Diagnostics and risk factors for bovine respiratory disease

by

Joaquin Baruch

D.V.S., University of the Republic, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

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Abstract

Diagnostic inaccuracy and unknown risk distributions for bovine respiratory disease (BRD) are some of the most important issues faced by the feedlot industry. Given these issues, the overarching themes of this thesis are on the evaluation of point-of-care diagnostic methods for BRD and on summarizing, synthesizing, and simulating the impact of risk factors and disease interventions on BRD morbidity. The objectives of the research chapters included in this thesis are to: 1) evaluate BRD disease progression using point-of-care diagnostic methods in a challenge study, 2) map the available literature on risk factors for BRD morbidity in beef cattle using a scoping review, 3) evaluate associations between vaccination programs and BRD morbidity in beef feedlot cattle, and 4) define BRD risk distributions based on cattle characteristics and disease interventions. When evaluating point-of-care diagnostic methods, we observed that computer-aided-stethoscope scores, oxygen saturation levels, and ultrasound-measured consolidation varied by study day and disease stage (viral or bacterial). Clinical illness scores and temperature measurements, commonly used as metrics for BRD diagnosis on the field, did vary by study day but may not be discriminative of disease stage. Lymphocyte, neutrophil, and fibrinogen concentrations, and lymphocyte/neutrophil ratio, demonstrated differences following bacterial inoculation and, therefore, could be used to assess disease progression. Metaphylaxis, vaccination, dietary supplements, and preconditioning programs were the most studied risk factors for BRD morbidity in beef cattle. Although unclear why these risk factors were studied more often than those related to animal management practices, sources of funding, and the potential market value of these interventions seem to be plausible explanations. Vaccination programs for BRD prevention implemented before feedlot arrival were associated with reduced BRD morbidity; however, vaccination programs implemented at feedlot arrival

were not. These associations indicate that preconditioning programs, which normally include vaccination before cattle arrive at the feedlot, might be better strategies for reducing BRD's burden to the beef industry. Metaphylaxis was the most effective intervention at feedlot arrival to reduce BRD morbidity, but its efficacy depended on the antimicrobial drug used and cattle characteristics. The lowest median BRD morbidities were estimated for tilmicosin, gamithromycin, tulathromycin, and tildipirosin, but estimations of BRD morbidity with other antimicrobials were similar to those obtained by feedlot arrival vaccinations on weaned animals. Therefore, whereas metaphylaxis can effectively reduce BRD morbidity, non-antimicrobial alternatives could be implemented to reduce the use of less effective antimicrobials. Overall, the results described in this thesis contribute to improving the knowledge on point-of-care diagnostics methods, availability of data on risk factors for BRD, effectiveness of vaccination timing on BRD morbidity, and defining BRD risk distributions based on cattle characteristics and disease interventions.

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Diagnostic inaccuracy and unknown risk distributions for bovine respiratory disease (BRD) are some of the most important issues faced by the feedlot industry. Given these issues, the overarching themes of this thesis are on the evaluation of point-of-care diagnostic methods for BRD and on summarizing, synthesizing, and simulating the impact of risk factors and disease interventions on BRD morbidity. The objectives of the research chapters included in this thesis are to: 1) evaluate BRD disease progression using point-of-care diagnostic methods in a challenge study, 2) map the available literature on risk factors for BRD morbidity in beef cattle using a scoping review, 3) evaluate associations between vaccination programs and BRD morbidity in beef feedlot cattle, and 4) define BRD risk distributions based on cattle characteristics and disease interventions. When evaluating point-of-care diagnostic methods, we observed that computer-aided-stethoscope scores, oxygen saturation levels, and ultrasound-measured consolidation varied by study day and disease stage (viral or bacterial). Clinical illness scores and temperature measurements, commonly used as metrics for BRD diagnosis on the field, did vary by study day but may not be discriminative of disease stage. Lymphocyte, neutrophil, and fibrinogen concentrations, and lymphocyte/neutrophil ratio, demonstrated differences following bacterial inoculation and, therefore, could be used to assess disease progression. Metaphylaxis, vaccination, dietary supplements, and preconditioning programs were the most studied risk factors for BRD morbidity in beef cattle. Although unclear why these risk factors were studied more often than those related to animal management practices, sources of funding, and the potential market value of these interventions seem to be plausible explanations. Vaccination programs for BRD prevention implemented before feedlot arrival were associated with reduced BRD morbidity; however, vaccination programs implemented at feedlot arrival

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Table of Contents

List of Figures

List of Tables

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Chapter 1 - **A literature review of the epidemiology of bovine respiratory disease in feedlot cattle in North America**

Introduction

Bovine respiratory disease (BRD) is a multifactorial disease complex that occurs in cattle and is characterized by upper and lower respiratory tract symptoms. Different viral and bacterial pathogens are associated with BRD, with environmental factors, management practices, and host-related characteristics, all contributing to its epidemiology (Lillie, 1974). Despite the efforts made in the past decades to reduce its impact, BRD is still considered the major source of morbidity and mortality in feedlot cattle worldwide, representing a great economic burden for the beef and dairy cattle industries in North America and elsewhere (Griffin, 1997; United States Department of Agriculture, 2013b).

With the increase in global demand for beef and the subsequent intensification of beef production (Miles, 2009), the challenges posed to the beef cattle industry are amplified, and new issues arise. As such, the industry needs to comply with food safety standards, while promoting a sensible use of antimicrobial drugs and committing to long-term environmental sustainability and animal welfare stewardship. At the same time, beef production must keep up with market demands by increasing its efficiency. Diseases have an impact on cattle production systems by reducing performance outcomes, which in turn increases land usage per pound of beef, cost of production, and leads to animal welfare issues. Improving animal health is, therefore, the best approach to tackle these challenges.

The first reports of BRD date to the beginning of the 1900s, when a disease associated with the transportation of calves from ranches to feedlot operations was reported and described as “shipping fever” (Farley, 1934). Since then, and despite many decades of research and efforts

towards the control of BRD, mortality and morbidity in cattle have not decreased significantly in the United States (US) (Smith, Step and Woolums, 2020). According to Loneragan et al. (2001), and using data from the 1990s, it has been estimated that 57% of cattle mortality at feedlot operations is due to BRD (Loneragan *et al.*, 2001). The same study reports an increase in mortality due to BRD from 1994 to 1999 in 121 feedlots (representing over 21 million cattle), while mortality due to other causes remained unchanged during the same time period (Loneragan *et al.*, 2001). Regardless of the impact of BRD on cattle mortality, the weight of beef carcasses has been increasing in recent years, showing that the feedlot industry has continued to improve its efficiency. Improvements in animal performance are likely related to advances in feed, implants, and genetics (Miles, 2009).

Risk factors and stressors predisposing to BRD are complex. They are mostly associated with transportation, commingling cattle, cold weather and wetness, extreme weather variation, stress-inducing interventions (e.g., dehorning, weaning, and castration of bulls), low weight, and metabolic distress (Taylor *et al.*, 2010; Smith, Step and Woolums, 2020). In feedlots, most BRD cases occur within the first 40 days after arrival, although variability on the temporal patterns of BRD occurrence is commonly observed and dependent on multiple epidemiologic factors (Babcock *et al.*, 2010).

A thorough understanding of the risk factors that can lead to increased BRD morbidity and improved diagnostic methods for BRD detection is paramount for the control and mitigation of the disease in feedlot cattle operations. The following sections of this chapter aim at reviewing the most important aspects of the epidemiology of BRD, provide background information for a better understanding of the current body of knowledge regarding BRD, and lay the groundwork for the research chapters that follow.

Causative agents of bovine respiratory disease

Multiple causative agents have been described as associated with the BRD complex, which in part explains the challenges related to diagnosis, vaccination, and control programs. Some of the agents involved in the epidemiology of BRD occur naturally as commensals in the respiratory tract of healthy cattle. Others are pathogens that impair the immune system, creating the conditions for ubiquitous agents to become opportunistic pathogens, complicating infection, and adding to its complexity (Aich, Potter and Griebel, 2009).

Viral pathogens are often associated with the epidemiology of BRD, with bovine herpesvirus type 1 (BHV-1) being the most common virus identified in the BRD complex (Griffin *et al.*, 2010; Caswell, 2014). There is a large body of research available focusing on BHV-1, and much research effort has been made to understand the role of this virus in cattle diseases after it was first isolated in 1956 (Madin, York and Mckercher, 1956). Besides causing lesions in the trachea and the upper respiratory tract in general, BHV-1 causes reproductive tract problems and conjunctivitis in cattle, which contributes to the importance of BHV-1 for the cattle industry (Ellis, 2009). Like most herpesviruses, BHV-1 has a latency period, and, in times of stress, it can be released from the lymph nodes to the upper respiratory tract (Kiorpes *et al.*, 1978). Transmission of BHV-1 occurs mostly via direct contact between animals, but aerosols are also considered a common route of transmission (Ellis, 2009).

Bovine viral diarrhea virus (BVDV) is also associated with BRD (Richer, Marois and Lamontagne, 1988), and two types of BVDV are related to BRD, BVDV-1 and BVDV-2. The presence of BVDV persistently infected animals (BVD-PI) in the feedlot is considered a risk factor for BRD (Hessman *et al.*, 2009). These are animals that were infected with BVDV during gestation and can act as super-spreaders after being introduced in the feedlot (Loneragan *et al.*,

2005). Most feedlot control strategies for this pathogen are performed by testing and identification of BVD-PI animals and by vaccination to protect uninfected animals from BVD-PI cattle (United States Department of Agriculture, 2013b). Tests for BVD-PI cattle are conducted at some points of sale using ear notch samples, but it is unclear to what extent this testing is currently performed in the industry. The role of BVDV in the BRD complex lies in its ability to produce immune suppression, which will increase the risk of lung colonization by bacterial pathogens.

Bovine respiratory syncytial virus (BRSV) is a *Pneumovirus* of the family *Paramyxoviridae,* first isolated in 1970, and in some accounts considered as the major etiological agent of BRD (Gershwin, 2008; Ellis, 2009). Bovine respiratory syncytial virus produces lung damage by generating an autoimmune host response, making animals more susceptible to other infections (Gershwin, 2008). Similarly to BHV-1, most of BRSV transmission occurs via direct contact between healthy and infected animals (Ellis, 2009). Both BRSV and BHV-1 play a key role in the epidemiology of BRD by initiating disease, whose severity depends on the influence of other stressors and the synergistic effect of other agents. An example is the combined effect of BRSV with *Histophilus somni* (HS), which increases the severity of disease when compared to the individual effect of each agent (Gershwin *et al.,* 2005).

Commensal bacterial pathogens are ubiquitous in the respiratory tract of healthy cattle, where they reside without normally causing disease. *Mannheimia hemolitica* (Mh) is likely one of the most common bacterial pathogens associated with BRD and is also a common commensal bacteria of the respiratory tract of cattle (Rice *et al.,* 2008). *Mannheimia hemolitica,* a Gram-negative, facultative anaerobic coccobacillus, has 12 serotypes based upon the ability to ferment arabinose. Serotypes A1 and A2 are usually found in the upper respiratory tract of cattle and

sheep, with serotype A2 being associated with respiratory disease in sheep and serotypes A1 and A6 being associated with BRD in cattle (Davies, Whittam and Selander, 2001). It has been reported that serotype A1 is the most commonly found in the lungs of diseased cattle at necropsy (Frank and Smith, 1983); this is the serotype usually used to initiate BRD in experimental challenge studies (Hanzlicek *et al.*, 2010; Theurer *et al.*, 2013). Although multiple components are related to the virulence of Mh, leukotoxin and lipopolysaccharide toxins are the most described factors due to their role in the inflammation process that occurs in the lung (Whiteley *et al.*, 1992).

Although Mh is considered the main bacterium associated with BRD, other commensal bacteria, such as *Pasteurella multocida* (Pm), are present in the nasal pharynx of healthy calves and also found in the lungs of BRD cases (Welsh *et al.*, 2004; Dabo, Taylor and Confer, 2008). According to Welsh et al., (2004), Pm may be increasingly affecting cattle, with serotype 3 (among the 16 serotypes identified for Pm) being the most commonly isolated pathogen from animals with pneumonia (Blanco-Viera *et al.*, 1995).

Histophilus somni (HS) is a Gram-negative pleomorphic rod and a commensal bacterium that also has been linked to BRD (Fulton, 2009). Similarly to Mh, the bacterial load of HS may increase under stressful conditions, further complicating BRD infection (Corbeil, 2008). Endotoxins and lipooligosaccharides are among the virulence factors associated with HS (Elswaifi, Scarratt and Inzana, 2012).

Because BRD is the result of a synergistic interplay between multiple pathogens, some of which also may be present as commensals, field diagnosis often relies on pathobiology and clinical signs observed in animals, rather than being based on pathogen identification. The

following section will, therefore, address pathobiology and clinical signs of the BRD complex in cattle.

Pathobiology and clinical signs of BRD

Enhanced by stress factors, viral pathogens associated with BRD damage the upper respiratory tract mucosa, allowing for bacterial proliferation and further colonization of the lungs, which leads to the development of fibrinous bronchopneumonia (Mosier, 2014). Depending on the immune response of the host, viral infection by BRSV, IBR, BVD, or parainfluenza may go unnoticed. Subsequently, if bacterial colonization develops, the disease condition may progress to BRD. Therefore, the study of the interactions taking place between viral pathogens, host animals, and bacteria are paramount for a better understanding of BRD and have been investigated in several challenge studies (Burciaga-Robles *et al.*, 2010; Molina *et al.*, 2013; Carlos-Valdez *et al.*, 2016).

Bacterial pathogens, which are protected in the form of biofilm during periods in which animals have a healthy, well-functioning immune system, may detach from the biofilm and disperse to new locations, colonizing the lower respiratory tract during periods of stress (Corbeil, 2008). Research has shown that stress may cause an increase from 40% to 80% in BRD mortality when animals are weaned just before shipment, as compared to preconditioned animals challenged with BHV-1 and Mh (Hodgson *et al.*, 2005). Stress-related factors that contribute to disease progression include transportation, mixing of animals, changes in feed and water practices, weaning, and weather conditions. As bacterial load, particularly Mh, continues to grow in the nasopharynx of sick calves, it is inhaled into the lungs, colonizing the lung mucosa and leading to the progression of BRD (Lillie and Thomson, 1972).

After bacteria colonize the lung tissue, the production of leukotoxin and lipopolysaccharides by Mh will lead to severe inflammation, initiating an immune response, and the production of fibrin. At the same time, the accumulation of neutrophils and macrophages in the alveoli reduces the available lung tissue, impairing oxygen exchange (Zecchinon, Fett and Desmecht, 2004). *Histophilus somni* initiates the same process, only via the production of immunoglobulin-binding proteins and lipooligosaccharides (Agnes *et al.*, 2013).

Establishing a case definition for BRD is challenging given the lack of specific clinical signs and sometimes asymptomatic-like presentation of BRD, with some animals presenting only lung consolidation found at slaughter (Rezac *et al.*, 2014). A general non-specific pattern of clinical signs includes increased respiratory rate, respiratory distress, decreased rumen fill, anorexia, nasal and ocular discharge, depression, and increased rectal temperature (Apley, 2006). Although these signs might be specific enough when all of them are present, different combinations can appear in animals at different stages of BRD, making it challenging to create a precise and repeatable case definition.

Given the potential synergistic effect of viral and bacterial pathogens, it is important to understand how these interact. If knowledge of these interactions is improved, diagnostic tests and treatment could be adjusted according to disease severity, and the use of antimicrobial drugs could be reduced, targeting their use towards specific pathogenic bacteria.

The identification of the definitive causative agent(s) of BRD is unfeasible due to the commensal nature of many of the agents involved. However, if bacterial etiology, rather than viral, can be determined, the use of antimicrobials becomes a valid treatment option. Furthermore, treating BRD viral infections in cattle using antimicrobial drugs, although not targeting the viral etiology, may help prevent subsequent bacterial colonization. For example,

Apley (2006) has reported that tulathromycin may remain active for eight days post-injection, protecting treated animals against bacterial colonization during that eight-day period. However, if this treatment is administered too early, it may have to be repeated once the antimicrobial coverage has passed, resulting in additional costs and frustration among feedlot operators due to treatment failure. Nonetheless, the distinction between viral and bacterial etiology of BRD is not so straightforward in field conditions, which makes antimicrobial use a complex challenge in feedlot operations. Similarly, therapeutic success may be dependent upon the time of treatment, which is related to the presence of clinical signs. While lung involvement is a good indicator of bacterial infection, consolidation is hard to determine in a clinical assessment without the aid of ultrasound equipment (except when large portions of lung are already affected, in which case therapeutic success is limited) (Apley, 2006).

Through disease challenge models, researchers have been able to describe the way diagnostic methods and BRD relate, a topic that will be addressed in a later section of this review. In challenge studies, in which the onset of disease is known, researchers can describe how animals develop BRD over time by challenging them with different pathogens. Although external validity may be limited in challenge studies, as the conditions of infection may not mirror what occurs during natural infection, challenge studies are sometimes the only option available for studying the development of clinical signs and the pathobiology of BRD over time. For example, researchers have shown that by challenging animals with BVD virus, animals develop clinical signs in two to 8 days (Grissett, White and Larson, 2015). Similarly, animals challenged with BHV-1 and BRSV would develop similar signs in two to five days (Grissett, White and Larson, 2015). Conversely, Mh inoculations lead to clinical signs in shorter times, with most animals showing signs one day after inoculation (Grissett, White and Larson, 2015).

These differences in time indicate that the type of pathogen might have an impact on the time of disease onset, making the study of disease progression relevant to improving BRD diagnostic practices. However, challenge studies using the most common combination observed in the field, BHV-1 and Mh (Griffin *et al.*, 2010; Caswell, 2014), are not frequently conducted, comprising a research gap.

Among the necropsy findings related to BRD, fibrinous purulent tracheitis is consistently found in animals with recent viral infections, whereas those signs may not be observed as the disease progresses and lower respiratory tract lesions are observed. Lower respiratory tract lesions are commonly related to cranioventral lung consolidation (Hanzlicek *et al.*, 2010), which is probably related to the location of Mh during bacterial migration to the lungs. The proportion of lung consolidation can range from small portions of 5% lung consolidation in mild cases to up to 50% in more severe cases of the disease (Hanzlicek *et al.*, 2010). Similarly, as the disease progresses and more neutrophils migrate to the lungs, fibrin is more commonly observed (e.g., a yellow serofibrinous exudate may be found in the thoracic cavity), as well as lung abscesses in animals with chronic BRD. Other organs remain unaffected, with no postmortem findings being reported.

BRD costs and burden of disease

Although it is difficult to establish the exact costs associated with BRD in the beef cattle industry, it is estimated that more than US$500 million are spent every year (NASS Cattle, 2007). Other sources have estimated that the feedlot industry has costs associated with BRD that range from $800 million to $1 billion USD (Griffin, 1997; Brooks, Raper and Ward, 2011).

Even though health-related costs are estimated to comprise no more than 8% of the total costs of a beef production cycle, these costs are potentially more easily modified than feed costs (Griffin, 1997). Costs associated with cattle processing upon arrival have been estimated to be between 5 and 15 USD per animal over the past 20 years, making up about 6% of total feedlot costs (Griffin, 1997). These costs may add up quickly if the interventions applied to all cattle upon arrival to the feedlot are ineffective or unnecessary, as is the case with BRD interventions applied to low-risk cattle that would not develop the disease if left untreated.

