to be the primary source of income, those that considered the operation as a supplemental source of income (IRR 0.48; CI 0.26, 0.87) had lower BRDC rates. Our study demonstrated unique associations between pre-weaning BRDC rates and cow-calf management practices, and identified several factors that may be useful indicators of pre-weaning BRDC rates in U.S. cow-calf production systems.

## **Introduction**

Bovine respiratory disease complex (BRDC) continues to be a significant health issue in the beef industry (Edwards, 1996; Smith, 1996; Snowden, 2006; Duff and Galyean, 2007; Fulton, 2009). In a national survey, over 33% of cow-calf producers agreed or strongly agreed that BRDC is economically important to their operation (USDA-APHIS:VS, 2009b). In the same study, respiratory disease was reported as the cause of death for 8.2% of the calves that died before weaning. In two multi-year studies, pre-weaning BRDC cumulative incidence within



herds ranged from 3.3% to 23.5% and 8.5% to 65.4% of calves born into a herd (Muggli-Cockett, 1992; Snowden et al., 2005, respectively). Calves that experienced pre-weaning BRDC were on average 16.5 kg and 7.7 kg lighter than their cohorts at weaning (Wittum et al., 1996; Snowden et al., 2005). Pre-weaning BRDC morbidity can negatively affect calf sale weight and can lead to pre-weaning mortalities, thereby reducing the weight and number of calves that cow-calf operators sell and impacting herd profitability.

Risk factors for BRDC have been well documented for feedlot cattle and pre-weaned dairy calves (Van Donkersgoed, 1993; Svensson et al., 2003; Lago et al., 2006; Svensson et al., 2006; Sanderson et al., 2008). However, pre-weaned beef calves are managed much differently from dairy calves; therefore, it cannot be assumed that pre-weaning BRDC risk factors would be similar to those in the dairy industry. There are no large-scale studies documenting management factors associated with pre-weaning BRDC in cow-calf herds.

The United States Department of Agriculture (USDA) National Animal Health Monitoring System's (NAHMS) 2007-08 Beef Survey generated data on management factors and disease occurrences for U.S. cow-calf herds (USDA-APHIS:VS, 2008). Our objective was to utilize these data to assess potential associations between cow-calf herd management practices and the rate of BRDC in pre-weaned beef calves. Documenting the cow-calf management factors that are associated with BRDC morbidity would allow further focus on management areas that are most likely to offer the largest positive impact on this important disease syndrome.

## **Material and methods**

### ***Survey design***

Data for this study were collected in two parts, Phases I and II (described below), of the USDA , NAHMS 2007-08 Beef survey (USDA-APHIS:VS, 2008). From a sampling frame obtained from the National Agricultural Statistics Service (NASS), which included all cow-calf operations in the 24 selected states, a stratified random sample of operations was selected. The survey was a single-stage design with sampling across strata (based on state and herd size) without replacement. Herd size on January 1, 2007 within state was the stratification variable. States included in this survey were Alabama, Arkansas, California, Colorado, Florida, Georgia, Idaho, Iowa, Kansas, Kentucky, Louisiana, Mississippi, Missouri, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, Oregon, South Dakota, Tennessee, Texas, Virginia, and Wyoming. States were chosen based on a goal that the survey represents at least 70% of the animals and cow-calf producers in the United States.

### ***Survey data***

A total of 4,001 operations were selected to participate in Phase I (General Beef Management Report) of the survey. The Phase I questionnaire contained 110 questions and was administered by trained enumerators from NASS between October 22 and November 30, 2007. Phase II of the survey (Veterinary Services Initial Visit) contained 51 questions and was administered by veterinary medical officers representing USDA-Animal Plant Health Information Service (APHIS) in January of 2008.

Of those selected during Phase I, 47.8% (1,033) provided complete information and were eligible to participate in subsequent studies. Of those eligible operations, 54.9% (567) consented

to participate in Phase II of the survey. Reasons for not participating in Phase II included: refused to participate (35.3%), were not contacted (7.8%), and had no beef cows on hand (2.0%). The dataset used for our study contains answers given by the 567 participants who completed both Phase I and II. Twenty four states (listed above) were included and these states include 87.8% (28.6 million) of the beef cows and 79.6% (603,000) of the cow-calf operations in the United States (USDA-APHIS:VS, 2009a).