According to Griffin (1997), animals that develop BRD gain 7% less weight, are 3% lighter upon shipment, and present 25% fewer grade choice, when compared with cattle that do not develop BRD (Griffin, 1997). These figures point to the complexity in the estimation of the true costs of BRD, which go beyond treatment costs. Griffin (1997) reported that the costs of BRD per animal were $111 in a Texas Ranch to Rail study. The use of metaphylaxis might reduce some health and performance costs, but it is itself costly, and new antimicrobial regulations may limit its application in the future (Wisener *et al.*, 2019).

Similarly, presence of lung lesions at slaughter are associated with lower average daily gain (ADG) during the feeding period; in one study, animals with lung lesions had an ADG of 3.05 lb. (95% CI 3.00 – 3.11 lb.) whereas animals with no lesions had an ADG of 3.28 lb. (95% CI 3.21 – 3.39 lb.) (Griffin, 1997). In addition, cattle with lung lesions had an average of 46 lb lower hot carcass weight (HCW) when compared to animals with no lung lesions (Griffin, 1997). Differences in weight gain reflect the morbidity effects of the BRD complex, as loss of appetite is a clinical sign of the disease.

There are also economic losses related to BRD that occur during the backgrounding phase of beef cattle production. As the number of BRD treatments increases, ADG decreases,

and the feed:gain ratio increases (Brooks, Raper and Ward, 2011). Specifically, Brooks et al., (2011) reported an ADG of 3.20 lb. per day for non-treated cattle, whereas animals receiving three treatments had 1.55 lb. of ADG. When animals are followed to slaughter, however, HCW only differed between non-treated animals and chronic BRD cases (Brooks, Raper and Ward, 2011). Specifically, chronic BRD animals presented 50 lb. lower HCW than non-treated cattle (Brooks, Raper and Ward, 2011).

The burden of disease for dairy cattle is also high. According to producer surveys, 12 – 16% of dairy cattle are affected by BRD (Guterbock, 2014). Slaughterhouse estimates report that 10% of slaughtered dairy cattle might have severe BRD lesions, indicating that the total BRD morbidity may be higher (Rezac *et al.*, 2014). In contrast to the high incidence of BRD in calves upon arrival to backgrounding or feedlot operations, most of BRD–related dairy cases occur in very young calves. In the adult dairy cattle, it is estimated that up to 30 million USD are lost due to BRD, not including the costs related to milk production and treatment (Guterbock, 2014).

In summary, BRD is the most common and costly disease to the feedlot industry, despite the improvements made by the development of new antimicrobials and vaccinations for treatment and prevention, and the use of preconditioning programs. The complex nature of BRD translates into difficulties in diagnosis and treatment, particularly in defining which animals should be treated.

Diagnostic methods for BRD detection

As previously indicated, the diagnosis of BRD represents a challenge for the feedlot industry due to its multifactorial nature and its non-specific clinical presentation. Current diagnostic practices in feedlot operations consist of observing animals daily for the detection of

clinical signs. Clinical signs include anorexia, depression, nasal and ocular discharge, lethargy, and non-reaction to external stimuli. Upon the identification of clinical signs, often pen riders isolate the animals presenting those clinical signs from the rest of the group for further investigation or examination. Often if rectal temperatures are above 104 degrees Fahrenheit (or a similar cutoff), the animals are considered BRD cases and proceed for treatment evaluation.

Many BRD affected animals are not observed to be symptomatic, which can be related to their prey nature and their instinct to hide signs associated with disease. Asymptomatic cattle are often only identified as BRD cases at slaughter, when lung consolidation is observed (Rezac *et al.*, 2014). Another issue in BRD detection is the lack of specificity of clinical signs. Clinical conditions other than BRD can be associated with depression and fever, which often leads to non-affected cattle being mistakenly diagnosed as BRD cases, thus decreasing the specificity of this diagnostic approach. It is estimated that sensitivity and specificity of diagnosis by detection of clinical signs is approximately 60% for both of these parameters (White and Renter, 2009). These values indicate that about 40% of affected animals remain unidentified and thus untreated for BRD, while 40% of non-affected animals may receive unnecessary treatment (White and Renter, 2009).

Improving diagnostic procedures for BRD detection is, therefore, an important area of research and a topic of interest for the feedlot industry. Many studies have addressed this issue, with one of the main challenges being correctly determining the onset of disease and identifying positive cases antemortem for test validation (due to the lack of a gold standard test). Although Bayesian methods can be used to estimate test characteristics using multiple imperfect tests and populations, most studies have relied on the use of challenge models to evaluate disease progression and diagnostic characteristics (Hanzlicek *et al.*, 2010; Amrine *et al.*, 2013; Fraser *et*

al., 2013; Carlos-Valdez *et al.*, 2016). Another issue to consider when evaluating BRD diagnostic methods is the turnaround time and the inability to conduct on-site diagnostic testing at the feedlot operation. Because cattle are highly susceptible to stress, time spent at hospital facilities is a key factor in treatment success (Apley, 2006). If the diagnostic test requires prolonged time at the hospital facilities, disease severity may increase, and the success of treatment may be compromised. These issues have been considered and have led to the development and testing of chute-side methods for BRD detection.

Whisper® is a computer-aided stethoscope, which through the recording and amplification of lung sounds and the use of an algorithm, produces a progressive score of severity from 1-5. The scores go from normal (score 1), mild acute (score 2), moderate acute (score 3), severe acute (score 4), to chronic (score 5). Although this method is relatively new, two studies have reported results related to its performance (Noffsinger *et al.*, 2014; Mang *et al.*, 2015). However, no studies have evaluated how these scores relate to lung consolidation and disease progression over time.

Given its ability to identify tissue density, ultrasound technology has been used for the determination of muscle development, liver damage, and, more recently, for aiding in the determination of lung consolidation (Buczinski, Forté and Bélanger, 2013; Buczinski *et al.*, 2014). By assessing lung consolidation in a noninvasive way, veterinarians can evaluate if there is sufficient lung involvement to support the hypothesis of a bacterial infection that would justify the use of antimicrobial drugs. Another alternative is to evaluate disease progression over time while animals are kept in hospital pens, monitoring those animals, and administrating treatment when it is deemed appropriate based on lung consolidation.

Facial thermography presents advantages over standard hands-on temperature measurements given the potential for using remote sensors in which temperatures can be measured at the pen. This technology has been studied before, and similar results to those obtained using rectal temperatures were observed (Schaefer *et al.*, 2007). Among the disadvantages of this technology, however, is the fact that elevated temperatures are not specific to BRD. Nonetheless, thermography could be used as a screening method to aid in detecting animals that may be missed during the examinations performed by pen riders and would thus not be subjected to rectal temperature measurements.

Blood leukocyte differentials could help in differentiating between viral and bacterial infections. As the viral infection progresses, leukocyte counts decrease (Jones and Allison, 2007). As bacterial infection develops in the lungs, and an acute inflammatory response is triggered, band neutrophils are liberated from the bone marrow to supplement the segmented neutrophils (Jones and Allison, 2007). Fibrinogen and plasma protein, have been studied as prior indicators of acute inflammation and could be useful for the detection of inflammatory processes. A disadvantage, however, is their non-specific relationship to BRD, meaning that changes in blood parameters occur with other diseases. Thus, a thorough assessment of clinical signs, rectal temperature, and potentially other diagnostic methods also may have to be used. Another traditional disadvantage related to leukocyte differentials is the need for shipping samples to be processed in the laboratory, which delays time until diagnosis and treatment.

As lung consolidation increases, the amount of tissue available for oxygen exchange decreases, which may influence the level of oxygen found in peripheral blood. Pulse oximetry is a common technique used in other species to evaluate respiratory capacity, but its application is often impractical in cattle, resulting in limited advances in its use. However, new technology to

measure oxygen saturation in cattle has been developed using a small-animal pulse oximeter probe.

Chute-side diagnostic methods may lead to the advancement of diagnostic practices for BRD detection due to the potential improvement of diagnostic accuracy and on-site use by feedlot operators. Another consideration for these new technologies is the need to have trained personnel in the field to conduct these tests. Whisper® stethoscopes, facial thermography, and pulse oximeters can be easily implemented by personal with little training, but the use of ultrasound equipment or the analysis of blood samples may require more advanced training. As technologies become available, and when the cost-benefit ratio is appropriate, feedlot operators may choose between these technologies to improve their economic return, while improving animal welfare. It is worth noting that when an animal is correctly identified as a BRD case, it can be treated in time and potentially gain more weight and have improved carcass performance. Nonetheless, costs of treatment, handling, and antimicrobial use have to be considered in the total costs. On the other hand, when a non-BRD animal is identified as BRD (i.e., false-positive), costs related to unnecessary treatment will be incurred with no benefit (unless the animal still benefits from treatment for unrelated causes). All these aspects must be assessed and considered before implementing new diagnostic methods in the field.

Risk factors associated with BRD

Most cattle stay in feedlots for periods of less than six months (United States Department of Agriculture, 2013a). Regardless, most BRD cases in both high and low-risk cattle occur during the first 40 days after feedlot arrival, which indicates that risk factors for BRD are often related to events that occur before, or immediately after, feedlot arrival (Babcock *et al.*, 2010).

Transportation

Bovine respiratory disease was initially described as "shipping fever" due to the relationship identified between shipments of freshly weaned calves to feedlot operations and the development of clinical signs (e.g., fever). However, "transportation" as a risk factor can be complex to define, as it can be associated with several factors, including distance traveled, placement within a truck, and weather conditions.

On average, cattle are shipped 339 miles to US feedlots (United States Department of Agriculture, 2013a). Distances traveled from ranches or sale barns to feedlots, regardless of their size, are similar, indicating that risk factors other than distance could explain the differences in BRD morbidity observed between larger and smaller feedlots (United States Department of Agriculture, 2013a). Sanderson et al. (2008) estimated that per every 1-mile increase in shipping distance from auction markets to feedlots, the incidence rate ratio of BRD increases by 0.1% (Sanderson, Dargatz and Wagner, 2008). Cernicchiaro et al. (2012) reported that as transport distance increases, BRD risk increases, with incidence rate ratios increasing on average by 59% to 91% when comparing groups of animals transported less than 250 miles to groups of animals transported more than 250 miles (Cernicchiaro, *et al.*, 2012a). However, these results may be dependent upon the region within North America, with Canada and the western area of the United States being affected in greater proportions with longer distances traveled, according to this study (Cernicchiaro, *et al.*, 2012a). Similarly, other studies have addressed issues related to transportation but have only focused on overall mortality outcomes (Ribble *et al.*, 1995; Warriss *et al.*, 1995).

Another transportation-related risk factor is animal placement within a truck, which may contribute to the incidence of BRD. In one study, the nose of the bottom deck presented a higher risk for cattle developing BRD compared to other parts of the truck (Wahrmund *et al.*, 2012). Another study reported that the middle part of the truck might be associated with a slightly higher BRD morbidity risk (White *et al.*, 2009).

Sex

Sex has been considered a risk factor for BRD, although results from different studies have reached conflicting conclusions. Sex is usually studied as a pen-level or lot-level variable rather than looking at individual animals. Some studies have reported that heifer pens are associated with a higher incidence of BRD compared to steers, and mixed pens were associated with a higher incidence of BRD compared to steer pens but similar to heifer pens (Sanderson, Dargatz and Wagner, 2008; Cernicchiaro *et al.*, 2012a, 2012b). Conversely, one study reported heifer pens were associated with a lower incidence of BRD when compared to steer pens (Cernicchiaro, Renter, *et al.*, 2012).

Explanations as to why one sex is more likely to be affected by BRD when compared to another are still unclear but may be interrelated with other factors. Castration of males, the "buller" syndrome (i.e., the repeated mounting and riding behavior), or the administration of melengestrol acetate to heifers for cycling inhibition could play a role in the differences observed, but no definitive answers have been found so far (Taylor *et al.*, 2010).

Body weight

Body weight is also often determined at the group level (cohort or pen weight) and is often associated with BRD risk. Lower body weights, especially of less than 600 pounds, tend to be the reference category for most studies, as lighter weights are associated with the highest risk for BRD. As body weight increases, the risk of BRD decreases (Sanderson, Dargatz and Wagner, 2008; Cernicchiaro *et al.*, 2012; Cernicchiaro *et al.*, 2012a, 2012b). This relationship is often described based on the correlation between body weight and age, which can be used as a proxy for tolerance to stressful events and immune system development.

Cohort size

Cohort (lot) size is often considered a proxy for the mixing of animals, with crowding or commingling of animals upon feedlot arrival being a known risk factor for BRD (Macdonald and Mcbride, 2009; Hay, *et al.*, 2016). Animals that are purchased in small cohorts are often pooled into the same pen or truck with other small cohorts. As animals from different origins are mixed, group stress levels increase as animals commingle and establish hierarchy among them. In addition, commingling may be associated with increase in pathogen exposure. Therefore, as cohort sizes increase, BRD morbidity risk tends to decrease (Cernicchiaro, *et al.*, 2012; Cernicchiaro, *et al.*, 2012b, 2012a; Hay, Morton, Schibrowski, *et al.*, 2016).

Health interventions

Preconditioning of cattle includes several practices applied to animals before feedlot arrival. Vaccination, weaning, castration, training to eat from feed bunks, and training to drink from water troughs are among those practices. Over 70% percent of feedlot operators have indicated that they view these practices as very effective for disease prevention (United States

Department of Agriculture, 2013a). Between 77 and 89% of feedlots report that some of their animals are subjected to some type of preconditioning program (United States Department of Agriculture, 2013a).

However, despite being considered valuable, according to the National Animal Health Monitoring System (NAHMS) (United States Department of Agriculture, 2013a), most of the information regarding the preconditioning of cattle is not available to feedlot operators before animal purchase. Prior knowledge of animal characteristics can be an area of market interest, as feedlot producers see it as extremely beneficial and thus may be willing to pay a premium for cattle where that type of information is known. Studies related to preconditioning programs are common in the literature and have been performed for almost 50 years with varying results (Woods, Pickard and Cowsert, 1972; Richeson *et al.*, 2012; Bailey *et al.*, 2015, 2016).

Vaccinations for BRD, either as part of a preconditioning program or not, are commonly administered to feeder cattle. According to NAHMS, 96.6% of feedlots with more than 1,000 animals administer some type of viral vaccination on arrival, with a BVD virus vaccine being the most commonly used type (United States Department of Agriculture, 2013b). However, the effectiveness of BRD vaccinations upon feedlot arrival has been questioned and addressed in multiple meta-analysis studies (Theurer, Larson and White, 2015; Snyder, Credille and Heins, 2019; O'Connor *et al.*, 2019a). A recent study addressed this issue, with the authors questioning whether there is enough evidence to support vaccination upon feedlot arrival (O'Connor *et al.*, 2019a). Results from this study indicated that there is little treatment-specific replication, given the large number of vaccine pathogens, timing, and number of doses administered. In other research, the benefit of delaying feedlot arrival vaccination has been investigated. While this approach would allow animals enough time to get used to the feedlot before receiving

vaccinations, results were not different when compared to vaccination upon feedlot arrival (Snyder, Credille and Heins, 2019).

Conversely, Theurer et al., (2015) pointed to the benefits of using five-way vaccines upon feedlot arrival, although this meta-analysis did not account for other BRD vaccines used in the studies, which could imply a potential confounding effect. In that same study, challenge studies performed to evaluate the effectiveness of vaccination programs tended to have better outcomes than natural infection, which would indicate that challenge studies may overestimate the benefit of vaccination (Theurer, Larson and White, 2015). In conclusion, despite the widespread use of vaccines, the effects of vaccination programs are contradictory, and no research synthesis methods have been conducted to evaluate the potential interactions between the administration of all BRD vaccines and time of weaning.

Metaphylaxis is the mass medication of a group of high-risk animals with an antimicrobial drug and is considered as the most effective and common BRD intervention used by feedlot operators (United States Department of Agriculture, 2013b; Abell *et al.*, 2016; O'Connor *et al.*, 2019b). Metaphylaxis is a convenient, easy to use practice that is often administered upon feedlot arrival. Multiple meta-analyses have shown that metaphylaxis is effective in reducing BRD morbidity (Abell *et al.*, 2016; O'Connor *et al.*, 2019b). One study has shown that tilmicosin, tildipirosin, tulathromycin, and gamithromycin were the most effective antimicrobial drugs for controlling BRD when compared to a control (O'Connor *et al.*, 2019b).

Knowing the impact of risk factors on BRD morbidity will help determine a risk definition for incoming cattle and point to a potential "toolbox" of interventions that feedlot operators can use to mitigate the impacts of BRD. Feedlot operators are faced with challenges regarding incomplete information on prior health interventions, which makes risk classifications

potentially inaccurate (Babcock *et al.*, 2013; Amrine, White and Larson, 2014). Therefore, further research on the impact of disease control measures and the potential interaction of such measures on BRD morbidity is warranted.

Research synthesis methods

There are many publications reporting the associations between risk factors and BRD, suggesting there is potential to summarize these associations, and when appropriate, obtain more robust and comprehensive estimates of effects. Research synthesis methods are methodological approaches used for combining the results from individual studies for supporting evidence-based decision-making (Tricco, Tetzlaff and Moher, 2011). These methods were first used in human medicine. However, they were soon after introduced to the veterinary sciences over the past decades, where they help provide an unbiased understanding of different outcomes, including disease control measures, disease prevalence, and other health-related estimates (Sargeant and O'Connor, 2020).

The research synthesis methods most commonly used in veterinary medicine are systematic reviews and scoping reviews. Systematic reviews aim to address a narrow research question by identifying, selecting, appraising, synthesizing, and summarizing the evidence gathered from the existing body of knowledge. This methodology is performed in a transparent, unbiased, and reproducible manner by following specific guidelines and by documenting all methodological steps. The quantitative pooling of results gathered using systematic reviews is performed to obtain an overall estimate of effect or association and is achieved via a meta-analysis, which comprises the statistical component of the systematic review (Sargeant *et al.*, 2006; O'Connor, Sargeant and Wang, 2014; Sargeant and O'Connor, 2014, 2020). However, not

all systematic reviews use a meta-analysis to summarize results, as results can be displayed, for example, using a forest plot without conducting a meta-analysis.

The PRISMA statement is a set of items that comprises a framework for reporting systematic reviews and meta-analyses, focusing on the standardization and strengthening of reporting according to the best available evidence (Moher *et al.*, 2009). A few examples have been cited throughout this chapter, including the study of the association between metaphylaxis use and BRD morbidity by O'Connor et al. (2019) and Abell et al. (2016), as well the study of the association between vaccination programs and BRD morbidity by Theurer et al. (2015).

In contrast to systematic reviews, scoping reviews address a broader question by mapping the available literature in a certain research area (Arkesey and O'Malley, 2005; Levac, Colquhoun and O'Brien, 2010). By following the methodological framework of scoping reviews, researchers identify data gaps that require further research and generate hypotheses, which can be further explored in systematic reviews and meta-analyses. Scoping reviews have been extensively used in the veterinary sciences for addressing topics as varied as the relinquishment of companion animals (Coe *et al.*, 2014), the potential zoonotic effect of certain pathogens (Wilhelm *et al.*, 2015), the evidence of human exposure to *Mycobacterium avium ssp.* (Waddell *et al.*, 2016), and One Health-related transdisciplinary research (Min, Allen-Scott and Buntain, 2013).

Within BRD research, there are a few publications using evidence synthesis methods to address topics related to vaccination, antimicrobial treatments, and metaphylaxis. However, there are other issues associated with the control of BRD that have yet to be explored. Indeed, the large number of publications addressing these topics represent a potential area in which research synthesis methods could be applied. Specifically, a scoping review can be used to identify

priority areas of research regarding risk factors for BRD to be further explored using systematic reviews and meta-analyses of the literature.

The meta-analysis methodology considers different sources of heterogeneity from the studies that are included in the analysis, exploring potential sources of confounding and bias and thus increasing the power of a systematic review. When the data gathered from a systematic review are too heterogeneous, it may not be possible to estimate a summary effect measure. In these situations, a meta-regression may be performed for exploring the sources of heterogeneity and thus better understand the data (Sargeant *et al.*, 2006; O'Connor, Sargeant and Wang, 2014; Sargeant and O'Connor, 2014).

Standard pairwise meta-analyses pool results from different randomized controlled trial studies (RCTs) evaluating the same interventions, providing a summary effect estimate of one intervention compared to the control (or another intervention). Examples of outcome measures that can be assessed using this methodology include estimating the prevalence of a disease (Ekong, Sanderson and Cernicchiaro, 2015) or the difference between two vaccination programs (Theurer, Larson and White, 2015). However, pairwise meta-analyses do not allow for the comparison of multiple interventions.

On the other hand, network meta-analyses (NMA) of RCTs allow researchers to create a network of trials in which at least two studies have had the same intervention studied (Dias *et al.*, 2013; Lin *et al.*, 2017; Hu *et al.*, 2019). Therefore, for example, if study 1 has evaluated intervention A versus B, and study 2 has evaluated intervention B versus C, we can presumably establish the relationship of A with B and B with C through direct evidence and the relationship between C and A through indirect evidence. This methodology has been used increasingly in veterinary medicine, with guidelines available for its interpretation (Hu *et al.*, 2019). One of the

key advantages of NMAs is that when connected networks are present (i.e., studies 1 and 2 connected through intervention B), researchers can make the most out of the available data, therefore improving the use of research synthesis methods.

Risk assessments

Risk assessment approaches, at least in some simple qualitative or semi-quantitative forms, have been used to assess BRD risks (Maier *et al.*, 2020). Quantitative risk assessments have been used in veterinary medicine for different purposes, including to evaluate the risk of introduction of foreign diseases (Foddai *et al.*, 2015) and to explore interventions for disease control programs (Smith *et al.*, 2010; Dodd *et al.*, 2011). By using a risk assessment methodology, researchers and policymakers can evaluate disease interventions, identifying the weaknesses of the surveillance system in place, estimate the risk of a potential disease introduction, or determine areas that are more at risk and are more amenable for mitigation strategies.

One example of the application of a risk assessment is the evaluation of preharvest and harvest interventions to reduce beef carcass contamination by *Escherichia coli* O157, reported by Dodd *et al.*, (2011). In this study, the authors concluded that fecal prevalence and fecal-to-hide transfer were areas of intervention that should be prioritized to reduce carcass contamination (Dodd *et al.*, 2011). Results from risk assessment studies may propel new research hypotheses and direct funding towards new areas of study, thus contributing to the advancement of scientific knowledge (Dodd *et al.*, 2011). Within BRD prevention and control, the application of more quantitative risk assessment models could play a key role in the research efforts directed towards the reduction of BRD morbidity in cattle. Risk assessment models can be used to evaluate the

impact that cattle characteristics and disease interventions have on outcomes related to BRD. Moreover, risk assessments may lead to findings that can potentially help feedlot producers generate appropriate risk category definitions for cattle, which would help optimize the application of interventions that are already being used, improve those interventions, or even point to new ones.

Conclusions and objectives

Several studies focusing on the assessment of diagnostic methods for BRD have been performed, as well as research on new technologies aimed at improving diagnostic approaches to BRD. Regardless, most feedlots still use diagnostic methods with relatively poor sensitivity and specificity. Chute-side diagnostic methods could help feedlot operators improve diagnostic accuracy for BRD, while prompting early detection and treatment of diseased cattle. This is an area of research that could benefit from the evaluation of disease progression using chute-side diagnostic methods.

Although there is a vast amount of literature on risk factors related to BRD morbidity, no comprehensive systematic approach has been followed to map the available data. By mapping these data, we could identify research gaps, potentially directing future efforts, and identifying research questions to address using primary research or systematic reviews and meta-analyses of the literature. The development of quantitative risk assessment models allows for the evaluation of cattle characteristics and disease interventions concerning the prevention of BRD in feedlot cattle, therefore improving the knowledge of risk categories for optimizing BRD control strategies. At the same time, the implementation of such models may provide answers that help generate scientific hypotheses and contribute towards new research in the field.

The following chapters of this thesis are structured as follows. In chapters 2 and 3, we evaluate the diagnostic performance of chute-side methods for detecting physiological and pathological changes as indicators of early BRD in calves experimentally inoculated with BHV-1 and Mh. In chapter 4, we aim at mapping relevant literature on risk factors for BRD morbidity in beef feeder cattle using a scoping review approach to identify research gaps and areas amenable to synthesizing through a systematic review. In chapter 5, we evaluate the association between vaccination programs for BRD prevention implemented pre-feedlot or upon feedlot arrival and BRD morbidity in beef feedlot calves using a network meta-analysis. In chapter 6, we estimate risks of morbidity for BRD in cohorts of feedlot cattle, based on cohort-specific characteristics using a Monte-Carlo simulation model. Lastly, in chapter 7, we reflect on the previous chapters, providing final remarks as to future research questions whose answers could potentially benefit the study of BRD and make a positive impact on the beef industry.

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Chapter 2 - **Performance of multiple diagnostic methods in assessing the progression of bovine respiratory disease in calves challenged with infectious bovine rhinotracheitis virus and *Mannheimia haemolytica***

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Abstract

The objective of this study was to evaluate the diagnostic performance of chute-side diagnostic methods for detecting physiological and pathological changes as indicators of early bovine respiratory disease (BRD) in calves experimentally inoculated with infectious bovine rhinotracheitis virus (IBR) and *Mannheimia haemolytica* (Mh). A challenge study was performed over 14 d in 30 Holstein steers [average weight (±SEM) = 211 kilograms (kg) ± 2.4 kg] inoculated on day 0 with IBR and on day 6 with Mh. Diagnostic methods included clinical illness scores (CIS), lung auscultation using a computer-aided stethoscope (CAS), rectal temperature, facial thermography, pulse oximetry, and bilateral thoracic ultrasonography. Animals were randomized into 1 of 5 necropsy days (days 6, 7, 9, 11, and 13) when the percentage of lung consolidation was estimated. The effect of study day on the results of the diagnostic methods and associations between each diagnostic method's values with lung consolidation measured at necropsy were determined with mixed models. Values for all diagnostic methods differed significantly ($P < 0.01$) by day. During the IBR phase (days 0 to 6) calves had "normal" to "moderate" CIS, whereas during the Mh phase (days 6.5 to 13) scores

were predominantly “severe” to “moribund.” Similarly, CAS scores were “normal” and “mild acute” during the IBR phase and “mild acute” to “moderate acute” after the Mh challenge. Oxygen saturation did not differ significantly between days 0, 1, 2, 4, and 6; however, significantly decreased 12 h after inoculation with Mh ($P < 0.05$). Mean lung consolidation between animal’s right and left side recorded by ultrasound was 0.13% (±0.07) before the inoculation with Mh. However, during the Mh phase, mean consolidation increased significantly over time ($P < 0.05$). The percentage of lung consolidation at necropsy ranged from 1.7% (±0.82) on day 6 to 55.4% (±7.49) on day 10. Clinical illness scores, rectal temperature, facial thermography, oxygen saturation, and ultrasonography were significantly associated ($P < 0.05$) with lung consolidation at necropsy. In addition, there was a significant trend ($P = 0.07$) between CAS and lung consolidation scores at necropsy. These chute-side diagnostic methods are useful for detecting disease progression on animals with early stages of BRD.

Introduction

Bovine respiratory disease (BRD) is the most common and costly health problem in the beef industry (Brooks *et al.*, 2011). Common ante-mortem diagnostic practices for in-field BRD diagnosis include clinical observations and rectal temperature; however, they have shown poor diagnostic performance. According to White and Renter (2009), the combination of clinical observations and rectal temperature has both a sensitivity and specificity near 60%, indicating that 40% of non-BRD animals receive treatment, whereas 40% of animals with BRD do not.

The applicability of tools for the diagnosis of BRD has been investigated using populations of cattle with naturally occurring disease (Schaefer *et al.*, 2007; Theurer *et al.*, 2013; Buczinski *et al.*, 2014; Noffsinger *et al.*, 2014; Mang *et al.*, 2015) or in experimentally inoculated animals (Hanzlicek *et al.*, 2010; Amrine *et al.*, 2013). Despite external validity limitations, a challenge approach allows the identification and investigation of the onset and progression of disease among study subjects. Moreover, by utilizing the combination of a viral and bacterial challenge, it is possible to assess the performance of these diagnostic methods at baseline, during a viral infection, and followed by a subsequent bacterial infection. The objective of this study was to evaluate the performance of chute-side diagnostic methods to measure physiological and pathological changes as indicators of early BRD in calves experimentally inoculated with infectious bovine rhinotracheitis virus (IBR) and *Mannheimia haemolytica* (Mh). This study is unique, as to the best of our knowledge, the combination of a viral and a bacterial challenge to study chute-side diagnostic methods for early detection of BRD has not been assessed before.

Materials and methods

Study design

This study was conducted from May 11th (day 0) to May 24th, 2017 at the Veterinary Biomedical and Research Center facility (VBRC), in Manhattan, KS. The VBRC Institutional Animal Care and Use Committee approved the protocol used in this experiment (approval number VAC17053B).

A challenge model utilizing a serial necropsy approach to observe point-in-time pulmonary lesions was used. Animals were randomized, using a random number generator in Microsoft Excel, into 1 of 5 necropsy days, on study days 6, 7, 9, 11, and 13. Study animals did not receive any antimicrobials throughout the study period. Blinding was not possible as all study subjects received the challenge and there were no controls.

Since multiple different diagnostic methods proposed in this study had not been evaluated in a challenge model previously, there were no preliminary data to utilize for calculating sample size. Thus, the simple descriptive statistics generated for each test, and relative to each other, and to clinical and necropsy data are unique and valuable for demonstrating proof-of-concept of early disease detection and for future hypothesis testing. Hypothesis testing using data from this study was used to determine whether test results postchallenge reflected a significant change (delta) from baseline (prechallenge/healthy). Utilizing a P value (or type-1 error rate) of 0.05 and repeated measures to maximize the value gathered from each animal subject, appropriate power (0.80) should be achieved in order to detect statistically significant differences at a level that is clinically relevant for respiratory disease management. Thus, 30 total animals each with baseline data (day 0) when healthy, to allow them to serve as their own "controls," and repeated measures enabled sufficient power to demonstrate statistical differences of clinically significant results.

Study subjects

Holstein calves (approximately 7 mo old) were procured from a calf ranch in north central Iowa. Inclusion criteria for enrollment of study subjects consisted of Holstein steers, homogenous in body weight, and in overall good health as determined by the site veterinarian (C.A.C.). After a 6-d acclimation period (days −6 to −1), a veterinarian (C.A.C.) performed a physical examination of all study subjects including a thorough inspection of the eyes, ears, nervous system, gastrointestinal tract, integument, musculoskeletal system, respiratory system, cardiovascular system, and body condition. Animals were excluded from the study if they were found to have abnormalities upon physical inspection or if their sera tested positive for Mh or IBR (i.e., microagglutination and virus neutralization tests for detection of Mh and IBR, respectively, were performed by the Texas A&M Veterinary Medical Diagnostic Laboratory). No other measurements were recorded before trial initiation (day 0) and, according to the animal supplier, there was no previous BRD treatment history for this group of animals at the farm of origin.

Cattle were housed in a single commercial feedlot-size pen (30 m wide by 38 m long) with a 4.5-m wide concrete apron, 26 m of concrete feed bunks, and a 1.8-m concrete waterer. Feed and water were provided ad libitum. Feed consisted of a grower ration including 59.5% wet distillers grain, 39% forage, and 1.5% mineral supplement with sodium monensin. Neither vaccination, metaphylaxis, nor antiparasitic treatment were administered at any point throughout the study timeline.

Inoculum preparation and administration

IBR inoculum. The IBR challenge virus [IBR CHV lot 05-08, Coopers from United States Department of Agriculture—Animal and Plant Health Inspection Service—Center for

Veterinary Biologics (USDA-APHIS-CVB)] was prepared by Central States Research Center's Veterinary Diagnostic Laboratory as per USDA-APHIS-CVB instructions and was stored at −70 °C until use. The inoculum was thawed at room temperature in a water bath before use.

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On study day 6 (after the morning's clinical and diagnostic evaluations were performed), cattle were restrained in a conventional pneumatic chute (Silencer Hydraulic Chute, Stapleton, NE) with their heads stabilized. An endoscope was introduced by K.F.L. into the right nasal passage of each animal and advanced to the first bronchial bifurcation by passing through the nasopharyngeal region and laryngeal folds into the trachea. The scope was maneuvered in an effort to administer the 60 mL of the Mh inoculum (1 × 106 CFU/mL) to all lung lobes, followed by 60 mL of sterile PBS and 60 mL of air.

Clinical observations

During the 14-d period, an experienced veterinarian (C.A.C.) assigned clinical illness scores (CIS) to animals daily at 0500 h, or twice a day at 0500 and 1400 h on days 6 and 7

(corresponding to observations 6.5 and 7.5). The CIS were defined as follows: 0 (normal), 1 (mild depression): may stand isolated with its head down or ears drooping, but will quickly respond to minimal stimulation, 2 (moderate depression): may remain recumbent or stand isolated with head down, may show signs of muscle weakness (standing cross-legged, knuckling, or swaying when walking), depression is obvious when stimulated, 3 (severe depression): may remain recumbent and reluctant to rise, or if standing isolated and reluctant to move; when moving, is ataxic, knuckling or swaying evident; head carried low with ears drooping; eyes dull, possible excess salivation/ lacrimation, obvious gauntness, and 4 (moribund): unable to stand; approaching death; highly unlikely to respond to any antimicrobial therapy (modified from Perino and Apley (1998)). Animals with a CIS of "moribund" were euthanized, and gross pathology and lung consolidation data were collected during necropsy.

After CIS were assigned, animals were taken to a closed barn (where the chute was located), therefore avoiding measurement's alterations due to weather conditions. Upon arrival to the barn, body weight was recorded using a chute-side scale on days 0, 6, 7, 9, 11, and 13. Measurements were recorded on pounds and later transformed to kilograms (kg). On the same days, the percentage of lung consolidation was recorded using an ultrasound machine (SonoScape S8 Exp, Shenzhen, China) with a Model C344 Curved Array transducer (frequency 5 to 2 MHz). Thoracic ultrasonography was performed on the left and right lung fields. The transducer was positioned to visualize the lung parenchyma between the ribs, beginning in the fourth intercostal space and moving caudally. An estimate of total lung consolidation was provided as a single numeric percentage for each side of the lung. Healthy lung tissues were anechoic, whereas pneumonic tissue was hyperechoic and visualized to depths as great as 6 cm. Pleural effusion was not documented as a unique parameter but recognized along with

consolidation in some of the animals. Animals were not shaved prior to the ultrasound assessment and vegetable oil was used as a lubricant.

On days 0, 1, 2, 4, 6, 6.5, 7, 7.5, 9, 11, and 13, the following measurements were recorded: lung sounds based on the use of a computer-aided stethoscope, rectal temperature, facial thermography, and oxygen saturation levels using pulse oximetry.

The computer-aided stethoscope (CAS; Whisper, Merck Animal Health, De Soto, KS) was placed approximately 5 cm caudal and dorsal of the humero-radial joint of the right-front limb. Lung and heart sounds were recorded—by C.A.C—for 8 s and a computer algorithm assigned a score; scores were defined as follows: 1 (normal), 2 (mild acute), 3 (moderate acute), 4 (severe acute), and 5 (chronic).

Rectal temperature was recorded using a digital GLA thermometer, whereas facial thermography pictures were taken using a thermography camera (FLIR E60, Burlington, VT) that has a thermal sensitivity of <0.05 °C. Thermography pictures were taken at an approximate distance of 50 cm, while cattle were restrained on the chute. Efforts were made to use a consistent distance and angle when taking these pictures. The maximum temperature was recorded from the medial canthus of the left eye. Measurements were recorded on degrees Fahrenheit and later transformed to degrees Celsius.

Oxygen saturation levels were measured by a veterinarian (J.B.) using a pulse oximeter (Masimo Rad-5, Irvine, CA). The instrument's probe was modified for this study by adding a semirigid plastic tube that allowed flexibility for intranasal measurements. Animals were restrained on the chute without sedation and the head was flexed to the side with the use of a halter. The instrument's probe was placed through either the right or left nostril until reaching the

nasal folds. After a few seconds, when the instrument returned a stable signal, oxygen level, heart rate, and perfusion index were recorded (only oxygen level results are presented).

Clinical illness scores were recorded first each day, whereas ultrasound was always the last measurement obtained; however, no specific measurement order was followed, with some of them being performed concurrently, when recording the other measurements. Team members recording data, however, were not aware of each other's measurements, hence minimizing review bias. No measurements were repeated if abnormal values were obtained, unless the instrument indicated the measurement was not of good quality (e.g., pulse oximetry or CAS). Efforts were made to ensure all instruments were working properly by pretesting them in a couple of animals before starting recording measurements.

Euthanasia and postmortem examinations

Animals were administrated xylazine (i.e., 0.3 mg/kg; AnaSed LA manufactured by VetOne, Boise, ID) intramuscularly, in the left neck, followed by the use of a penetrating captive bolt as a stunning method before exsanguination. During necropsy, a macroscopic evaluation of the main systems, as well as gross pathology of the upper and lower respiratory tract, was performed by a trained veterinarian (K.F.L.). A percentage of consolidation was then assigned to each lung lobe upon visual, palpation, and incision inspection. Those individual percentages were inputted into a formula to compute an overall lung consolidation percentage as follows: overall lung consolidation = (0.053 × cranial segment of left cranial lobe) + (0.049 × caudal segment of left cranial lobe) + (0.319 × left caudal lobe) + (0.043 × accessory lobe) + (0.352 × right caudal lobe) + (0.061 right middle lobe) + (0.060 × caudal segment of right cranial lobe) + (0.063 × cranial segment of right cranial lobe) (Fajt *et al*., 2003).

Data analyses

Statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC) and STATA 12 (Stata Corp., College Station, TX) software. Generalized linear mixed models (GLMM) were fitted to assess the effect of study day on the different diagnostic methods. Outcomes consisted of 1) CIS (categories 0 to 4), modeled using a multinomial distribution, cumulative logit link, maximum likelihood with a Laplace approximation estimation method, and a random intercept for animal with an unstructured covariance structure; 2) body weight, 3) rectal temperature, and 4) facial thermography (all recorded on a continuous scale), were modeled using a Gaussian distribution, identity link, pseudo-likelihood estimation technique and a random residual with a heterogeneous first-order autoregressive (ARH-1) covariance structure for animal; 5) ultrasound consolidation (average percentage between right and left sides) and 6) oxygen saturation (percentage) were modeled with a beta distribution, logit link, a pseudo-likelihood estimation technique, and a random residual with an ARH-1 covariance structure for animal; and 7) computer-aided stethoscope scores (categories 1 to 4; no scores of 5 were observed) were modeled using a multinomial distribution, a cumulative logit link, a maximum likelihood with a Laplace approximation estimation method, and a random intercept for animal. Study day (with categories from 0 to 13) was modeled as a fixed effect in all models. Pairwise comparisons between study days were estimated and the P values were adjusted using a Tukey–Kramer method for multiple comparisons.