Data validation for Phase I was performed by each state's NASS personnel, and validation for Phase II was performed by USDA's Center for Epidemiology and Animal Health staff (USDA-APHIS:VS, 2008). Additional data validation was completed by the NAHMS staff after the data sets from each state were all combined. All participants' data were statistically weighted to reflect the population from which they were selected. The weights included the inverse of the probability of being selected and adjustments for nonresponse (Dargatz and Hill, 1996).

### *Statistical analysis*

All data analyses were performed using Stata 10.1 (StataCorp, College Station, TX), which provides a unique platform (svy) for assessing survey data with weighted responses. The outcome variable of interest for each herd was the rate of respiratory disease in calves between birth and weaning, hereafter referred to as BRDC rate. Herd-level rates were defined using reported counts of calves treated with an antimicrobial for respiratory disease and total calf-days at risk from birth to weaning. Calf-days at risk for each herd were initially determined using the reported number of calves born alive multiplied by the reported average number of days from birth to weaning. Calf-days at risk were then adjusted based on any calf mortalities and respiratory morbidities occurring prior to weaning. The reported count of calf mortalities for

three times periods (birth to 24 hours, 24 hours to three weeks, and three weeks to weaning) were contained in the data set. Morbidity was not reported in these periods; only a total count for illness occurring pre-weaning. All calf-days for calves dying within 24 hours were subtracted from the initial calf-days at risk estimate. For calf mortalities within other time periods and for all pre-weaning morbidities, calves only contributed one half (mid-point) of their calf-days at risk from their respective time period.

There were 804 survey responses initially considered as independent variables for this study. Some variables (e.g., type of animal identification used) were not retained for analysis given lack of plausibility for potential associations with the outcome of interest. Descriptive analyses including histograms and scatter plots were completed on each variable. Categorical variables where one level represented more than 95% of the herds were excluded from further analysis. Contingency tables were used to assess the similarities of variables among herds. For variables with >95% of the herds answering similarly to multiple related questions, a single variable was created, to represent responses to these related questions. For example, animals administered bovine viral diarrhea virus (BVDV) vaccine and animals administered infectious bovine rhinotracheitis (IBR) vaccine were included in one variable representing vaccination with both BVDV and IBR (versus those that vaccinated for only one or neither). After these initial data evaluation steps, which included assessments of plausibility, descriptive statistics and correlations among variables, and combining some variables into single variables, 86 independent variables were used for further analysis.

Associations between BRDC rate and independent variables were evaluated in weighted analyses using negative binomial regression models with Taylor series linearized variance estimation and single sampling unit centered at the grand mean (Dargatz and Hill, 1996; Stata,

2007). Each independent variable was initially assessed separately in bivariable models. Independent variables were retained for further analysis if the bivariable  $p$  value was  $\leq 0.30$ . Continuous independent variables were assessed for the assumption of linearity by categorizing into quantiles. If the quantiles were non-linear over each interval, the variable was entered into the model using the categorical coding. To check for potential multicollinearity, associations among variables that were retained following the bivariable analysis ( $p \leq 0.30$ ) were assessed using chi-square and Spearman's correlation for binary and nominal/ordinal variables, respectively. In addition, collinearity was assessed by adding one covariate at a time to the bivariable analyses of other possible collinear variables.

Variables remaining following the bivariable analysis were entered into a multivariable negative binomial regression model and manual backward selection were used to select independent variables significantly associated ( $p \leq 0.05$ ) with the outcome. In addition, herd size was assessed as a potentially important predictive variable and potential confounder. Each variable not retained during the initial backward elimination process was later reoffered to the model to assess significance and to check for confounding. Any variable, reoffered to the final model that was statistically significant or resulted in a coefficient change of  $\geq 20\%$  for any other variable was retained in the model. This process continued until no variables reoffered to the model were eligible to be retained. After the final model was determined, comparisons among levels within variables were made using adjusted Wald tests.

## **Results**

### ***Study population***

There were 443 operations that had calves born alive during the study period and reported data on pre-weaning calf respiratory disease. For the studied herds, the median number of beef cows on hand was 128 (range 2-5847) and the mean was 269.7 (standard deviation (SD) 467.2). The median percentage of the herd over 10 years of age was 10.6 (range 0-82.6) and the mean was 13.5 (SD 13.6). Median percentage of cows under 5 years of age was 43.1 (range 0-100) and the mean was 42.9 (SD 21.6). For our study herds, the median number of calves born alive was 125 (range 1 – 6,400). For the calves born alive, the mean percentage of calves affected by respiratory disease was 3.0% (SD 7.1), and the mean days from birth to weaning was 207.2 (SD 35.5) with a median of 210 (range 90 - 300). The mean number of mortalities from birth to 24 hours was 3.1 (SD 6.5) with a median of 1.0 (range 0 - 70). The mean number of mortalities from 24 hours to 3 weeks of age was 3.3 (SD 7.9) and the median was 1 (range 0 -70), and for 3 weeks of age until weaning the mean number of mortalities was 4.0 (SD 10.4) with a median of 2 (range 0 - 125). Mean number of calf-days at risk was 53, 260 (SD 95, 655) with a median of 25, 905 (range 180 – 1,280,000). Mean BRDC rate was 1.5 (SD 3.7) cases per 10,000 calf days and the median was 0.18 (range 0 – 75.0). Figure 1 displays the distribution of herd BRDC rates.