Generalized linear mixed models were fitted to assess the association between each diagnostic technology with lung consolidation percentages (obtained at necropsy). The outcome of each model consisted of the percentage of lung consolidation obtained at necropsy, which was modeled using a beta distribution, logit link, and a pseudolikelihood estimation technique. Fixed

effects consisted of the different diagnostic methods evaluated. Only data from diagnostic methods recorded on the day of the necropsy (when lung consolidation was measured) were considered for this analysis.

"Normal" CIS were not observed on necropsy days, whereas "severe" and "moribund" scores were collapsed due to few observations in the latter category. Similarly, CAS classified as "severe acute" or "Chronic" were not observed on necropsy days and therefore not included. Continuous predictors (i.e., rectal temperature, facial thermography, and oxygen saturation) that did not meet the linearity assumption were categorized into 3 quantiles (33% of observations in each category). Ultrasound results were separated for left and right lung and categorized into 3 categories based on biological criteria (0%, 1% to 15%, and greater than 15%).

Results

Study subjects

All the study calves tested negative for IBR virus neutralization, Mh antibodies and presented acceptable overall health before the initiation of the study; all 30 initially selected animals were enrolled in the study. On day 10, 2 calves that were assigned a CIS corresponding to "moribund" were euthanized outside (earlier) the randomization protocol. One calf died before the morning observation on day 10. Similarly, 2 moribund animals (CIS of 4), scheduled to be euthanized on day 13, were euthanized earlier, on day 11 (**Supplementary Table 1**).

Clinical observations

Descriptive statistics for all measurements are depicted in **Supplementary Table 2**. All diagnostic measurements significantly varied by study day ($P < 0.01$). All animals during the first 3 d of the study were assigned CIS considered "normal" (CIS of 0). On day 3 and until the challenge with Mh on day 6, animals were also assigned "mild," "moderate," and "severe" CIS.

After Mh inoculation, higher CIS were recorded, and after day 9, “moderate,” “severe,” and “moribund” scores were assigned (**Table 1**). Clinical illness scores on day 0 were significantly different ($P < 0.05$) from scores on the viral phase (only days 5 and 6), and from scores assigned on the bacterial phase (days 6.5, 7, 7.5, 8, 9, 10, 11, 12, and 13).

Mean body weight at the beginning of the study was 211 kg (+/− 2.4 kg). However, body weight was subsequently observed to decrease throughout the study to an average of 181 kg (+/− 5.0 kg) on the last day (**Table 2**).

The model-adjusted mean percentages of lung consolidation (based on ultrasound; average between animal’s right and left lung fields) was 0.1% (±0.07) on day 0, and 0.1% (±0.08) on day 6. Lung consolidation significantly increased after Mh inoculation (day 6) until day 11 (**Table 2**).

No “chronic” scores (5) were recorded by CAS throughout the study. During the viral phase of the challenge (days 0 to 6), “normal” (1), “mild acute” (2), and “moderate acute” (3) scores were recorded. After the bacterial challenge (day 6), higher scores ($P > 0.05$) were observed (**Table 3**). CAS on days 4, 7, 7.5, 9, and 11 were significantly different ($P < 0.05$) from scores on day 0.

Mean rectal temperatures between days 0 and 6 ranged from 39.1 °C to 40.5 °C. Between day 6.5 (12 h after Mh inoculation) and until the last day of the study, temperatures ranged from 39.8 °C to 41.7 °C (**Table 2**). Mean facial thermography temperatures between days 0 and 6 ranged from 37.4 °C to 39.8 °C. Temperatures ranged from 36.4 °C to 40.4 °C between day 6.5 and until the last day of the study. Model-adjusted mean rectal and facial thermography temperatures are depicted in **Table 2**.

Model-adjusted mean percentage of oxygen saturation did not significantly ($P > 0.05$) differ among days 0, 1, 2, 4, and 6 (viral phase; **Table 2**). Oxygen saturation significantly decreased 12 h (day 6.5) after inoculation with Mh ($P < 0.05$). Mean percentage of oxygen saturation on days 7, 9, 11, and 13 significantly differed ($P < 0.05$) from values recorded on days 0, 1, 2, and 4 (**Table 2**).

Euthanasia and postmortem examination

Fibrinous to mucopurulent tracheitis, consistent with IBR infection, was observed in calves at necropsy on day 6 (pre-Mh challenge). Among calves euthanized on days 7 to 13 (post-Mh challenge), lungs progressively showed severe consolidation of the cranioventral portions. Fibrinous pleural adhesions and fibrinous to purulent pleuritis were also observed. Necrotic laryngitis and normal to necrotic tracheitis were also observed at necropsy on days 7 to 13.

Model-adjusted mean (±SEM) lung consolidation at necropsy was 1.7% (±0.8%) on day 6, and 55.4% (±7.5%) on day 10 (Tables 2 and 4).

When testing associations between each diagnostic method relative to lung consolidation measured during necropsy, our sample size was reduced to 27 as no diagnostic tests were performed on 3 animals (2 were euthanized due to a “moribund” CIS and one died; all on day 10). Associations between each diagnostic method with lung consolidation at necropsy are depicted in Figure 1. Clinical illnesses scores, lung consolidation based on ultrasound, rectal temperature, facial thermography temperature, and oxygen saturation were significantly associated ($P < 0.05$) with lung consolidation at necropsy. There was a significant trend ($P = 0.07$) between CAS and lung consolidation scores at necropsy.

Discussion

We successfully demonstrated the applicability of chute-side diagnostic methods to measure physiological and pathological changes as indicators of BRD in calves, seronegative to both IBR and Mh, experimentally inoculated with IBR and Mh. Using a 2-pathogen model, we induced clinical symptoms and pathological findings consistent with IBR and Mh infections which allowed us to evaluate the performance of chute-side methods for early detection of BRD in calves.

Single-pathogen challenge studies have been previously performed to evaluate the detection capabilities of CIS (Amrine *et al*., 2013), necropsy lung consolidation (Hanzlicek *et al*., 2010), and rectal temperature (Hanzlicek *et al*., 2010; Theurer *et al*., 2013; Grissett *et al*., 2015). By challenging animals with a pathogen(s), the onset and progression of the disease can be observed (Hanzlicek *et al*., 2010; Amrine *et al*., 2013; Hanthorn *et al*., 2014; Confer *et al*., 2016).

During the viral phase of the challenge (days 1 to 6), CIS corresponding to "normal," "mild," and "moderate" were observed in calves and were consistent with another report of IBR infection (Grissett *et al*., 2015). Similarly, typical upper respiratory tract lesions were observed in the 5 animals necropsied by day 6. Later in the study, during the bacterial phase, CIS of "severe" and "moribund" were observed: likely, the potential immunosuppression and pathological process produced by the initial IBR challenge facilitated the disease progression observed after the Mh challenge (Griffin *et al*., 2010; Caswell J. L. 2014). Although the lack of blinding could have biased our measurement of CIS, it is difficult to implement blinding in challenge studies when animals become morbid (Sargeant *et al*., 2010).

As previously reported by Schaefer et al., (2007), rectal and facial thermography temperatures significantly increase with the onset of disease. However, in our study, there also was a subsequent decrease in mean temperatures as the disease progressed and lung consolidation increased. These decreased temperatures were daily averages based on a relatively small number of animals available by the end of the study, which were severely ill or moribund and were no longer exhibiting a febrile response to infection. Therefore, animals that were approaching death, with greater consolidation percentages, had lower temperatures.

The use of auscultation has been previously investigated as a tool for evaluating lung diseases such as BRD (Dedonder *et al*., 2008). Compared with traditional auscultation, CAS represents an objective method for identifying lung pathology including consolidation (Mang *et al*., 2015; Zeineldin *et al*., 2016). Mang *et al*. (2015) found that CAS had a sensitivity of 92.9% (95% credible interval = 0.71%–0.99%) and a specificity of 89.6% (0.64%–0.99%) when classifying animals as sick if a "mild-acute" score or greater was recorded. In our study, CAS scores were associated with lung consolidation recorded at necropsy. During the viral phase (days 1 to 6), 37% to 66% of our study animals had a "mild-acute" (2) score or higher, which is consistent with an IBR infection. However, onethird of the study calves also had a CAS score of 2 ("mild-acute") before inoculation occurred (day 0). Thus, in this type of calves, roughly one-third may have physiological or anatomical characteristics consistent with a CAS score of 2 regardless of health status. In which case, the CAS score 2 definitions as "mild-acute" may be a misnomer. Unfortunately, we cannot differentiate whether this is a case of test misclassification or it is an artifact of how the CAS algorithm defines categories based on a latent continuous distribution (where numerically close values may be classified as 2 distinct categories).

Imaging can also be used to objectively evaluate lung pathology. Although ultrasound does not completely reach the cranio-ventral area where typical BRD lesions occur (Buczinski *et al*., 2014), lung consolidation percentages based on ultrasound were significantly associated with necropsy findings. However, as not all commercial cattle operations have access to an ultrasound machine or trained personnel, its applicability is limited.

As the amount of consolidated lung tissue increased, other changes were detected such as decreased levels of oxygen saturation. No significant changes in the mean levels of oxygen saturation were observed during the first 6 d of our study, likely due to the absence of lung lesions during the IBR challenge. Pulse oximetry was evaluated previously (Coghe *et al*., 1999), however, not in comparison to lung pathology. Animals in the present study showed the highest mean percentage of consolidation at necropsy (55.4%) on day 10. At day 13, however, mean values were lower (23.3%) likely due to the fact that our necropsy randomization plan was altered and most moribund animals were euthanized before schedule.

Although challenge studies offer a useful model for studying the pathogenesis of infectious diseases by allowing to induce the onset of disease and follow the evolution of clinical and physiological parameters that are associated with the underlying pathological process, their external validity is compromised. Hence, study findings cannot be directly extrapolated to cattle managed under natural, field conditions. This study could have benefited from having multiple observers recording subjective measurements such as CIS or ultrasound. However, precision for most if not all diagnostic methods employed in this study has already been established. To minimize information bias, all subjective and objective measurements were recorded by rigorously trained, qualified, and experienced personnel.

Our results indicate that the use of one, or a combination of these chute-side diagnostic methods, could improve BRD confirmation. Clinical illness scores are considered a subjective tool, as they rely on the visual appraisal of animals, and are nonspecific relative to BRD. Rectal temperature and facial thermography on the other hand, although objective in nature, do not detect BRD specific changes. Newly received animals stressed due to the changes in environment, management, or diet, among other factors, could be inaccurately classified as depressed or may experience non-BRD-related increments in temperature. Ultrasound, CAS, and pulse oximetry, in contrast, provide objective measurements of lung consolidation through the evaluation of changes in imaging, sounds, and level of oxygen that occur during the BRD pathological process. Given their significant association with disease progression and pathological changes in the respiratory tract, a combination of these chute-side diagnostics can be implemented to improve the specificity of the BRD case definition as well as for early diagnosis of BRD. Practical considerations for potential implementation of these diagnostics in commercial cattle production systems include the operation's infrastructure, cost of instruments, and level of training, among other factors, required to perform these tests.

We conclude that the diagnostic methods evaluated in this study may be useful indicators of early BRD in calves infected with IBR and subsequently with M. haemolytica. In our challenge study, rectal temperature and facial thermography data differed between days when animals were diseased when compared with healthy, but it did not appear that these tools would be useful for differentiating between viral and bacterial (following viral) infections. This can be important to consider given that antimicrobials for BRD are not efficacious for treatment or control of viral infections. Alternatively, the computer-aided stethoscope, pulse oximeter, and ultrasound may be useful ante-mortem approaches that reflect bacterial pathology (i.e., lung

consolidation) when compared with healthy animals or those only with initial IBR-related disease. Given the importance of BRD in cattle production systems, and the inaccuracy of diagnostic approaches commonly used in the field, further evaluation of these potentially useful diagnostic technologies seems warranted.

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Table 2.1. Percentage of clinical illness scores observed in calves inoculated with IBR and *Mannheimia haemolytica* by study day.

	Study Day															
Clinical Score	0	1	2	3	4	5	6	6.5	7	7.5	8	9	10	11	12	13
Normal	100	100	100	90	23	0	0	0	0	0	0	0	0	0	0	0
Mild	0	0	0	10	63	77	77	44	32	5	10	0	0	0	0	0
Moderate	0	0	0	0	13	23	17	44	56	85	70	60	43	8	60	40
Severe	0	0	0	0	0	0	7	12	12	10	20	25	43	75	40	60
Moribund	0	0	0	0	0	0	0	0	0	0	0	15	14	17	0	0
Total Calves[1]	30	30	30	30	30	30	30	25	25	20	20	20	14	12	5	5

[1]Calves were randomly assigned to be euthanized on study days 6, 7, 9, 11 and 13. On day 10, 2 animals were euthanized (CIS = "moribund") and 1 died before clinical illness scores were recorded.

Table 2.2. Model-adjusted means and standard errors (SEM) of pre- and post-mortem clinical measurements in calves inoculated with IBR and *Mannheimia haemolytica*, by study day.

	Study Day												
	Mean (SEM)												
Variable, unit	0	1	2	4	6	6.5	7	7.5	9	10	11	13	*P*-value[4]
Body weight, kg	211[a]	-	-	-	202[b]	-	195[cd]	-	203[abc]	-	190[d]	182[d]	<0.01
	(2.4)	-	-	-	(2.3)	-	(2.7)	-	(4.8)	-	(4.1)	(5.0)	
Ultrasound, %[1]	0.1[d]	-	-	-	0.1[d]	-	5.1[c]	-	17.0[b]	-	23.2[a]	18.5[ab]	<0.01
	(0.07)	-	-	-	(0.08)	-	(0.82)	-	(1.59)	-	(2.52)	(2.33)	
Rectal temperature, °C	39.1[a]	38.9[a]	40.3[f]	40.9[cd]	40.5[bf]	41.7[e]	40.7[cbg]	41.2[d]	39.8[abfg]	-	40[bf]	39.5[abcf]	<0.01
	(0.05)	(0.06)	(0.08)	(0.06)	(0.08)	(0.09)	(0.09)	(0.08)	(0.31)	-	(0.24)	(0.43)	
Facial thermography temperature, °C	38.1[a]	37.4[bc]	38.5[a]	39.8[de]	39.4[def]	40.4[g]	39.2[f]	39.7[d]	38.6[abef]	-	38.5[abdef]	36.4[d]	<0.01
	(0.10)	(0.11)	(0.14)	(0.14)	(0.11)	(0.15)	(0.11)	(0.09)	(0.35)	-	(0.53)	(0.38)	
Oxygen saturation, %[2]	97.9[a]	97.6[a]	98.1[a]	97.8[a]	96.5[ab]	94.4[cd]	95.4[bcd]	95.8[abd]	91.9[bcd]	-	91.2[c]	92.8[bcd]	<0.01
	(0.33)	(0.31)	(0.37)	(0.35)	(0.38)	(0.45)	(0.44)	(0.64)	(1.88)	-	(1.82)	(1.74)	
Lung consolidation, %[3]	-	-	-	-	1.7[d]	-	13.0[c]	-	42.9[ab]	55.4[a]	40.2[ab]	23.3[bc]	<0.01
	-	-	-	-	0.82	-	3.62	-	5.74	7.49	4.80	4.80	
Total Calves[5]	30	30	30	30	30	25	25	20	20	3	12	5	

[1]Ultrasound (%): mean percentage consolidation between animal's left and right side.
[2]Oxygen saturation measured by a pulse oximeter.
[3]Percentage of lung consolidation measured during necropsy. We imputed individual lobe consolidation into the following formula: Lung consolidation = (0.053 X left cranial lobe) + (0.049 X left caudal lobe) + (0.319 X left cardiac lobe) + (0.043 X accessory lobe) + (0.352 X right cranial posterior lobe) + (0.061 X right middle lobe) + (0.060 X right caudal) + (0.063 X right cranial anterior lobe). (Fajt *et al*., 2003).
[4]Indicates a statistical difference among study days.
Different letter superscripts within a row indicate statistically significant differences (*P*-value < 0.05).
[5]Calves were randomly assigned to be euthanized on study days 6, 7, 9, 11 and 13. On day 10, 2 animals were euthanized (CIS = "moribund") and 1 died.

Table 2.3. Percentage computer-aided stethoscope scores recorded in calves inoculated with IBR and *Mannheimia haemolytica*, by study day.

	Study Day										
Whisper Score	0	1	2	4	6	6.5	7	7.5	9	11	13
Normal	67	60	57	33	63	40	32	30	15	8	60
Mild acute	33	40	43	63	37	56	40	25	55	58	40
Moderate acute	0	0	0	3	0	0	24	45	30	33	0
Severe acute	0	0	0	0	0	4	4	0	0	0	0
Chronic	0	0	0	0	0	0	0	0	0	0	0
Total Calves[1]	30	30	30	30	30	25	25	20	20	12	5

[1]Calves were randomly assigned to be euthanized on study days 6, 7, 9, 11 and 13. On day 10 two animals were euthanized (CIS = “moribund”) and one died.

Table 2.4. Percentage of consolidation in each lobe, and overall lung consolidation, recorded at necropsy.

ID	Day[1]	Rt crn ant	Rt crn post	Rt mid	Rt cau	Acc	Lt crn	Lt cau	Lt card	Overall[2]
1	6	0	0	0	0	0	0	1	0	0.3
2	6	0	0	0	0	0	0	0	0	0.0
3	6	1	1	0	0	0	0	0	0	0.1
4	6	0	0	0	0	0	0	0	0	0.0
5	6	0	0	0	0	0	1	0	0	0.1
6	7	5	0	20	0	0	0	0	0	1.5
7	7	50	5	90	5	100	1	0	10	15.5
8	7	10	0	90	20	90	0	0	5	17.3
9	7	40	0	90	5	80	0	0	0	13.2
10	7	10	0	40	40	10	0	0	5	17.8
11	9	100	30	100	20	100	1	0	1	25.6
12	9	100	100	100	40	100	90	20	100	52.8
13	9	50	10	100	80	90	0	0	20	42.9
14	9	10	0	90	50	90	60	50	90	51.1
15	9	100	5	90	40	100	60	10	90	41.3
16	10	90	20	100	70	100	10	20	20	49.8
17	10	30	0	100	20	100	100	30	90	38.6
18	10	20	10	80	80	100	100	60	100	68.5
19	11	20	0	100	60	100	5	5	100	39.5
20	11	40	5	90	40	100	90	20	100	42.7
21	11	70	0	100	40	100	1	10	90	36.5
22	11	100	50	100	60	100	5	5	100	47.6
23	11	30	10	100	70	100	40	10	100	47.7
24	11	60	10	100	30	100	0	0	0	25.3
25	11	50	0	100	70	100	0	0	10	38.7
26	13	20	5	50	40	100	5	0	2	23.4
27	13	70	0	100	80	100	20	40	70	60.2
28	13	0	0	100	20	100	0	5	5	19.3
29	13	70	0	100	10	80	80	5	100	28.2
30	13	0	0	0	0	70	0	1	0	3.3

Rt crn ant = Right cranial anterior. Rt crn post = Right cranial posterior. Rt mid = Right middle. Rt cau = Right caudal. Acc = Accessory. Lt crn = Left cranial. Lt cau = Left caudal. Lt car = Left cardiac.
[1]Day of necropsy.
[2]Overall lung consolidation = (0.053 X left cranial lobe) + (0.049 X left caudal lobe) + (0.319 X left cardiac lobe) + (0.043 X accessory lobe) + (0.352 X right cranial posterior lobe) + (0.061 X right middle lobe) + (0.060 X right caudal) + (0.063 X right cranial anterior lobe) (Fajt *et al*., 2003).

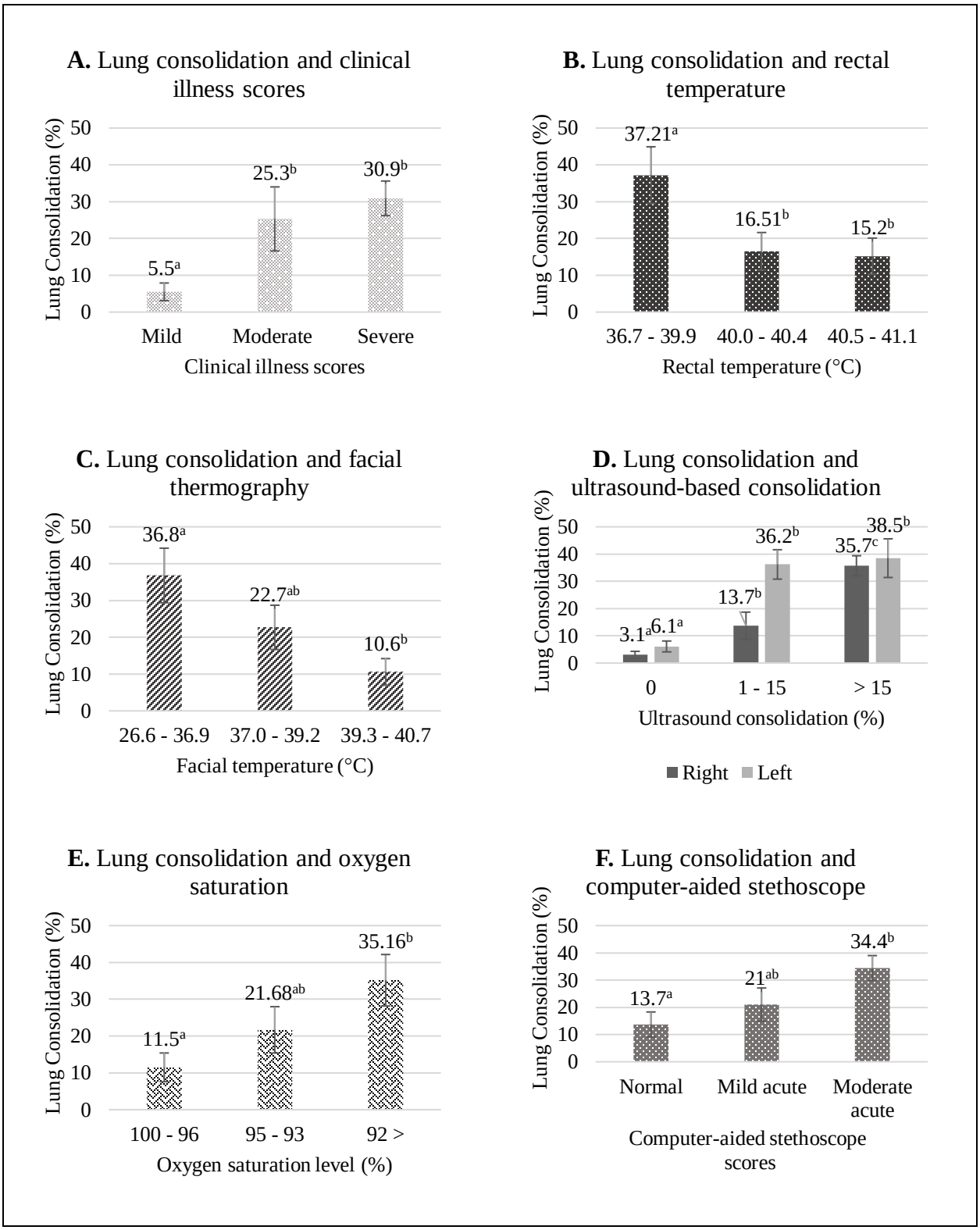

Figure 2.1. Graphs depicting associations between pre-mortem clinical measurements with lung consolidation, in %, measured during necropsy of 27 calves inoculated with *IBR* and *Mannheimia haemolytica.*

Graphs depict model adjusted means for lung consolidation by **A.** clinical illness scores, **B.** rectal temperature, **C.** facial thermography, **D.** ultrasound-based consolidation measured on the animal's right and left sides, **E.** oxygen saturation, and **F.** computer-aided stethoscope scores. Error bars represent standard errors of the mean. Different letter superscripts within a graph indicate statistically significant differences (P-value < 0.05).

Chapter 3 - **Assessment of bovine respiratory disease progression in calves challenged with bovine herpesvirus type-1 and *Mannheimia haemolytica* using point-of-care and laboratory-based blood leukocyte differential assays.**

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Abstract

Blood leukocyte differentials can be useful for understanding changes associated with bovine respiratory disease (BRD) progression. By improving turnaround time, point-of-care leukocyte differential assays (PCLD) may provide logistical advantages to laboratory-based assays. Our objective was to define associations between PCLD and laboratory-based determination of blood leukocyte differentials with BRD progression in steers challenged with bovine herpesvirus type-1 and *Mannheimia haemolytica*. Thirty Holstein steers (average weight of 211 Kg $\pm$ 2.4 Kg) were inoculated intranasally on day zero with bovine herpesvirus type-1 and intrabronchially on day 6 with *Mannheimia haemolytica*. Blood leukocytes differentials were measured using both assays from study day zero to 13. Linear mixed models were fitted to evaluate the associations between: 1) the type of assay (laboratory-based or PCLD) with respect to leukocyte, lymphocyte, and neutrophil concentrations, 2) study day with cell concentrations, and 3) cell concentrations with lung consolidation measured at necropsy. Point-of-care leukocyte, lymphocyte, and neutrophil concentrations were significantly associated ($p < 0.05$) with their respective cell concentrations obtained from the laboratory-based leukocyte differential. Cell concentrations reported by both assays differed significantly ($p < 0.05$) over time, indicating shifts from healthy to viral and bacterial disease states. Lymphocyte concentrations, lymphocyte/neutrophil ratios

obtained from both assays and band neutrophil concentrations from the laboratory-based assay were significantly associated ($p < 0.05$) with lung consolidation. Therefore, these measurements may enhance assessments of disease severity. PCLD may be a useful alternative to assess BRD progression when laboratory-based leukocyte differentials are impractical.

Introduction

In the United States, cattle are commonly affected by bovine respiratory disease (BRD), a multifactorial disease complex that causes an estimated burden of 3 billion USD annually[10]. In feedlots, the economic impact associated with BRD may include costs associated with negative performance (e.g., average daily gain) and carcass characteristics (e.g., hot carcass weight, yield grade), treatment and prevention, and death[8]. In addition to BRD's significant economic impacts in the feedlot industry, the dairy, backgrounding, and cow-calf industries are also significantly impacted[3,12].

A common practice for BRD diagnosis consists of assessing clinical signs of individual animals and measuring rectal temperature[1]. This practice, however, has poor diagnostic sensitivity and specificity, both estimated at approximately 60%[18]. Refining case definition and diagnosis for BRD is necessary to improve the animal's health and well-being as well as promote the judicious use of antimicrobials. Although reports of diagnostic practices for BRD are common in the literature, determining the onset of disease is still challenging due to lack of benchmark ante-mortem diagnostic tests. To address this issue, several authors have used challenge studies to evaluate the performance of diagnostic assays[5,7,13].

Although several combinations of pathogens can cause BRD, one of the most important combinations observed on the field consists of a viral infection with bovine herpesvirus (BHV-1) followed by lung colonization with *Mannheimia haemolytica* (Mh)[6,11]. Blood leukocyte differentials have been used to aid diagnostics, clinical assessments, monitoring of disease or therapeutic actions, and in particular, to assess BRD in other challenge studiess[4,5,7,13,15]. However, blood leukocyte differentials have not been used to evaluate the progression of disease following a BHV-1/Mh challenge.

Consistent with acute infectious processes, prior research has demonstrated an increase in leukocyte and neutrophil concentrations and a decrease in lymphocyte concentration upon infection with BRD causative pathogens[4,5]. The determination of blood leukocyte differentials could help veterinarians and producers in mitigating the impacts of BRD, but it is yet unclear how these data change as the disease process progresses and lung consolidation increases. Leukocyte differentials are usually measured by submitting blood samples to trained personnel in a clinical pathology laboratory, which can create logistical challenges for field case management. Point-of-care systems have been developed to provide a more rapid, chute-side measurement alternative[9]. Therefore, our objective was to define associations between point-of-care and laboratory-based determination of blood leukocyte differentials with BRD progression in steers challenged with bovine herpesvirus type-1 and *Mannheimia haemolytica*.

Materials and methods

This study was conducted from May 11th (study day zero) to May 24th, 2017, at the Veterinary Biomedical and Research Center facility (VBRC), in Manhattan, Kansas. The study protocol was approved by the VBRC Institutional Animal Care and Use Committee (approval number VAC17053B).

Study design and study subjects

Information regarding study design, characteristics, and management of study subjects for this challenge study has been reported previously for a concurrent study[2]. Briefly, 30 Holstein steers with an average body weight of 211 Kg (SEM=2.4 Kg) were enrolled in the study, which was carried out over 14 days (study days zero to 13). Steers tested negative to Mh and BHV-1 antibodies (based on microagglutination and virus neutralization tests for detection of Mh and

BHV-1, respectively, which were performed at the Texas A&M Veterinary Medical Diagnostic Laboratory) and had no prior history of BRD treatment or clinical signs. A veterinarian inspected the animals upon arrival to the study facility (study day -6), and for the following 6 days after arrival to ensure they were healthy before trial initiation. Animals were housed at the VBRC research facility in a single feedlot-size pen (30 m wide by 38 m long with 26 m of concrete feed bunks and a 1.8-m concrete waterer) and were fed a growing ration. Animals were randomly selected to be euthanized on study days 6 to 13, and lung consolidation scores were assigned to lung lesions by a veterinarian during necropsy[2]. In addition, animals were observed, and clinical illness scores assigned[2], daily or twice a day on study days 6 and 7. Moribund animals were necropsied outside the randomization process, and lung consolidation was recorded[2].

On study day zero, animals were restrained on a pneumatic chute without sedation and the BHV-1 inoculum was administered into each nasal passage using an atomizer (Model 163, De Vilbiss Healthcare, LLC., Port Washington, NY). On study day 6, after blood samples were collected (see below), animals were restrained and inoculated with the Mh isolate (serotype one). The Mh inoculum was administrated while animals were restrained in the chute with their heads stabilized. An endoscope was introduced into the right nasal passage until reaching the first bronchial bifurcation where the Mh inoculum was administrated[2]. In summary, the study timeline was as follows: animals were deemed healthy through study day zero, the viral challenge was administered on study day zero, the bacterial challenge on study day 6, and animals were followed up to study day 13[2].

Sampling

On study days 0, 1, 2, 4, 6, 6.5, 7, 7.5, 9, 11, and 13, animals were restrained in the chute and their heads haltered to collect blood samples by means of jugular venipuncture using 20-gauge

needles and EDTA vacutainer tubes. Samples were placed on ice and transported to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) within 3 hours of the first animal being sampled. After arrival to the KSVDL, samples were processed for a complete blood count with leukocyte differential, including segmented neutrophil, band neutrophil, lymphocyte, monocyte, eosinophil, and basophil concentrations, by the clinical pathology laboratory (laboratory-based leukocyte differential; LLD) measured by a medical technologist who was blinded to the animal's disease status. A second blood sample was obtained, by means of jugular venipuncture, using a specialized EDTA tube/needle—provided by the instrument's manufacturer—and immediately aliquoted to a single slide deck and inserted in the point-of-care leukocyte differential (PCLD) instrument (QScout MLD® test, Advanced Animal Diagnostics, Inc., Durham, NC). Samples evaluated by the PCLD were run using the manufacturer's settings, the results were obtained after 2 minutes, and the data were stored. The PCLD data included total leukocyte, total neutrophil, and lymphocyte concentrations.

Data analyses

Statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC) and STATA 12 (Stata Corp., College Station, TX) software. Descriptive statistics (mean, median, standard deviation, and range) were performed for all outcomes by study day.

Linear mixed models (LMMs) were fitted using the GLIMMIX procedure in SAS to account for repeated measures when evaluating the association between the blood parameters reported by the 2 assays with study day. Leukocyte, lymphocyte, segmented neutrophil, total neutrophil/lymphocyte ratio, basophils, eosinophil, band neutrophil, monocyte, spun hematocrit, hematocrit calculated, hemoglobin, cellular hemoglobin, fibrinogen, and plasma protein were modeled as continuous outcomes using a Gaussian distribution, identity link, and a residual

pseudo-likelihood (RSPL) estimation technique. Study day (with categories from zero to 13) was included as a fixed effect in all models. A random residual with a heterogeneous first-order autoregressive covariance structure at the animal level was included. Models were checked for outliers by using a |2| SD cut-off in the studentized residuals. Model homoscedasticity and normality were assessed via visual appraisal of scatterplots and histograms of standardized residuals. Leukocyte, lymphocyte, segmented neutrophil, total neutrophil/lymphocyte ratio, and monocyte concentrations were log 10 transformed to improve model fit and back-transformed for interpretation. After attempting several transformations, outcomes for which the residuals did not meet the normality assumption, namely basophil, eosinophil, and band neutrophil concentrations, were transformed into categorical variables. Based on the references intervals provided by the KSVDL, variables were categorized as follows: basophils concentration "within reference interval" (<0.2 k/µL) and "outside of reference interval" (≥0.2 k/µL), eosinophil concentration "within reference interval" (<1.6 k/µL) and "outside of reference interval" (≥1.6 k/µL), and band neutrophil concentration "within reference interval" (<0.2 k/µL) and "outside of reference interval" (≥0.2 k/µL). These outcomes were modeled using a binary distribution, logit link, and an RSPL estimation technique. A random residual term with an autoregressive moving average (ARMA (1, 1)) covariance structure at the animal level was fitted to account for repeated measures. *P*-values for pairwise comparisons between study days were adjusted using a Tukey-Kramer method for multiple comparisons.

Leukocyte, lymphocyte, and neutrophil concentrations were reported for both LLD and PCLD assays. Therefore, 3 LMMs were fitted using the GLIMMIX procedure in SAS to evaluate the association between the results from the PCLD and the LLD. Outcomes for each of the 3 models, respectively, were leukocyte, lymphocyte, and segmented neutrophil concentrations

reported by LLD modeled with a Gaussian distribution, identity link, and an RSPL estimation technique. Fixed effects included leukocyte, lymphocyte, and total neutrophil concentrations reported from the PCLD and study day. An interaction term between the 2 main effects (cell count and study day) also was included in each of the 3 models. A random residual with a heterogeneous first-order autoregressive covariance structure at the animal level was used for each model. Model fit and multiple comparisons adjustments were performed as described above.

Eleven generalized LMMs were fitted to assess the associations between leukocyte, lymphocyte, neutrophil, total neutrophil/lymphocyte ratio, plasma protein, and fibrinogen obtained from the LLD and the PCLD with lung consolidation scores obtained at necropsy. For the LLD, band and segmented neutrophil concentration were used in different models, whereas for the PCLD—where segmented and band neutrophil concentrations were not differentiated—total neutrophils were used. In each model, lung consolidation scores, recorded as a proportion of consolidated tissue in the lung, were modeled as the outcome with a beta distribution, logit link, and an RSPL estimation technique using the GLIMMIX procedure in SAS. Estimated lung consolidation proportions were then converted to percentages for interpretation purposes. Fixed effects corresponded to leukocyte, lymphocyte, segmented neutrophil, band neutrophil, total neutrophil/lymphocyte ratio, plasma protein, and fibrinogen obtained from the LLD, and leukocyte, lymphocyte, total neutrophil, and total neutrophil/lymphocyte ratio obtained from the PCLD, for the 11 models, respectively. Fixed effects on each model were categorized into 3 categories (33% of observations on each category) for the PCLD and LLD. Categories were defined as follows: leukocytes (< 8.1 k/µL; 8.1 – 12.2 k/µL; > 12.2 k/µL), lymphocytes (< 2.9 k/µL; 2.9 – 5.1 k/µL; > 5.1 k/µL), segmented or total neutrophils (< 4.4 k/µL; 4.4 – 6.5 k/µL; >

6.5 k/µL), band neutrophils (< 0.2 k/µL; 0.2 – 0.7 k/µL; > 0.7 k/µL), total neutrophil/lymphocyte ratio (< 0.9; 0.9 – 2.3; > 2.3), plasma protein (< 7.0 g/dL; 7.0 – 7.6 g/dL; > 7.6 g/dL), and fibrinogen (< 700 mg/dL; 700 – 900 mg/dL; > 900 mg/dL).

Results

On enrollment, all study calves tested negative for BHV-1 and Mh[2]. Bovine respiratory disease was successfully induced with all animals showing clinical signs of progressive severity up to study termination[2]. Clinical signs, necropsy findings, and results from other diagnostic methods have been reported in detail elsewhere[2]. Briefly, up to study day 6, animals did not present clinical signs of disease or presented mild depression, whereas, after study day 6, animals presented moderate and severe depression, with some animals being moribund[2]. Fibrinous to mucopurulent tracheitis with almost no lung consolidation was observed on study day 6, whereas, after study day 6, the fibrinous to mucopurulent tracheitis decreased, but lung consolidation increased to an average maximum of 55.4% on study day 10[2]. Descriptive statistics (means, standard deviations, and ranges) for blood parameters during the study period are depicted in **Supplementary tables 1 and 2.**

Associations of LLD-blood parameters by study day

Model-adjusted mean leukocyte concentrations were higher than the reference interval (5.0 – 10.0 k/µL) during study days 0, 1, 2, and 4. However, the mean concentration significantly decreased by study day 6 when compared to study days zero or one (**Table 1**). Mean leukocyte concentration significantly increased on study day 6.5 when compared to study day 6 (when animals were inoculated with Mh; **Table 1**). Mean leukocyte concentration significantly increased on study day 7 when compared to study day 6.5; however, concentrations on study days 7.5, 9, 11, and 13 did not differ when compared to study day 6.5 (**Table 1**). Throughout the

study, mean lymphocyte concentrations remained within the reference interval (2.5 – 5.0 k/µL; **Table 1**). Mean lymphocyte concentration significantly decreased from study day zero to study day one; means for study days 6.5, 7, 7.5, 9, and 11 were significantly lower than study day zero (**Table 1**). Means for segmented neutrophil concentrations were within the reference interval (1.0 – 5.0 k/µL) except for study days one and 7, when concentrations were above, and study day 13 when mean concentration was below the reference interval. Mean segmented neutrophil concentration significantly decreased from study day zero compared to study day 6; however, 12 hours after the bacterial inoculation (study day 6.5), the mean concentration significantly increased when compared to study day 6 (**Table 1**). Segmented neutrophil concentrations decreased after study day 7. Specifically, study days 7.5, 9, and 11 each had significantly lower concentrations when compared to study day 7 (**Table 1**). The mean ratio between total neutrophils and lymphocytes significantly increased from study day 6 to study day 6.5. Specifically, mean lymphocyte concentration was double the neutrophil concentration on study day 6, whereas on study day 6.5, neutrophil concentrations were 70.0% higher than lymphocyte concentrations (**Table 1**). The mean ratio remained above one from study day 6.5 until study day 13, indicating that the predominant cells were the total neutrophils. Mean monocyte concentrations were above or at the reference interval (0.0 – 0.8 k/µL) every day until study day 9. However, on study day 11, the concentration significantly decreased, to within the reference interval, when compared to study day 9 (**Table 1**).