### ***Analysis results***

Of the variables assessed in the initial bivariable analyses, 21 were found to be associated ( $p \leq 0.30$ ) with the outcome variable (Table 1). Crude incidence rate ratios (IRR) for these variables provided unadjusted estimates for the magnitude and direction of effects for factors affecting calf respiratory disease rates (Table 1). In the bivariable analyses, several

variables of interest were found not to be associated ( $p > 0.30$ ) with BRDC rates. Herd size, divided in quartiles, was not associated with BRDC rate. Herd demographics, as described by the proportion of cows in the herd under five years of age, was found not to be associated with BRDC rate. Disease management variables, including how often the calving pen was used to house sick cows, vaccinating 22 day old to weaning-aged calves with a four-way viral vaccine that included infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), parainfluenza (PI-3), bovine respiratory syncytial virus (BRSV), consultation by a veterinarian on disease prevention, number of defined breeding seasons, and the separation of cow-calf pairs from pregnant cows also were not associated with BRDC rates. General management practices including mean days from birth to dehorning, the use of creep feed, the use of artificial insemination and body-condition scoring also were not associated with the outcome variable.

The final multivariable model included six independent variables that were significantly associated with herd-level BRDC rates (Table 2). To assess the form of the model that best fit the data (Poisson or negative binomial), the dispersion parameter point estimate ( $\alpha$ ) and 95% confidence intervals were evaluated. If the confidence interval did not include 0, the negative binomial regression model was deemed the most appropriate (Heeringa et al., 2010). Although the reported average number of visits by outsiders was significantly associated with the outcome, the direction of effects varied and there were no differences between the referent category (zero visits by outsiders per month), and effects for herds reporting 1-2 visits ( $p = 0.256$ ), 3-5 visits ( $p = 0.316$ ), 6-30 visits ( $p = 0.147$ ) or  $>30$  visits ( $p = 0.668$ ). However, comparison between herds reporting 1-2 visits per month had higher BRDC rates compared to both 3-5 visits ( $p = 0.008$ ) and 6-30 visits ( $p = 0.001$ ). Similarly BRDC rates were lower in herds recording 3-5 visits ( $p = 0.020$ ) and 6-30 visits ( $p = 0.001$ ) compared to herds reporting  $>30$  visits per month. No

difference was found between 1-2 visits and >30 visits per month ( $p = 0.273$ ). Compared to single breed herds, two-breed cross ( $p = .005$ ) and three-breed cross ( $p \leq 0.001$ ) herds had higher BRDC rates. When compared to single breed herds, composite herds tended to have higher BRDC rates ( $p = 0.051$ ). Additionally, herds reporting the cow-calf operation was a secondary source ( $p = 0.015$ ) of income had lower BRDC rates compared to primary source income herds. No difference was found between other source of income herds and primary source ( $p = 0.087$ ) income herds or secondary income herds ( $p = 0.522$ ).

## **Discussion**

Using survey data representative of a large percentage of the U.S. cow-calf industry, we were able to generate unique information on management practices associated with pre-weaning BRDC rates. In the final model, six variables were found to be associated with pre-weaning BRDC rates (Table 2). These effects may be indicative of pathogen exposure levels within the herd, genetic or breed components to the disease syndrome, or may simply represent general management characteristics of certain cow-calf operations that affect rates of BRDC in pre-weaned calves. However, since we cannot assess the temporal relationships among these associations relative to onset of BRDC and given the cross-sectional design of our study, it is not appropriate to assume causal relationships. The associations that we observed will require further evaluation into mechanisms for associations and potential disease mitigation opportunities, however, we have provided a unique assessment of potential risk factors for pre-weaning BRDC in U.S. cow-calf herds that may be useful to profile or evaluate herds that could have higher BRDC rates.

The mean cumulative risk of pre-weaned calf respiratory disease (3.0%) in our data was higher than that reported in one previous U.S. study (Wittum et al., 1994a) , but slightly lower



than another (Dewell, 2006). One reason our mean percentage may have been higher than the 0.5% reported by Wittum *et al.* is that they used a shorter period of time at risk (45 days) in their analysis (Wittum *et al.*, 1994a). The approximate days to weaning in the Dewell *et al.* study was 200 days, which is comparable to our study; however, their risk estimate (4.6%) was based on a much smaller number of calves located in a single herd (Dewell, 2006). Combined, these studies indicate that while pre-weaned BRDC risk is relatively low, it still may vary among different subsets of the national population. Neither of the previous studies reported a BRDC incidence rate; thus Figure 1 displays unique data for U.S. cow-calf herds.

The multivariable model of data from the survey population identified six management practices that were significant indicators of pre-weaning BRDC rate. Operations that reported feeding antibiotics to unweaned calves to prevent respiratory disease had higher BRDC rates than those that did not; however, it is possible that this association is demonstrating reverse causation. Because of the cross sectional design of this study, we do not know the historical BRDC experience in our study herds. Herds may have begun feeding antibiotics in response to a recent or current BRDC outbreak, or feeding antibiotics may have been used for prevention in the survey year in response to previous year's BRDC experiences. Little research investigating the benefit of feeding antibiotics to prevent BRDC in pre-weaned calves has been published, but the practice is thought to be beneficial for post-weaned calves in some instances (Duff and Galylean, 2007). Although we report the term "prevention" as per the wording of the survey question, this may actually have been an antibiotic used for disease control since very few feed grade antibiotics are labeled for BRDC prevention and producers may consider prevention and control as equivalent. Care must be exercised when evaluating the association between feeding

antibiotics and BRDC rate because of the possibility of reverse causation and the potential for this observed association to be due to other unmeasured factors.

Rates of BRDC were lower for herds importing bred heifers, but importing weaned steers had a positive association with BRDC rate. Producers that market bred heifers are likely to practice complete health programs because of the high value of breeding stock and germ plasm (Chenoweth and Sanderson, 2001). These complete health programs may include vaccination or other preventive health measures, and managing genetics to affect risk factors such as dystocia, and udder and teat conformation, which have been associated with pre-weaned BRDC (Wittum et al., 1994a; Stokka, 2010). It is possible that lower BRDC rates could be observed when herds marketing bred heifers concentrate on managing risk factors such as dystocia and udder conformation, which results in lower BRDC rates among herds that import this class of animal. Exposure to BRDC pathogens by contact with other populations, especially populations derived from multiple source herds, is believed to be a pre-weaning BRDC risk factor (Stokka, 2010). It is possible that steer-purchasing also occurs through marketing channels where exposure to multiple pathogens is likely (Edwards, 2010). The higher BRDC rates among herds importing steers could be due to pre-weaned calves being exposed to different BRDC pathogens or increased pathogen concentrations. The temporal relationships between importation of bred heifers or steers and exposure to unweaned calves cannot be assessed with our data, but our results suggest that importing some classes of cattle could potentially impact, either negatively or positively, the BRDC rates for pre-weaned calves in cow-calf operations.

Although few studies have documented its importance, biosecurity has been cited as an important management practice for pre-weaning BRDC control as it may reduce BRDC pathogen introduction and transmission to calves (Callan and Garry, 2002). Pathogen

transmission can occur not only from animal to animal contact, but also from environmental or fomite contamination; therefore, controlling entrance to a cow-calf facility by outsiders may be a logical biosecurity practice (Callan and Garry, 2002). Although the categorical variable representing the number of visits to the herd by outsiders was significant in our final model, we found no difference in pair-wise comparisons among herds that had zero visits per month by outsiders and those reporting one or more visitors per month. It is possible that most visits do not involve contact with either outside animals or herd animals, but are instead non-livestock associated visits. Although our data set would not allow us to assess the respondents' biosecurity practices, it is also possible that respondents who allow outside visitors are cognizant of biosecurity as a disease control method and have effective biosecurity programs in place. In comparisons among herds that had at least one visit per month, we found that herds with 1-2 visits per month or >30 visits per month had higher BRDC rates compared to herds with 3-5 or 6-30 visits per month. Perhaps these effects indicate that herds with few visitors are less concerned with biosecurity than herds with moderate numbers of visitors. These results also may indicate that large numbers of visits may overwhelm some biosecurity programs making them less effective than when implemented in herds with moderate numbers of visits. The overall variability in effects among different levels of the variable representing the reported number of herd visits and the lack of specificity in defining types of visitors (e.g., employees, veterinarians, cattle buyers), combined with the cross-sectional nature of the data, make it extremely difficult to determine potential mechanisms for observed associations. Further, when evaluating the association between outsider visitors and BRDC from our data set, caution is warranted because of the possibility that number of visits is a proxy for other unmeasured factors.