Model-adjusted mean hematocrit values (both calculated and spun values) and hemoglobin significantly decreased from study day zero to study day 6 (**Table 1**). However, mean hematocrit values significantly increased on study days 11 and 13 when compared to study day 6 (**Table 1**). Mean fibrinogen values increased throughout the study, reaching its peak on the

last day of the study. Specifically, study day zero was significantly lower than study day 6, and each of the last 3 study days were significantly higher than any other study day (**Table 1**). Plasma protein values decreased from study day zero to study days 4 and 6 (**Table 1**). However, plasma protein values significantly increased when comparing study days 6.5 to study days 11 or 13 (**Table 1**).

The probability of having basophil concentrations above the reference interval (≥0.2 k/µL) did not differ between study days 0, 1, and 2 (**Table 2**). However, from study day 4 to study termination, no basophils concentrations above the reference interval were observed. Animals did not present eosinophils concentrations above the reference interval (≥1.6 k/µL) during the study period; therefore, no models were fitted, and only descriptive results are depicted in **Supplementary table 1**. Prior to study day 6, no animals had band neutrophil concentrations above the reference interval (>0.2 k/µL). The probability of animals having band neutrophil concentrations above the reference interval significantly increased when comparing study day 6 with study day 6.5 (12 hours after the bacterial challenge; **Table 2**). From study days 6.5 to study day 13, the probability of animals having band neutrophil concentrations above the reference interval ranged from 0.42 to 0.85, showing no significant differences between study days 6.5, 7, 7.5, 9, 11, and 13 (**Table 2**).

Associations of PCLD-blood parameters by study day

Model-adjusted mean leukocyte concentrations from the PCLD decreased through the first 6 days of the study, with mean concentration on study day 6 being significantly lower than on any of the previous study days (**Table 3**). However, when comparing study day 6 to study day 7, the mean concentrations significantly increased (**Table 3**). Mean lymphocyte concentrations

did not vary significantly by study day during the first 6 days of the study, but 12 hours after the inoculation with Mh (by day 6.5), the mean concentration significantly decreased when compared to study day 6 (**Table 3**). Study days 9, 11, and 13 each had significantly lower mean lymphocyte concentrations than study days 0, 1, 2, and 6 (**Table 3**). Mean total neutrophil concentrations were significantly decreased on study days 2, 4, and 6 when each was compared to study day zero (**Table 3**). Mean total neutrophil concentration significantly increased 12 hours after the Mh inoculation (study day 6.5 compared to study day 6; **Table 3**). Conversely, mean total neutrophil concentration did not differ between study days 7.5, 9, 11, and 13. The mean ratio between total neutrophils and lymphocytes significantly increased from study day 6 to study day 6.5. On study day 6, the predominant cells were the lymphocytes, whereas on study day 6.5 (12 h after the Mh inoculation), the predominant cells were the neutrophils. During the rest of the study, the predominant cells continued to be neutrophils, except for study day 9, in which the predominant cells were the lymphocytes.

Associations between PCLD with LLD results, and with lung consolidation

Leukocyte, segmented neutrophil, and lymphocyte concentrations reported by the PCLD assay were significantly associated with leukocyte, total neutrophil, and lymphocyte concentrations measured by the LLD assay, respectively (**Table 4**). In addition, results from all 3 models included significant interaction terms, indicating that the associations for each the cell types (i.e., leukocyte, lymphocyte, or neutrophil) as recorded by the different assays significantly depended on the time of the measurement (**Table 4**).

Given that moribund animals were necropsied early, our sample size was reduced to 27 for evaluating the associations between blood parameters and lung consolidation. Mean

leukocyte concentrations (on the day of necropsy) reported by the LLD and the PCLD assays were not significantly associated ($p = 0.10$ and $p = 0.36$, respectively; **Figure 1**) with the proportion of lung consolidation. Similarly, segmented neutrophil and total neutrophil concentrations reported by the LLD and the PCLD assays, respectively, were not significantly associated ($p = 0.26$ and $p = 0.42$, respectively; **Figure 1**) with the proportion of lung consolidation. Conversely, band neutrophil concentration reported by the LLD, and lymphocyte concentrations and total neutrophil/lymphocyte ratio from both assays were significantly associated with the proportion of lung consolidation (all $p < 0.05$; **Figure 1 and 2**). Plasma protein levels were not significantly associated with the proportion of lung consolidation ($p = 0.14$), but fibrinogen levels were ($p = 0.03$; **Figure 2**). As lymphocyte concentrations (reported by both assays) increased, the proportion of lung consolidation decreased (all $p < 0.05$). Conversely, as band neutrophil and fibrinogen levels reported by the LLD assay and the total neutrophil/lymphocyte ratio obtained from both methods increased, the proportion of lung consolidation increased (all $p < 0.05$).

Discussion

This study illustrates how blood leukocyte differentials as measured by a point-of-care and laboratory-based assays vary during BRD's progression in a population of steers challenged with BHV-1 and Mh. Variations in the neutrophil and lymphocyte ratio were observed within hours after calves, already infected with BHV-1, were inoculated with Mh. Thus, changes in this ratio could contribute to strategies for assessing disease progression relative to viral and bacterial involvement. In addition, band neutrophil concentrations increased, whereas lymphocyte concentrations were low when animals were severely ill, and lung consolidation was high. Similarly, increasing fibrinogen concentrations were observed towards the end of the study and

were associated with high lung consolidation. Therefore, timely data on band neutrophil, lymphocyte, or fibrinogen concentrations could be useful, perhaps in conjunction with other clinical data, to enhance BRD case management relative to disease severity.

The mean total leukocyte concentrations were above the reference interval at study initiation, which could be attributed to the animal's age, as 6 to 7 month-old calves have higher leukocyte concentration than adult cattle[14], or to a catecholamine-induced response due to animal handling[17]. As the viral phase of the study (study day 1 to 6) progressed, panleukopenia (depression of all white blood cells) was observed. Similar to our results, other authors[15] found a slight decrease in leukocyte concentration by study day 4, after BHV-1 inoculation. In our study, leukocyte concentration increased 24 hours after the bacterial inoculation but decreased after 36 hours. Previous research[4,13] also has indicated an increase in leukocyte concentration 24 hours after a Mh inoculation. Cattle have a small reserve pool of neutrophils in their bone marrow[17]. Thus, in times of acute inflammation, the leukocyte count will transiently increase during the release of neutrophils from the reserve pool[17]. Once that storage pool is depleted, the leukocyte count will decrease as the tissue demand overwhelms the bone marrow's ability to respond, which is consistent with the leukocyte count decrease 36 hours after the start of the bacterial phase of the study (after study day 7.5).

Lymphopenia is commonly observed in acute inflammatory disease processes as experienced in BRD[14]. During our study, lymphocyte concentrations slightly decreased during the BHV-1 challenge, then significantly decreased 12 hours after bacterial infection, likely in response to the Mh inoculation on already immunosuppressed animals[14]. Possibly, in healthy calves, exposure to low doses of Mh may be eliminated naturally, however in sick calves, a large inoculation dose of Mh would produce an acute inflammatory process that explains the observed

lymphopenia[16]. Contrary to our results, another study[13] reported no differences in lymphocyte concentrations in Mh challenged animals; in their study, animals were only inoculated with Mh, and likely the disease process was not as acute or severe as the one observed after a BHV-1/Mh challenge.

Infections with BHV-1 cause inflammation in the upper respiratory tract and neutropenia is commonly observed 48 hours after an initial inflammatory process[14]. In our study, neutrophil concentration (segmented for the LLD and total for the PCLD) decreased at the end of the viral phase (study day 6) compared to healthy animals (day zero), which could be explained by the increase of upper-respiratory-tract inflammation due to the inoculation with BHV-1[2]. Likewise, others[15] found a decrease in neutrophil concentrations 4 days after inoculation with BHV-1. However, when the inflammatory process continues, such as when bacterial infection occurs, neutrophilia can also be observed[14]. Segmented neutrophils migrate into the lungs within hours of a bacterial insult[14], which could explain the peak of neutrophil concentration observed in the blood 12 hours after the Mh inoculation. Similarly, others[4,5] have found an increase in segmented neutrophils 24 hours after a Mh inoculation on animals previously exposed to bovine viral diarrhea virus.

Band neutrophils are released from the bone marrow when an animal experiences an acute inflammatory process, as observed in BRD's progression[14,19]. During the viral phase of our study (study days zero to 6), the percentage of animals with abnormal band neutrophil concentration was less than 3.0% but then increased to 59.0% 12 hours after animals were challenged with Mh. This sudden increase in band neutrophils concentration above the reference interval, which was sustained until study termination, could be attributed to the acute inflammatory process produced by the bacterial challenge. Contrary to our results, another study

found no differences over time in band neutrophil concentration in Mh challenged animals[13]. Thus, in our study, the inflammatory stimulus and tissue demand may have been sufficiently severe to utilize the storage pool of neutrophils and mobilize band neutrophils from the bone marrow[14], which may make these reliable indicators of disease progression.

Erythrocytes transport oxygen in the blood, and an increase in the number of erythrocytes frequently occurs in chronic respiratory diseases as part of a mechanism to compensate for respiratory failure[14]. However, there are apparently no changes in erythrocyte-related parameters upon the initial stages of BRD[13]. In our study, hematocrit and hemoglobin concentrations decreased during the viral infection (until study day 6) and then increased after the inoculation with Mh, but the mean values were never outside of the reference interval, likely preventing these parameters from being useful for diagnostic or prognostic decisions. Moreover, severely ill cattle also may eat and drink less, resulting in a higher hematocrit due to dehydration.

Large amounts of serofibrin are commonly found in the lungs of animals that died or were euthanized due to BRD [2,13], and hence, fibrinogen and total plasma proteins have been studied in detail as markers for acute response to inflammatory processes[14]. In our study, fibrinogen increased significantly throughout the study period, with the highest concentrations being observed, most notably after the Mh inoculation. Given that fibrinogen is a positive acute-phase protein, and its production is upregulated when inflammatory processes are generated, it is not surprising that fibrinogen increased during the bacterial phase of the study. Similarly, others[7,13] have found increasing fibrinogen values throughout their study when inoculating animals with *Mycoplasma bovis* or Mh. Plasma proteins in our study decreased from the start of the study to 36 hours after the Mh inoculation. The subsequent increase in plasma protein that occurred later in the study could be an indirect effect due to the increases in fibrinogen. Our

study results are similar to a previous study[12] and are consistent with an increase in the animals' inflammatory process due to BRD's progression. Therefore, fibrinogen and plasma proteins could be appropriate indicators for monitoring disease progression over time.

Leukocytes, lymphocytes, and neutrophils, as measured by PCLD, were positively and linearly associated with the corresponding blood parameters recorded by LLD. Although our study was not designed to compare the PCLD and LLD assays formally, the strong linear associations indicate that they provide similar results. The PCLD has the advantage of improving turnaround time by obtaining results within minutes, reducing logistical problems of shipping samples and having sick and healthy animals commingled while awaiting results. Similarly, reducing these logistical problems improves animal well-being by decreasing the time between diagnosis and treatment, increasing in turn, treatment success. However, these advantages need to be assessed with the cost/benefit to producers or veterinarians, which was beyond the scope of this study.

High lymphocyte concentrations were associated with low percentages of lung consolidation measured at necropsy, and as lung consolidation increased, lower concentrations of lymphocytes in blood were observed as they migrate to the lungs during disease progression. High band neutrophils were associated with high percentages of lung consolidation. Specifically, when band neutrophil concentration was within the reference interval, consolidation was significantly lower than when concentration exceeded that interval, which is consistent with a release of band neutrophils from the bone marrow due to the acute inflammatory process associated with lung consolidation[14]. Band neutrophils, however, were only reported by the LLD assay; PCLD does not differentiate segmented versus band neutrophils as this must be done by visual assessment of a blood smear, which might be a limitation given that segmented (reported

by the LLD) and total (reported by the PCLD) neutrophils were not associated with lung consolidation. Higher total neutrophil/lymphocyte ratios (reported by LLD and PCLD) and fibrinogen (reported by LLD) were associated with higher lung consolidation, which is consistent with increasingly acute inflammation and an accumulation of fibrin in the lungs. The fact that total neutrophils (reported by PCLD), segmented neutrophils (reported by LLD), leukocyte (reported by LLD and PCLD), and plasma proteins (reported by LLD) were not significantly associated with the percentage of lung consolidation may have been due to the limited sample size available for these analyses. Whenever possible, future studies should increase the number of animals in which both leukocyte differentials and lung consolidation scores are recorded.

Although researchers commonly use challenge studies to reproduce disease in a naïve population, they do not capture the variation that occurs under field conditions, compromising external validity. However, when the onset of the disease is hard to identify, which is the case for BRD, a challenge study can reduce misclassification bias and enable researchers to assess disease progression. Because hematological profiles of cattle can vary based on physiological (e.g., age, sex, diet, stress, reproductive status, hydration) and environmental characteristics (e.g., ambient temperature and wind), having study subjects with similarities in demographic factors and environmental exposures is advantageous to reduce unexplained variability and more accurately estimate the magnitude of associations.

In conclusion, the results of this study indicate that lymphocytes, total neutrophils, and neutrophil/lymphocyte ratios presented differences at the moment of the bacterial challenge, whereas band neutrophils and fibrinogen increased over the study time. These changes, together with their associations with lung consolidation, may be useful as indicators of BRD progression

by differentiating between viral and bacterial infections. Because changes in blood leukocyte differentials in cattle can be associated with diseases other than BRD, diagnostic or prognostic evaluations should consider hematological results in combination with clinical assessment and application of additional diagnostic procedures. Despite its lack of specificity, leukocyte differentials could contribute valuable information toward diagnosis and/or prognosis, and to decisions on interventions, such as administration of antimicrobial treatment based on disease progression. Except for a few assay differences (i.e., differentiation of band and segmented neutrophils, and assessment of plasma protein and fibrinogen by the LLD), the performance of both assays was comparable in terms of magnitude and direction of associations between blood cells recorded by either assay, over time and with respect to changes in lung consolidation. Further evaluation of the PCLD or similar systems seems appropriate given the relevance of BRD and the need for point-of-care diagnostic assays with a fast turnaround time to aid in early diagnosis and disease management.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Table 3.1. Model-adjusted means (and standard error of the mean) of clinical pathology parameters reported by a laboratory-based assay in calves inoculated with BHV-1 on day zero and *Mannheimia haemolytica* on day 6, by study day.

		Study day											
		0	1	2	4	6	6.5	7	7.5	9	11	13	p^3
Cell type[1], unit	Ref lim[2]	Model-adjusted mean (SEM)											
Leukocytes, k/µL	5.0-10.0	**11.9^{a}**	**11.9^{a}**	**10.5^{bd}**	**10.2^{bcd}**	9.4^{de}	9.1^{de}	**11.4^{ab}**	9.1^{df}	9.2^{abdf}	6.7^{cef}	6.2^{abdf}	<0.01
		(1.0)	(1.0)	(1.0)	(1.0)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.3)	
Lymphocytes, k/µL	2.5-7.5	5.5^{a}	4.7^{bc}	4.6^{ab}	4.8^{ac}	5.4^{ab}	2.7^{d}	3.5^{f}	3.1^{df}	2.6^{df}	2.5^{df}	2.9^{abdf}	<0.01
		(1.0)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.2)	
Seg neutrophils, k/µL	1.0-5.0	4.9^{ace}	**5.2^{ae}**	4.7^{ace}	4.0^{ac}	2.7^{b}	4.1^{acd}	**5.6^{e}**	4.1^{abd}	3.1^{bda}	2.0^{bc}	**0.8^{abe}**	<0.01
		(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.2)	(1.3)	(2.0)	
Neutrophils/lymphocyte	No ref	0.9^{ae}	1.1^{ace}	1.0^{ae}	0.8 ^{ae}	0.5^{b}	1.7^{cd}	1.8^{d}	1.4^{ade}	1.6^{de}	1.1^{abde}	0.5^{ab}	<0.01
		(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.2)	(1.2)	(1.3)	(1.4)	
Monocytes, k/µL	0.0-0.8	**1.1^{abdg}**	**1.3^{de}**	0.8^{cgh}	**1.0^{abdh}**	**0.9^{abdh}**	**1.3^{ebi}**	**1.1^{abdh}**	0.8^{ac}	0.8^{abhi}	0.3^{f}	0.6^{abfh}	<0.01
		(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.2)	(1.3)	
Hct (spun), %	26.0-42.0	35.4^{ai}	35.5^{ai}	34.4^{adi}	33.1^{beh}	32.8^{cdfg}	32.4^{cgh}	34.4^{aef}	34.9^{aef}	33.8^{efgi}	36.9^{ae}	38.4^{a}	<0.01
		(0.6)	(0.6)	(0.5)	(0.5)	(0.6)	(0.6)	(0.6)	(0.7)	(1.2)	(1.2)	(1.3)	
Hct calculated, %	24.0-46.0	32.3^{abc}	32.2^{ac}	31.1^{b}	29.9^{de}	29.7^{de}	28.7^{e}	30.5^{abd}	31.1^{abd}	31.2^{abde}	33.2^{abcd}	36.5^{c}	<0.01
		(0.5)	(0.5)	(0.5)	(0.4)	(0.5)	(0.5)	(0.6)	(0.6)	(1.2)	(1.3)	(1.4)	
Hemoglobin, g/dL	8.0-15.0	12.5^{ac}	12.3^{acd}	11.8^{be}	11.6^{be}	11.6^{bde}	11.3^{bf}	11.9^{ae}	12.1^{aeg}	12.1^{aefg}	12.8^{aeg}	13.3^{cg}	<0.01
		(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.4)	(0.5)	(0.4)	
Cell hemoglobin, g/dL	No ref	12.4^{ae}	12.2^{ae}	11.8^{bf}	11.4^{cdg}	11.4^{cf}	11.0^{d}	11.6^{afg}	11.9a^{fg}	11.9^{adfg}	12.7^{aefg}	13.6^{e}	<0.01
		(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)	(0.4)	(0.5)	(0.4)	
Fibrinogen, mg/dL	300-700	423.3^{d}	460.0^{cd}	443.3^{d}	453.3^{d}	553.3^{cb}	564.8^{cb}	609.9^{b}	576.5^{bc}	**759.7^{a}**	**814.6^{a}**	**868.2^{a}**	<0.01
		(25.5)	(25.5)	(25.5)	(25.5)	(25.5)	(27.5)	(27.6)	(30.3)	(30.2)	(36.6)	(38.6)	
Plasma protein, g/dL	7.0-9.0	7.6^{a}	7.4^{bcd}	7.5^{ab}	7.4^{bde}	7.2^{cf}	7.0^{ghi}	**6.9^{gi}**	**6.9^{hi}**	7.1^{ceg}	7.4^{acej}	7.6^{adf}	<0.01
		(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	

[1]Seg neutrophils=Segmented neutrophils, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, Hct=Hematocrit, Cell hemoglobin=Cellular hemoglobin.
[2]Reference intervals, as provided by the Kansas State Veterinary Diagnostic Laboratory.
[3]Indicates statistical difference among study days.
Different letter superscripts within a row indicate statistically significant differences ($p < 0.05$).
Bold values indicate concentrations outside of the reference intervals.

Table 3.2. Model-adjusted probabilities (and standard errors) of animals presenting basophil and band neutrophil concentrations outside of the reference limits reported by a laboratory-based assay in calves inoculated with BHV-1 on day zero and *Mannheimia haemolytica* on day 6, by study day.