Our multivariable model demonstrated that breed management may play a role in pre-weaning BRDC rates (Table 2). We found operations with two-way crosses, three-way crosses or composites had higher BRDC rates compared to single-breed herds, which is in contrast with results from a study where BRDC incidence was greater among single breed pre-weaned calves compared to two-way, three-way or composite calves (Snowder et al., 2005). However, their study was completed on a large U.S. federal research operation with similar management practices for both single and multiple breed groups. It is possible the single breed operations in our study were primarily purebred operations and enhanced disease prevention, or that more intensive treatment regimens were practiced given the perceived added value of purebred calves. Additionally, in our survey a definition for crossbred or composite was not provided; therefore, there may have been a difference in the breed makeup between our study population and the Snowder *et al.* study. Because of the national scope of our study, it is unlikely that similar genetics were contained between the single-breed herds. It is more likely that other management practices, unique to single or multiple breed herds, which can affect BRDC rates were present.

Respondents that reported the operation was not the primary source of income but a supplemental source of income had lower BRDC rates. Primary income source herds derive most of their income from the sale of calves (Wikse et al., 1994). Because of the potential added importance of calf-sales in these herds, it is possible that an increased BRDC recognition intensity occurred resulting in more pre-weaned calves being treated for respiratory disease. In our study population, primary income also was associated with greater herd size (data not shown). Increased population size also may be associated with greater within herd exposure to BRDC pathogens, which is considered to be a risk factor for pre-weaning BRDC (Stokka, 2010). Additionally, a lack of labor quantity has been associated with increased pre-weaning BRDC

(Stokka, 2010), and labor deficiencies could be more common in primary income source herds. Because primary income source herds may have different pathogen exposure levels or disease recognition and control strategies, and are likely managed much differently than herds considered for supplemental income or other (hobby) purposes, income category may be a proxy for relationships among several risk factors that affect BRDC rates.

Although the survey design and implementation should result in data effectively representing a large proportion of U.S. cow-calf herds, the external and internal validity of our study may be affected by several study limitations. Because the survey design was complex, we used a weighted analysis of the sample population to potentially provide representation of the target population (Levy and Lemeshow, 2008; Heeringa et al., 2010). We recognize that the assumptions required when using non-response weighting, if incorrect, may result in biased estimates of effects. However, if the effects are unbiased, the weighted analysis allows for better external validity. The participants volunteered to participate in the survey; therefore, a self-selection bias may have been present. In evaluating our final model, we compared it to an unweighted model (data not shown) and found relatively similar estimates of effects. Internal validity may have been affected by the accuracy of our statistical models. Unfortunately, limitations in available survey analysis software for weighted analyses prevent extensive evaluations of model fit (Heeringa et al., 2010). However, we demonstrated that a negative binomial distribution was clearly more appropriate for our data than a Poisson distribution. The additional variability may have been due to absence of hierarchical variables.

For these national survey data, there was no uniform case definition for BRDC; reported were the number of calves treated with antimicrobials for respiratory disease. Because the study population included many cow-calf operations throughout the U.S., the case definition and

disease recognition skills among participants likely differed. The potential misclassification in our study may or may not have been differential with respect to risk factors of interest. Because the outcome variable was the number of pre-weaned calves treated for respiratory disease and not the number of first BRDC treatments, some calves may have been counted more than once. In addition, due to the retrospective nature of the survey (operators were asked to recall events that occurred over the course of the previous year), recall bias may have affected both the number of BRDC events that occurred and the management practices that were present. Similarly, because the days at risk were calculated considering mortalities that occurred within specific time frames, some timing misspecification may have occurred. Combined with potential inaccuracies in the reported average weaning days, these data may have led to differential or non-differential misspecification of the rate exposure (calf-days at risk).

## **Conclusion**

Several cow-calf management practices were associated with herd-level pre-weaning BRDC rates in a survey of U.S. cow-calf herds. Feeding antibiotics to pre-weaned calves was positively associated with BRDC rates, but it is possible that this practice was a response to, rather than a cause of BRDC events. The effects of importing cattle from outside sources were found to be significantly associated with BRDC rates, but the direction of effects was dependent on the class of animal imported. Surprisingly, when compared to herds reporting no outside visitors to herds with at least one visitor per month no difference in BRDC rates were found. However, among herds that reported outside visitors, the number of visits differentially affected the herd level BRDC rates. Additionally, breed management practices and the economic purpose of the cow-calf operation were found to be associated with pre-weaning BRDC rates. Due to the cross-sectional nature of our survey data, we cannot determine whether any of the associated

management practices preceded or followed BRDC events. However, enhancing our understanding of these risk factors may help in identifying future disease mitigation opportunities and provides unique profiles of U.S. cow-calf herds that are associated with increase rates of BRDC in pre-weaned calves.

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**Table 5-1 Results of bivariable negative binomial regression analyses demonstrating management practices that were associated<sup>a</sup> with the rate of pre-weaned calf respiratory disease in U.S. cow-calf herds**

Variable	Response	N	IRR <sup>b</sup>	95% Confidence Interval (IRR)
Vaccinate 22 day old to weaned calves for <i>Mannheimia/Pasteurella</i>	No	293		
	Yes	149	0.55	0.30, 1.01
Vaccinate cows with four way viral vaccine (IBR, BVD, PI3, BRSV)	No	260		
	Yes	182	1.61	0.68, 3.80
Unweaned calves fed antibiotics to prevent respiratory disease	No	408		
	Yes	35	2.65	0.89, 7.92
Number of times calves vaccinated for respiratory disease from birth to weaning <sup>c</sup>	0	67		
	1	111	2.82	1.04, 7.69
	2	175	2.79	1.09, 7.18
	≥3	52	0.99	0.36, 2.76
Import unweaned calves with dams	No	418		
	Yes	25	2.99	1.00, 8.93
Import bred heifers	No	402		
	Yes	41	0.32	0.13, 0.77
Import weaned steers	No	412		
	Yes	31	2.83	0.82, 9.74
During an average month, the number of visits to the operation made by outside visitors <sup>d</sup>	0	33		
	1-2	79	2.02	0.37, 11.05
	3-5	92	0.49	0.10, 2.43
	6-30	114	0.39	0.08, 1.82
	>30	116	1.03	0.22, 4.83
Best describes genetic make-up of calves	Single breed	122		

	Composite	32	2.32	1.08, 4.99
	2 Breed X	196	3.09	1.37, 6.97
	3 Breed X	93	4.23	1.70, 10.53
At least one live calf with horns was born this year	No	234		
	Yes	209	1.92	0.84, 4.36
Mean weaning weight of replacements (kg)	<228	130		
	229-250	37	0.76	0.27, 2.15
	>250-273	126	2.11	0.71, 6.33
	>273	43	1.00	0.40, 2.51
Average days from birth to weaning	<180	132		
	181-210	162	1.51	0.64, 3.56
	211-230	40	2.23	0.41, 12.02
	231-315	109	0.53	0.23, 1.18
The same people buy the operation's calves each year	No	209		
	Yes	232	1.78	0.83, 3.84
The cow-calf operation is a primary, supplemental or other source of income	Primary	209		
	Supplemental	209	0.68	0.26, 1.80
	Other	25	0.31	0.08, 1.19
Percent of operator's total hours worked (on and off operation) is devoted to the cow-calf operation	<25	113		
	26-50	115	0.79	0.32, 1.94
	51-99	78	2.43	0.69, 8.55
	100	137	0.70	0.29, 1.69
Use estrus synchronization	No	349		
	Yes	94	1.51	1.08, 2.13
Use pregnancy ultrasound	No	395		
	Yes	48	1.35	0.82, 2.32

Use pelvic measurement	No	381		
	Yes	62	3.46	1.11, 10.76
Hours cows allowed in labor before intervention is initiated	$\leq 2$	261		
	$> 2$	141	0.57	0.25, 1.31
One or more cows treated for any disease except respiratory disease	No	243		
	Yes	196	1.58	0.69, 3.65
One or more cows treated for respiratory disease	No	373		
	Yes	66	1.98	0.61, 6.42

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<sup>a</sup> $p \leq 0.30$

<sup>b</sup>IRR : incidence rate ratio

<sup>c</sup>Vaccinating with one or more of the following : IBR, BVDV, PI3, BRSV, *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*

<sup>d</sup> Visitors included : employees, veterinarians, neighbors, nutritionists, commercial haulers, etc.