	Study day											
	0	1	2	4	6	6.5	7	7.5	9	11	13	p[1]
Cell type	Mean-adjusted probability (SE)											
Basophils outside vs. within reference limits[2]	0.20	0.26	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33
	(0.07)	(0.08)	(0.06)	-	-	-	-	-	-	-	-	
Band neutrophils outside vs within reference limits[3]	0.00	0.00	0.00	0.00	0.03 [a]	0.59 [b]	0.79 [b]	0.46 [ab]	0.85 [b]	0.76 [b]	0.42 [ab]	<0.01
	-	-	-	-	(0.03)	(0.10)	(0.08)	(0.11)	(0.08)	(0.12)	(0.21)	

[1]Indicates statistical difference among study days.
[2]Basophils outside of reference limits (≥0.2 k/µL) vs. within reference limits (<0.2 k/µL).
[3]Band neutrophils outside of reference limits (≥0.2 k/µL) vs. within reference limits (<0.2 k/µL).
Different letter superscripts within a row indicate statistically significant differences ($p < 0.05$).

Table 3.3. Model-adjusted means (and standard error of the means) of blood leukocyte differentials measured by the point-of-care leukocyte differential assay in calves inoculated with BHV-1 on day zero and *Mannheimia haemolytica* on day 6, by study day.

	Study day											
	0	1	2	4	6	6.5	7	7.5	9	11	13	p^1
Cell type, unit	Model-adjusted mean (SEM)											
Leukocytes, k/µL	14.1^{a}	14.2^{ag}	12.9^{ae}	11.9^{beg}	10.7^{df}	10.4^{bdfg}	13.6^{ae}	10.6^{bcd}	10.8^{ade}	7.9^{cf}	7.7^{adef}	<0.01
	(1.0)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.3)	
Lymphocytes, k/µL	7.0^{a}	6.8^{a}	6.6^{ac}	6.4^{ac}	6.1^{ac}	4.5^{b}	5.3^{cd}	3.9^{e}	3.9^{be}	2.4^{f}	2.8^{bdef}	<0.01
	(1.1)	(1.1)	(1.1)	(1.0)	(1.0)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.3)	
Total neutrophils, k/µL	6.7^{ad}	6.8^{af}	5.6^{bd}	4.7^{b}	3.9^{c}	5.2^{ab}	7.4^{df}	5.9^{abd}	6.2^{abd}	4.9^{abcd}	3.6^{abcd}	<0.01
	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.2)	(1.3)	
Neutrophil/lymphocyte	1.0^{ab}	1.0^{abf}	0.9^{ac}	0.7^{c}	0.6^{h}	1.4^{bd}	1.6^{de}	2.0^{def}	0.9^{dg}	1.2^{eg}	1.5^{abcf}	<0.01
	(1.1)	(1.1)	(1.1)	(1.0)	(1.0)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	(1.1)	

[1]Indicates statistical difference among study days.
Different letter superscripts within a row indicate statistically significant differences ($p < 0.05$).

Table 3.4. Model-adjusted means (k/µL) and standard error of the mean (SEM) for the 3 models evaluating the associations between 1) leukocytes, 2) neutrophils, and 3) lymphocytes reported by a point-of-care leukocyte differential (PCLD) and a laboratory-based leukocyte differential (LLD; outcome) assays while accounting for study day and an interaction between its respective cell count and study day. Calves were inoculated with BHV-1 on day zero and *Mannheimia haemolytica* on day 6.

LLD-Leukocytes					LLD-Neutrophils					LLD-Lymphocytes				
		Leuk Mean	Leuk SEM	p^1			Neut Mean	Neut SEM	p^1			Lymph Mean	Lymph SEM	p^1
PCLD-Leuk[2]		1.1	1.0	<0.01	PCLD-Neut[2]		1.2	1.0	<0.01	PCDL-Lymph[2]		1.1	1.0	<0.01
Study day	0	Ref	Ref	<0.01	Study day	0	Ref	Ref	<0.01	Study day	0	Ref	Ref	<0.01
	1	1.0	1.1			1	1.5	1.2			1	0.8	1.2	
	2	0.9	1.1			2	1.9	1.2			2	0.6	1.2	
	4	0.9	1.1			4	1.0	1.2			4	0.6	1.2	
	6	0.9	1.1			6	0.9	1.3			6	0.8	1.2	
	6.5	1.0	1.1			6.5	1.3	1.2			6.5	0.4	1.3	
	7	1.3	1.1			7	2.0	1.2			7	0.7	1.2	
	7.5	0.9	1.1			7.5	1.4	1.2			7.5	0.9	1.3	
	9	0.8	1.1			9	0.7	1.5			9	0.4	1.3	
	11	0.6	1.1			11	0.5	1.5			11	0.4	1.3	
	13	0.4	1.2			13	0.0	2.9			13	0.3	1.4	
PCLD-Leuk*day	0	Ref	Ref	<0.01	PCLD-Neut*day	0	Ref	Ref	<0.01	PCLD-Lymph*day	0	Ref	Ref	0.04
Leuk.	1	1.0	1.0		Neut.	1	0.9	1.0		Lymph.	1	1.0	1.0	
	2	1.0	1.0		Neut.	2	0.9	1.0		Lymph.	2	1.1	1.0	
Leuk.	4	1.0	1.0		Neut.	4	1.0	1.0		Lymph.	4	1.1	1.0	
Leuk.	6	1.0	1.0		Neut.	6	1.0	1.0		Lymph.	6	1.1	1.0	
Leuk.	6.5	1.0	1.0		Neut.	6.5	1.0	1.0		Lymph.	6.5	1.1	1.0	
Leuk.	7	1.0	1.0		Neut.	7	0.9	1.0		Lymph.	7	1.0	1.0	
Leuk.	7.5	1.0	1.0		Neut.	7.5	0.9	1.0		Lymph.	7.5	1.0	1.1	
Leuk.	9	1.0	1.0		Neut.	9	1.0	1.1		Lymph.	9	1.1	1.1	
Leuk.	11	1.0	1.0		Neut.	11	1.0	1.1		Lymph.	11	1.1	1.1	
Leuk.	13	1.1	1.0		Neut.	13	2.3	1.4		Lymph.	13	1.2	1.1	

[1]Indicates statistical significance of a variable
[2]Fixed effects included in the model as continuous. Leuk= Leukocytes (k/µL), Neut= Neutrophils (k/µL), Lymph=Lymphocytes (k/µL).
Ref=Reference category.

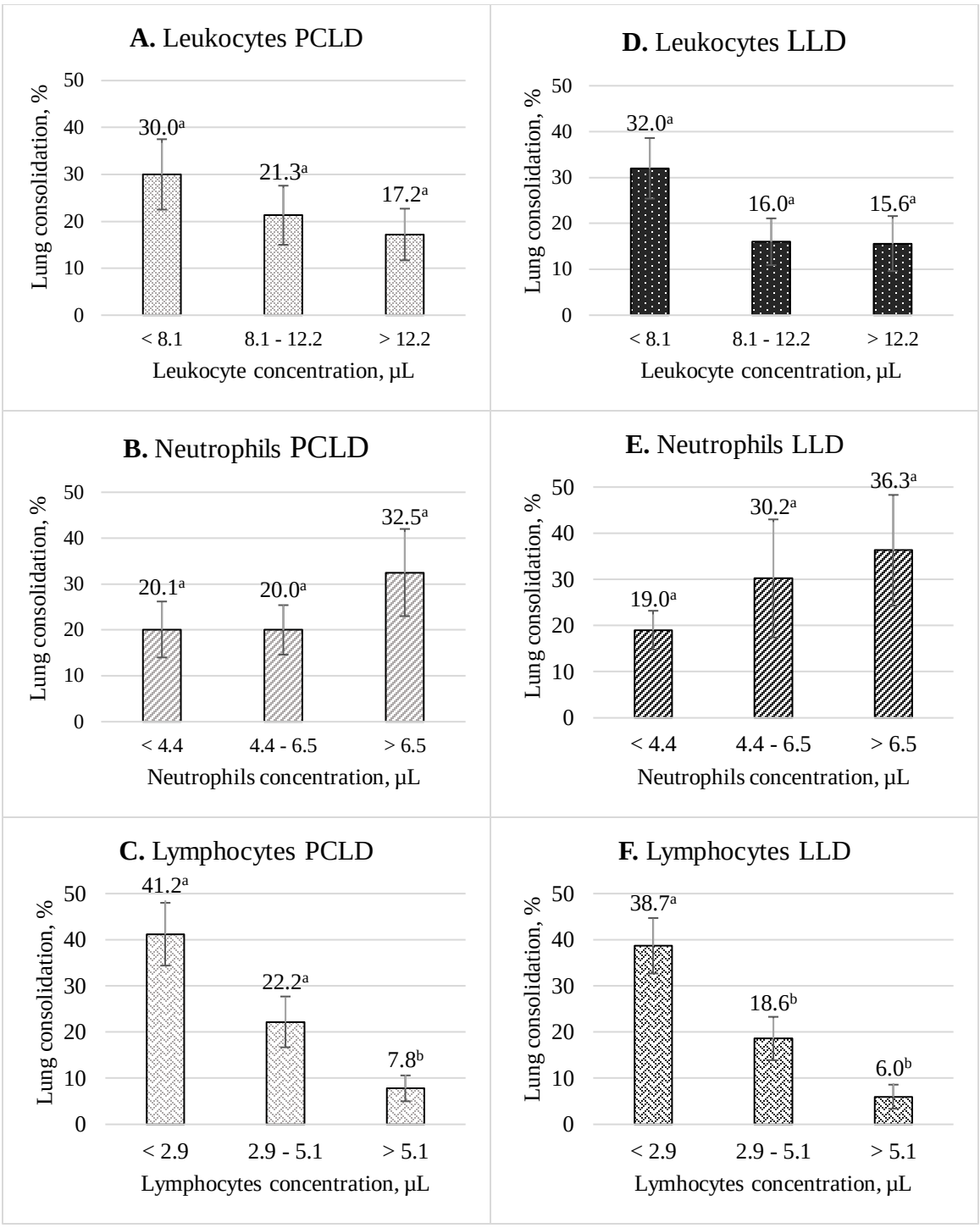

Figure 3.1. Graphs depicting associations between blood leukocyte differentials measured by a laboratory-based blood leukocyte differential (LLD) and a point-of-care blood leukocyte differential (PCLD) assays with lung consolidation, in %, measured during necropsy.

A. Leukocytes concentration from PCLD, **B.** Total neutrophils concentration from PCLD, **C.** Lymphocyte concentration from PCLD, **D.** Leukocytes concentration from LLD, E. Segmented neutrophils concentration from LLD, and F. Lymphocyte concentration from the LLD. Error bars represent standard error of the mean. Different letter superscripts within a graph indicate statistically significant differences ($p < 0.05$).

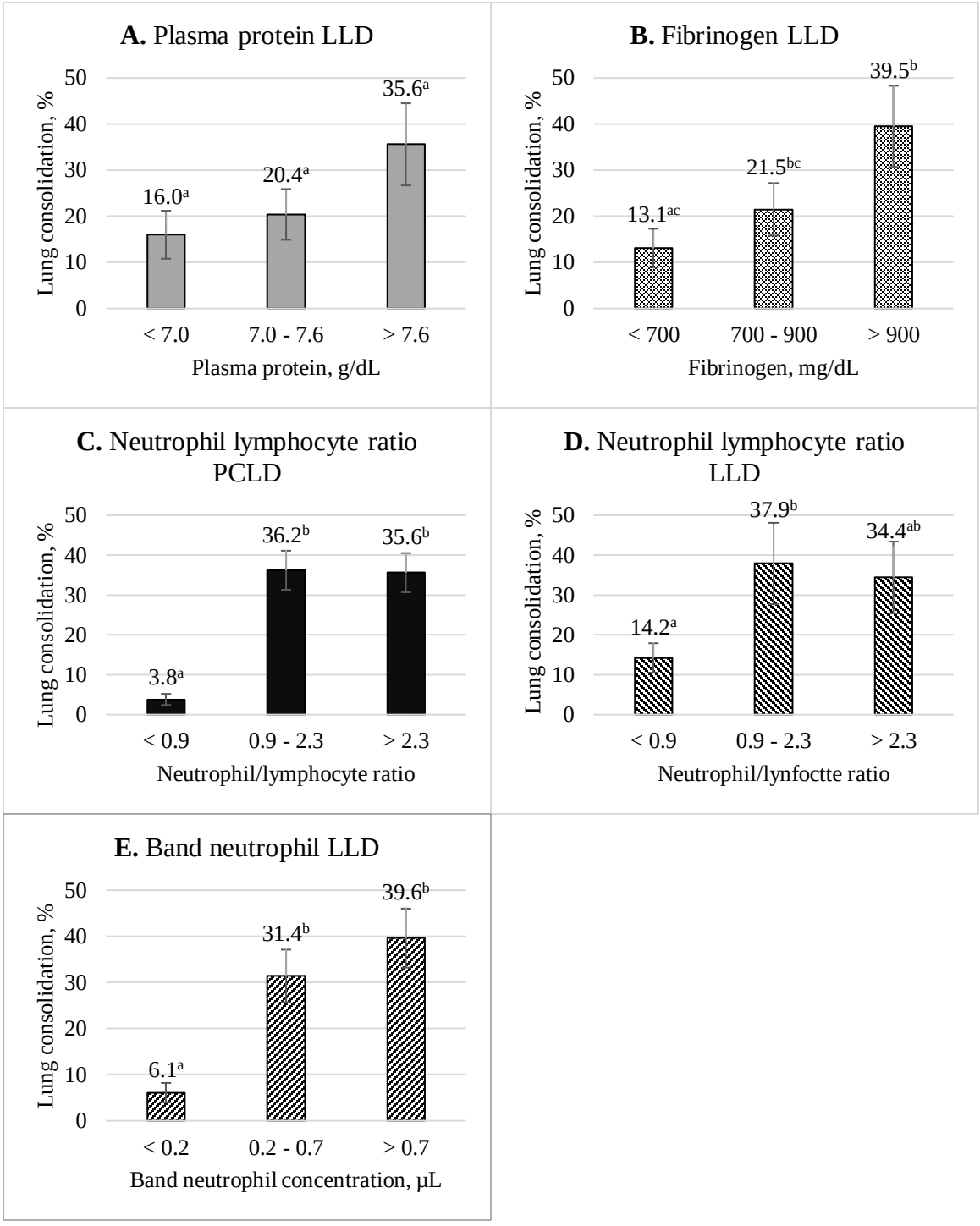

Figure 3.2. Graphs depicting associations between blood parameters measured by a laboratory-based blood leukocyte differential (LLD) and a point-of-care blood leukocyte differential (PCLD) assays with lung consolidation, in %, measured during necropsy.

A. Plasma proteins from LLD, **B.** Fibrinogen from LLD, **C.** Neutrophil/lymphocyte ratio from PCLD, **D.** Neutrophil/lymphocyte ratio from LLD, and E. Band neutrophil concentration from LLD. Error bars represent standard error of the mean. Different letter superscripts within a graph indicate statistically significant differences ($p < 0.05$).

Chapter 4 - **Mapping risk factors for bovine respiratory disease in beef cattle: a scoping review**

Manuscript prepared as per Animal Health Research Reviews guidelines.

Abstract

Bovine respiratory disease (BRD) has multiple infectious agents and risk factors contributing to its complexity. Scoping reviews are structured literature reviews used to map the availability of data on a broad subject and to identify research priorities and data gaps. Our objective was to use a scoping review approach to map relevant literature on risk factors for BRD morbidity in beef cattle, identify research gaps, and determine potential areas amenable for conducting a systematic review. Our research question was, "What quantitative data on risk factors are available for BRD morbidity in beef cattle worldwide?" A search was performed on Agricola, Pubmed, CAB, and Scopus databases in April 2017. While no restriction for publication year or country were applied, exclusions were included for diseases other than BRD, risk factors for BRD outcomes other than morbidity, challenge studies, studies conducted on dairy, veal calves, or bulls and publications written in languages other than English. From the 2,213 publications obtained, 141 publications pertained to risk factors for BRD morbidity. Publications from which data were extracted included observational and experimental studies published between 1970 and 2017, with 2008 as the median year of publication. Ninety-three percent of the publications were from studies conducted in the United States, Canada, or Australia, with 81% of the studies reporting BRD morbidity in feedlot operations. Key risk factors associated with BRD morbidity identified in our review included metaphylaxis, vaccination, preconditioning programs, and dietary supplements. Other well-known risk factors, such as shipping distance, sex, season, commingling, and initial body weight, were not commonly reported. Undertaking this scoping

review allowed us to map relevant literature on risk factors for BRD morbidity, which, in turn, will help us streamline research efforts by prioritizing areas where information is lacking, and conducting systematic reviews and meta-analyses in key research themes.

Keywords. Bovine respiratory disease, risk factors, scoping review.

Introduction

Although a significant disease of cattle globally, in North America alone, bovine respiratory disease (BRD) costs the beef industry one billion dollars annually (Griffin, 1997). Even though risk factors for BRD have been studied for several decades and the technology for disease detection and management has improved, some authors report that BRD's incidence might be increasing rather than decreasing (Loneragan *et al.*, 2001; Miles, 2009).

Risk factors for BRD have been previously described using traditional literature reviews (Taylor *et al.*, 2010); however, this methodology does not follow a systematic and replicable approach, leading to potentially biased and non-reproducible results. Systematic reviews and meta-analysis have been conducted to evaluate the effect of antibiotic use in feedlot cattle (O'Connor *et al.*, 2013; Abell *et al.*, 2016) or the effect of different types of vaccination on BRD morbidity (Theurer, Larson and White, 2015; Snyder, Credille and Heins, 2019). A scoping review is another review method that aims to map key themes in a research area that is complex in nature or has not been explored in detail by conducting a structured review of available evidence (Arkesey and O'Malley, 2005). To date, no scoping review has been conducted to map and understand the extent of literature regarding risk factors for BRD morbidity.

A better understanding of the impact of risk factors on disease morbidity could aid veterinarians in providing recommendations to farmers regarding best practices to reduce BRD morbidity. Decreasing BRD morbidity will help decrease its economic burden, improve animal welfare, and favor antimicrobial stewardship. Directing our research efforts towards areas where knowledge is lacking, and for those areas in which there is sufficient information, synthesizing

the available body of evidence through systematic reviews and meta-analysis will help us reduce the burden of BRD through evidence-based practice

The objective of the present study was to use a scoping review approach to map relevant literature on risk factors for BRD morbidity in beef cattle, identify research gaps, and determine potential areas amenable for conducting a systematic review.

Materials and methods

This scoping review was conducted using guidelines described elsewhere (Arkesey and O'Malley, 2005; Daudt, Van Mossel and Scott, 2013). A three-fold approach was followed to identify: 1) the extent of relevant research, 2) possible areas where sufficient information exists, making them amenable to conducting full systematic reviews, and 3) gaps in the literature, concerning risk factors for BRD morbidity in beef cattle.

Research team, question, and search strategy

The team consisted of three veterinary epidemiologists with ample experience in BRD research and research synthesis methods and a graduate student in epidemiology. The research question, and its PICO elements, consisted of: "what quantitative data on risk factors (Intervention) are available for BRD morbidity (Outcome) in beef cattle (Population) worldwide?" Risk factors were compared (Comparison) to controls or other risk categories established as the reference. Databases were screened using "BRD or BRDC or Bovine Respiratory Disease" and "Beef Cattle" search terms, choosing the combination that produced the greatest number of publications. Four electronic databases were searched (PubMed, Agricola, CAB, and Scopus), with search restrictions (academic journals and English language) set for all databases except PubMed. Final searches were conducted on April 8th, 2017. All citations were

exported to a citation manager software (RefWorks, Ann Arbor, Michigan, USA) and, after that, exported to excel where duplicates were removed manually.