**Table 5-2 Results of multivariable negative binomial regression model demonstrating management practices associated with the rate of pre-weaned calf respiratory disease in U.S. cow-calf herds**

Variable	<i>p</i>	Level	$\beta$	S.E. ( $\beta$ )	IRR <sup>a</sup>	95% Confidence Interval (IRR)
Intercept			-8.89	0.59		
Unweaned calves fed antibiotics to prevent respiratory disease	0.008	No	Referent			
		Yes	1.24	0.46	3.46	1.39, 8.60
Import bred heifers	0.013	No	Referent			
		Yes	-0.93	0.37	0.40	0.19, 0.82
Import weaned steers	0.022	No	Referent			
		Yes	0.96	0.42	2.62	1.15, 5.97
During an average month, the number of visits to the operation made by outside visitors <sup>b</sup>	0.001	0	Referent			
		1-2	0.72	0.63	2.06	0.59, 7.13
		3-5	-0.56	0.55	0.57	0.19, 1.70
		6-30	-0.78	0.53	0.46	0.16, 1.31
		>30	0.23	0.52	1.26	0.45, 3.51
Best describes genetic makeup of calves	0.001	Single breed	Referent			
		Composite	0.82	0.42	2.27	1.00, 5.16
		2 Breed Cross	0.86	0.31	2.36	1.30, 4.29
		3 Breed Cross	1.39	0.37	4.00	1.93, 8.31

The cow-calf operation is a primary, supplemental or other source of income (i.e. pleasure)	0.029	Primary	Referent				
		Supplemental	-0.74	0.31	0.48	0.26, 0.87	
		Other	-1.17	0.68	0.31	0.08, 1.18	

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<sup>a</sup> IRR : incidence rate ratio

<sup>b</sup> Visitors included : employees, veterinarians, neighbors, nutritionists, commercial haulers, and others.

Dispersion parameter ( $\alpha$ ) estimate 3.39 (95% Confidence interval 2.53, 4.54)

## **CHAPTER 6 - Conclusion**

Bovine respiratory disease complex (BRDC) is a multifactorial disease common in both pre- and post-weaning calf populations. This disease syndrome continues to plague the beef industry, annually costing hundreds of millions of dollars, despite many years of study and development of multiple preventive health products and programs. That BRDC remains important to the beef industry after many years of research reinforces the complexity of this disease syndrome and the difficulty in effectively reducing impacts of a complex disease. A multifaceted epidemiologic research approach, taking into account all facets of the epidemiologic triad, including calf, pathogen, and management, is necessary to prevent and reduce the health and economic effects of this complex disease syndrome.

Fundamental to the control of BRDC are multiple component preventive health programs. Programs are often designed using research from multiple studies demonstrating the effectiveness of a single component (e.g., a single type of vaccine). I hypothesized that different component combinations (e.g., vaccine-parasiticide combinations) may have variable effectiveness. Because calves are usually administered programs consisting of multiple preventative health measures and not single components, I designed a study investigating potential differences in calf health and performance between multiple component programs. The programs in my study differed in both the types of components and their mode of administration. One program consisted of all injectable components and the other utilized topical or oral components and only one low-dose injectable (subcutaneous) product. My work showed a lower percentage of calves (47.8%) in the all-injectable program experienced BRDC morbidity compared to the calves in the one-injectable program (59.7%). This research provides evidence that the effectiveness of BRDC prevention opportunities do differ between programs.

Additionally, by assessing only non-morbid calves, I found the all-injectable program calves (1.23 kg) gained more daily weight compared to the one-injectable group (1.16 kg). My results indicated that differences in calf aversion to program administration did occur. A lower percentage of calves in the one-injectable program (39.8%) vocalized compared to 47.8% of the calves in the all-injectable group. Vocalization is believed to be associated with animal welfare; thus, my research may indicate potential welfare differences between programs. In addition, calf activity as measured by pedometers differed throughout the early feeding period, with the one-injectable program calves taking fewer steps in each 24 hour period. This is important because it provides evidence that a carry-over of aversion to initial administration may occur, or that multiple injection effects are not short-term. Behavior as measured by the percentage of time spent lying down was higher for the one-injectable program calves on certain days. This group also had higher BRDC morbidity risk. I have also shown (Chapter 3) that calves spent less time lying down post-*Mannheimia haemolytica* inoculation. Findings contained in my two research projects provide evidence that monitoring posture may be an important method for BRDC recognition. My research provided information to the beef industry that differences do exist between preventive health programs as expressed through health, performance and behavior.

No BRDC preventive health program has been shown to be completely effective; therefore, the disease continues to occur. It is believed the success of the treatment for BRDC depends on how early during the course of disease that the treatment is initiated. Unfortunately, disease recognition is difficult, largely because calves are prey animals and are able to mask clinical illness. Additive to this difficulty is the requirement of calf-care-givers to observe large groups of calves and subjectively assess the clinical state of each animal. I found that commonly employed BRDC recognition tools, such as physical examinations, were unable to discern health



from disease state. Additionally, I found that blood metabolites were unable to distinguish health from disease. However, I also noted changes in the amount of daily time that calves spent lying down and standing. The importance of this is unknown, but the results suggest that investigating postural changes in calves for use as diagnostic tool is warranted. I also found that calf activity, as measured by pedometers, was able distinguish health from disease. My research identified calf activity as a practical method for assessing BRDC disease state and I was able to show that some commonly used techniques, such as physical examinations and blood component parameters are not useful BRDC recognition tools.

Through the many years of BRDC research, the list of associated pathogens has changed. The class Mollicutes, and more specifically *M. bovis*, are just one of many newly identified BRDC pathogens. Because *M. bovis*' role in BRDC has just recently been discovered, little epidemiologic research has been completed. Thus, data from my study are critical to our understanding of this organism. I found that Mollicutes is ubiquitous and dynamic in some weaned calf populations. Although I found no association between nasal prevalence and BRDC morbidity and mortality, prevalence was associated with calf weight gain. This provides important information suggesting that when Mollicutes organisms are isolated from the upper airway, calf performance may be reduced. I also provided evidence that some calves are exposed to *M. bovis* before arrival to stocker units, and that seroconversion during the early feeding period is common. My findings suggest that exposure to *M. bovis* may have already occurred at the time of arrival to the feedlot. The seroconversion results are important for veterinarians and producers when designing vaccination programs, because it indicates that even highly stressed calves can mount immune responses to *M. bovis* during the early feeding period. Additionally, I found that those calves that seroconverted had gained 0.35 kg/day compared to

0.49 kg/day for those calves that did not seroconvert. That I found no difference in health outcomes between seroconversion status but a difference in performance provides evidence that exposure and immune reaction to this organism may have negative production impacts. I provide important evidence that Mollicutes and *M. bovis*, even if not associated with calf health, can affect production within some calf populations.

Identifying BRDC risk factors is as important as demonstrating differences in health programs, identifying accurate BRDC recognition tools, and determining the prevalence and impacts of new BRDC-associated organisms. Until my final research project, BRDC risk factors had not been described for pre-weaned beef calves in U.S. cow-calf operations. By using a data set from a survey of U.S. cow-calf production systems, I identified several management practices as risk factors for BRDC. I found that feeding antibiotics to pre-weaned calves was associated with higher BRDC rates. My study would not allow us to discern if feeding antibiotics was for prevention or control, and I suggest that this effect may be a measure of the response to BRDC rather than a factor leading to BRDC. My research also showed that importing specific classes of animals may affect BRDC rates, but the direction and magnitude of these effects is dependent on the class of animals imported. These results provide evidence that special precautions may be warranted when importing some classes of animals. I provided evidence that for herds reporting at least one outside visitor to the operations, moderate numbers of visitors was associated with lower BRDC rates compared to very few or many. These findings may suggest that operations with a moderate number of visitors may practice effective biosecurity, but many monthly visitors adversely affect some biosecurity programs. Single breed herds were found to have lower BRDC rates compared to two and three breed herds. This is important because it may indicate that single breed herd health programs contain general management practices that reduce BRDC.

Additionally, the economic reason for the existence of the cow-calf operation was associated with the herd BRDC rate. It is plausible that greater BRDC detection intensity may be present in herds that are the primary source of income compared to supplemental source of income. Due to the cross-sectional nature of my survey data, it cannot be determined whether any of the associated management practices preceded or followed BRDC events. However, enhancing our understanding of these risk factors may help in identifying future disease mitigation opportunities and provides unique profiles of U.S. cow-calf herds that are associated with increase rates of BRDC in pre-weaned calves.

My research has brought forth unique information concerning pre-weaning and post-weaning BRDC. I have provided evidence that differences in health, performance and behavior do exist between some health programs; thus, field trials utilizing randomized program (rather than product) assignments for identifying differences between programs is justified. Additionally, I identified calf activity and posture as accurate indicators of BRDC. I also have illustrated Mollicutes and *M. bovis* may be important organisms associated with the performance of post-weaned calves. Lastly, I have provided the first assessment of management practices as risk factors for pre-weaning BRDC in U.S. cow-calf herds.

# Appendix

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