Inclusion-exclusion criteria and relevance screening

Relevance screening of abstracts and full publications was conducted using the criteria depicted in **Table 1**. To calibrate and standardize the screening process, two reviewers (NC and JB) performed the relevance screening of 30 randomly selected abstracts. After the screening of those abstracts, a Kappa statistic was calculated to determine their agreement beyond chance for retaining abstracts for further data extraction. The Kappa statistic between reviewers was determined to be 0.74 and deemed substantial. Upon calibration of the screening tool, one reviewer (JB) proceeded to screen all abstracts, followed by weekly discussions with a second reviewer (NC) to resolve potential conflicts.

Data characterization and synthesis of results

A data extraction tool was created and tested with four full-text publications. After calibration, and to maintain consistency, a single reviewer (JB) proceeded to extract data from all relevant full-text publications. Data extracted included:

(1) Study characteristics: publication year, country, production stage (cow-calf, backgrounding, feedlot), study setting (commercial, research), study design,

(2) Risk factors studied (**Table 2**), and

(3) Outcomes: BRD morbidity (number of animals experiencing BRD—publication defined case definition—over the total number of animals), BRD mortality (number of BRD-associated deaths over the total number of animals), overall mortality (number of all-cause deaths over the total number of animals), retreatment (number of BRD cases that required subsequent treatment

for BRD over the total number of BRD cases), average daily gain, gain to feed ratio, dry matter intake, hot carcass weight, and days on feed.

Risk factors were categorized based on common characteristics to identify research themes. For example, distance traveled from sale barn to feedlot and placement within a transportation truck were categorized as transportation. For those research themes with the largest number of publications, publication year, study setting, study design, and health and performance outcomes reported were described in detail. Likewise, because a publication may have more than one study, and a study has more than one randomization group, treatment arms were extracted as "groups."

Results

Search results and study characteristics

A total of 2,213 citations were obtained after the initial search (Pubmed = 381, Agricola = 258, CAB = 929, and Scopus = 645), from which 588 were duplicates (**Fig. 1**). After duplicate removal and relevance screening of the abstracts, 274 publications were deemed appropriate for full publication screening, of which 141 were considered for data extraction (**Fig. 1**). Year of publication ranged from 1970 to 2017, the median was 2008, and 90% of the publications were published after 1993. Although some publications were from studies conducted in Europe and South America, the majority of publications were from studies conducted in the United States, followed by Canada and Australia (**Table 3**). Most publications reported BRD morbidity after feedlot arrival and/or during the backgrounding period. Whereas most studies were conducted in commercial or research feedlots, non-feedlot research facilities were also commonly used (**Table 3**).

Though the majority of publications pertained to experimental studies (**Table 4**), observational study designs were also common, and most of them pertained to cohort studies (**Table 4**). Secondary outcomes regarding overall mortality, BRD-specific mortality, and retreatment were commonly reported (**Table 4**). Except for days on feed and hot carcass weight, other performance outcomes were also commonly reported (**Table 4**).

In total, 17 different BRD morbidity case definitions were reported in the 141 publications. Most of these differences, however, were attributed to slight variations in cut-points used for rectal temperature. When those slight temperature differences are ignored, 65.4% of publications reported a case definition that used a combination of clinical signs and rectal temperature (**Table 5**). Fifteen percent of the publications did not use rectal temperature as part of their case definitions, relying only on clinical signs (**Table 5**). In addition to these variations, 13.4% of the publications did not provide details regarding a BRD case definition, and 5.6% reported using a feedlot or veterinarian definition but did not provide details (**Table 5**).

Risk factors

Although 21 risk factor themes were identified in the 141 publications (**Table 2**), metaphylaxis, vaccination, dietary supplements, and preconditioning programs represented 83.0% of publications (**Table 2**). Other risk factors, collectively reported on 25.5% of publications, included age or body weight, sex, genetic traits, hereditability, breed, and dam reproduction management practices (**Table 2**).

Metaphylaxis

The number of publications reporting on the association between metaphylaxis use with BRD morbidity doubled in the period 2007 to 2017 compared to 1997 to 2007. These studies primarily consisted of controlled trials (95.0%), where BRD morbidity was reported after feedlot

arrival (83.0%). In addition to BRD morbidity, the majority of publications reported overall mortality, BRD mortality, and retreatment rates (**Fig. 2**). Performance outcomes were commonly reported in those publications related to metaphylaxis, with average daily gain and gain-to-feed ratio being the most common (**Fig. 2**). The most commonly reported metaphylactic antibiotics were tilmicosin, tulathromycin, and oxytetracycline (**Table 6**).

Vaccination

Over time, publications related to vaccination programs for BRD morbidity have been increasing. Ninety percent of studies reporting the association between vaccination and BRD morbidity were experimental, while 10.0% were observational. Thirty-six percent of the publications reported BRD morbidity before feedlot arrival and 64% after arrival. Whereas BRD mortality and overall mortality were commonly reported in association with vaccine programs, performance outcomes were not (**Fig. 2**). A 5-way vaccine (including a combination of infectious bovine rhinotracheitis (IBR), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea (BVD) type I and II, and parainfluenza type-3 (PI3)) was the most commonly reported, followed by BRSV and *Mannheimia haemolytica* (**Table 7**). However, from the 13 groups in which the use of a 5-way vaccine was studied, only five publications had exclusively used a 5-way vaccine upon arrival (Duff *et al.*, 2000; O'Connor *et al.*, 2001; Macgregor *et al.*, 2003; Seeger *et al.*, 2008; Richeson *et al.*, 2015). Therefore, most of the available data on the association of 5-way vaccines and BRD morbidity could potentially be confounded with other BRD vaccines. BRSV and *Mannheimia haemolytica* vaccines were common in the literature in past decades, whereas 84.6% of the publications related to 5-way vaccines were published in the last ten years.

Dietary supplements

Dietary supplements include a wide array of interventions, which can be added to the diet or administered systemically (i.e., injectable vitamins), and were grouped by common characteristics (**Table 8**). Publications related to the association between uses of dietary supplements and BRD morbidity have doubled in the last ten years compared to before 2007. Eighty-five percent of the publications pertained to experimental studies, with the majority (85.7%) of the publications reporting BRD morbidity after feedlot arrival. Whereas overall mortality was reported commonly, BRD specific mortality was not (**Fig. 2**). Average daily gain and gain-to-feed ratio were commonly reported outcomes, whereas days on feed and hot carcass weight were not. Minerals added to the diet, feed components, and feed additives were the most commonly studied (**Table 8**).

Preconditioning programs

Publications on preconditioning programs included weaning, castration, bunk broke (i.e., allowing animals to eat concentrated feed before feedlot arrival), vaccination, dehorning, or their combination (**Table 9**); 72.2% of these publications were published in the last 20 years. Weaning and castration were included in most of the programs reported (**Table 9**). Most publications reported BRD morbidity after feedlot arrival, but some studies followed animals from backgrounding to the feedlot (reporting BRD morbidity in both). Out of the 18 publications related to preconditioning programs, 30% reported BRD morbidity before feedlot arrival and 88.8% after feedlot arrival. From these 18 publications, health outcomes other than BRD morbidity were not commonly reported, but dry matter intake and average daily gain were reported in most publications (**Fig. 2**).

Discussion

This scoping review mapped published literature on risk factors for BRD morbidity in a structured, and unbiased way. Publications on metaphylaxis were the most common, followed by vaccination, dietary supplements, and preconditioning. These findings suggest that, frequently, publications focus on antimicrobial strategies or combinations of vaccines, immunity enhancers, and stress-reducing methods for BRD prevention.

Not surprisingly, most publications pertained to studies conducted in North America and Australia, which could be attributed to BRD's impact on the North American and Australian feedlot cattle industry (Griffin, 1997). This geographic distribution, in part, may also reflect our exclusion criteria, as studies published in languages other than English were excluded. Brazil, Argentina, and Mexico also have large feedlot operations, and it seems plausible that some publications may have been available but excluded if they were published in Portuguese or Spanish. Likewise, among beef cattle, feedlot cattle are affected the most by BRD, which explains why most publications pertained to studies reporting morbidity after feedlot arrival. In this review, experimental studies, which provide a greater quality of evidence than observational studies, were commonly reported. This result reflects the risk factors with the largest number of publications, as metaphylaxis and vaccination protocols can be randomized easily and require randomized control trials for approval. Despite experimental studies' higher level of evidence, observational studies may play a key role in studying risk factors in which funding is less commonly available (Sanderson, Dargatz and Wagner, 2008; Cernicchiaro *et al.*, 2012a, 2012b).

Metaphylaxis use was the most studied risk factor for BRD, which is consistent with its effectiveness for BRD morbidity reduction and its widespread use by the feedlot industry (O'Connor *et al.*, 2019b). According to the National Animal Health Monitoring System

(NAHMS) (United States Department of Agriculture, 2013), 93% of feedlots with more than 8,000 animals use this intervention on at least some cattle, which in turn may motivate the private sector's funding sources to conduct studies related to metaphylaxis. Tilmicosin and tulathromycin were the most commonly studied metaphylactic antibiotics, which aligns with the time that these antimicrobials have been available and previous studies of effectiveness (O'Connor *et al.*, 2019b). A systematic review of metaphylaxis drugs has already been conducted, and given the increasing volume of publications on the subject, an update may be warranted as new studies become available.

In metaphylaxis studies, commonly reported health-related outcomes, were primarily overall mortality rather than BRD specific mortality, which may be due to the lack of resources or expertise to perform post-mortem diagnoses. Overall mortality, however, might not be an appropriate proxy for BRD mortality, and when available, BRD specific mortality should be reported. Retreatment was also reported, perhaps because the response to treatment has important implications for animal welfare, economics, and judicious use of antimicrobials. Besides average daily gain in body weight, performance outcomes were not commonly reported. Other performance outcomes may be more logistically difficult to measure in some settings; for example, hot carcass weight requires following animals to slaughter, and feed to gain is often only measurable with pen-level studies.

According to NAHMS, approximately 96% of feedlots with more than 1,000 animals use some type of vaccination on at least some cattle upon arrival to the feedlot (United States Department of Agriculture, 2013), which might explain why vaccines have been studied extensively. Readily available funding from the private sector, and/or the fact that studies of non-antimicrobial practices for the reduction of BRD morbidity may be on the rise (Wisener *et al.*,

2019), may explain why vaccine publications studies were commonly found. Besides BRD morbidity prevention, some vaccines claim a reduction in BRD severity, which could potentially be assessed by a reduction in mortality. It is unclear why performance outcomes other than average daily gain were not commonly reported, as these outcomes could indicate an economic advantage to the adoption of vaccines. Consistent with its widespread use in feedlots, viral vaccinations were the most commonly reported; however, according to a recent meta-analysis, there might be limitations in their efficacy (O'Connor *et al.*, 2019b). Although many vaccination studies were found, little replication of specific treatments was observed, which can be attributed to the number of possible vaccine and vaccination-timing combinations available. Although there are other reviews on this topic (Theurer, Larson and White, 2015; O'Connor *et al.*, 2019a), this topic seems amenable to conducting a systematic review given the number of publications.

Publications related to preconditioning programs, an intervention that has been recommended for decades (Woods, Mansfield and Webb, 1973; Wieringa, Curtis and Willoughby, 1976), were commonly reported in this review and appear to be on the rise. This result may suggest that there is an increasing interest in understanding the pre-arrival issues that may have an impact on BRD morbidity. Although these programs are not new, the growing regulatory pressure regarding the use of antimicrobials may increase the relevance of these publications.

Health outcomes other than BRD morbidity were not commonly reported in association with preconditioning, which is surprising given the impact that some of these programs may have on improving animal health in general. Conversely, performance-related outcomes were reported commonly, perhaps because weaning, castration, and being trained to eat from a feed bunk may provide an extra economic incentive to feedlot producers. Variations in the type of

program used can be easily attributed to the lack of a standardized definition of preconditioning (Woods, Mansfield and Webb, 1973). Preconditioning programs are a topic amenable for systematic reviews. However, few replications of combinations of specific treatment characteristics (treatment-specific replication) might prevent a quantitative assessment.

Similar to preconditioning, publications on dietary supplements are on the rise in recent years. This result may be attributed to their potential to increase performance outcomes while potentially reducing BRD. Performance outcomes have a substantial weight in the overall decision making of a feedlot producer. There could be circumstances in which BRD morbidity is not significantly different between two interventions, but the intervention's cost might be justified if it improves performance. Few treatment-specific replications were observed, which may limit the possibility of conducting a systematic review on this topic.

Data gaps related to transportation, sex, cohort size, feedlot structure characteristics (feedlot size, number of water troughs, number of adjacent pens), BVD persistently infected (PI) animals, and commingling were identified through this scoping review. This result is not surprising given the complexity of studying some of these risk factors. Given the lack of a product to be marketed, sources of funding for these factors may be scarce compared to funding available to support vaccination or metaphylaxis research. Despite transportation being investigated for many years (Ribble, Meek, Shewen, *et al.*, 1995; Warriss *et al.*, 1995), few publications were identified in our study. Further, several factors are considered within transportation including distance traveled (Sanderson, Dargatz and Wagner, 2008; Cernicchiaro *et al.*, 2012a), truck placement (White *et al.*, 2009; Wahrmund *et al.*, 2012), or body weight loss during transport (Cernicchiaro *et al.*, 2012b).

Publications in the area of vaccines for BVD prevention were common, as 96.6% of feedlots with more than 1,000 animals use them on at least some of their animals (United States Department of Agriculture, 2013). However, the association between exposure to BVD-PI animals in a cohort with BRD morbidity was only reported in a few publications. Since BVD has been identified as a risk factor for BRD for many years (Richer, Marois and Lamontagne, 1988), it is surprising that few risk factor studies were found, and yet most vaccination studies used some type of BVD vaccine. These few studies would mean that most efforts have been oriented towards prevention with vaccination rather than understanding BVD's association with BRD. These results may be related to publication bias, given the potential lack of motivation for veterinarians and feedlot operators to study risk factors that have no market value. These areas of research with data gaps could benefit from more publications in order to understand how non-antimicrobial related risk factors could aid on BRD prevention.

A search of the grey literature was not conducted, and a language-related exclusion criterion was applied, which might have biased the number of publications obtained. Although BRD is an important problem in the dairy industry (Guterbock, 2014), our study focused on risk factors for BRD in beef cattle only, as there may be several differences in management practices between production types that warrant their separation. Similarly, challenge studies were excluded. Challenge studies tend to overestimate the efficacy of some interventions and may not be an accurate representation of field disease or intervention effectiveness (Theurer, Larson and White, 2015). Some studies, which were not included in this review, were related to BRD but only reported mortality outcomes (Martin *et al.*, 1980, 1981, 1982; Ribble, Meek, Janzen, *et al.*, 1995; Ribble, Meek, Jim, *et al.*, 1995), blood parameters (Burke *et al.*, 2009; Eitam *et al.*, 2010),

and changes in antibody titers for BRD-pathogens (Lehmkuhl and Gough, 1977; Solis-Calderon *et al.*, 2007), which did not meet our inclusion criteria.

Risk factors for BRD were mapped, and data gaps and potential areas amenable for conducting systematic reviews were identified through this scoping review. Metaphylaxis, vaccination, dietary supplements, and preconditioning were commonly published and amenable for conducting systematic reviews. Conversely, areas of research related to animal management were not commonly published and seem to warrant further research. Continuous mapping of risk factors for BRD will enhance the use of available resources to conduct research in a targeted way. Targeted research is needed to develop more quantitative assessments of risk to aid the progress on risk management of BRD morbidity in beef cattle.

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Table 4.1. Inclusion and exclusion criteria for a scoping review of risk factors for bovine respiratory disease (BRD) morbidity.

Criteria	Inclusion	Exclusion
Population		
Species	*Bos taurus*, cattle	*Bos indicus*, species other than cattle
Production type/stage	Feedlot, backgrounding, pre-feedlot arrival, sale-barn, feedlot arrival, finishing/feeder cattle Commercial and research settings	Veal and dairy
Sex	Steers, heifer, and bulls castrated at feedlot arrival	None
Age	Any	None
Body weight	Any	None
Breed	Beef breeds and beef crosses	Dairy breeds
Intervention		
	Risk factors/exposures for BRD: weaning, commingling, bunk broke, preconditioning, body weight, origin, vaccination, dehorning, sex, castration, weather, shrink, transportation distance, shipping duration, metaphylaxis, ration, environment, and management	None
Outcomes		
	Health outcomes: BRD morbidity, BRD mortality, overall mortality, retreatment Performance outcomes: days on feed, gain to feed ratio, average daily gain, dry matter intake	Studies not reporting BRD morbidity
Other		
Study design	Experimental and observational studies reporting naturally occurring BRD	Challenge trials, laboratory studies, and *in vitro* studies
Publication type	Peer-reviewed original research articles	Non-peer-reviewed articles, conference proceedings, and review articles
Publication year	Any	None
Region/Country	Worldwide	None
Language	English	Other than English

Table 4.2. Frequency distribution of risk factors reported from a scoping review of risk factors for bovine respiratory disease (BRD) morbidity.

Risk factor	Description	N[1]	%[2]
Metaphylaxis	Mass administration of antimicrobials to a group of cattle in a prophylactic way	41	29.1
Vaccination	Vaccination programs for BRD prevention that are not part of a preconditioning program	30	22.0
Dietary supplements[3]	Diet components, feeding programs, time on feed, vitamins, and supplemental minerals	27	19.1
Preconditioning	Combinations of pre-feedlot arrival vaccines, castration, weaning, and ration	18	12.8
Age & body weight	Age or body weight categories and their associations with BRD	8	5.7
Sex	Individual animal sex or pen sex (steers, heifers, mixed-sex pen)	8	5.7
Genetics	Genetic traits and hereditability associated with BRD	8	5.7
Breed	Different breeds and their association with BRD	6	4.3
Cow-calf management[4]	Cow-calf reproduction and farm management (i.e., dams and calves)	6	6.4
Transport	Risk factors related to the transportation of animals	5	3.5
Weather	Weather/season related factors (time of the year, wind speed, temperature)	5	3.5
BVD-PI[5]	BVD-PI in cohort, exposure, and adjacent pen	4	2.8
Cohort size	Number of animals that are purchased together but not necessarily placed in the same pen	4	2.8
Implant	Use of hormonal implants	4	2.8
Castration	Castration timing, but not as a part of a preconditioning program	3	2.1
Feedlot and pen characteristics	Feedlot size and pen characteristics (e.g., number of water troughs)	3	2.1
Mixing of animals	Mixing of animals at purchase or when arriving at the feedlot (commingling)	3	2.1
Source of origin	Animal's place of origin (states within the United States or Canada)	3	2.1
Risk code	An ordinal category system used by feedlot personal to classify BRD morbidity risk	2	1.4
Other	Horned animals, arrival year, feedlot as the primary source of farmer's income (yes vs. no), and pathogens isolated at arrival	8	5.7

[1]N = number of publications.
[2]Percentage of publications.
[3]Administered through feed or systemically.
[4]Cow-calf management: reproduction practices (n = 4), farm management (n = 3).
[5]Persistently infected with bovine viral diarrhea virus (BVD-PI).

Table 4.3. Frequency distribution of study characteristics from a scoping review of risk factors for bovine respiratory disease morbidity.

Study characteristic	N[1]	%[2]
Publication year		
< 1974	3	2.1
1974 to 1984	6	4.3
1985 to 1995	12	8.5
1996 to 2006	28	19.9
2007 to 2017	92	65.3
Country		
United States	98	69.5
Canada	23	16.3
Australia	10	7.1
Italy	2	1.4
Mexico	1	0.7
Portugal	1	0.7
Spain	1	0.7
England	1	0.7
Argentina	1	0.7
Not reported	3	2.1
Production stage		
Feedlot	114	80.9
Backgrounding	21	14.9
Cow-calf	7	5.0
Backgrounding and feedlot [3]	1	0.7
Not reported	4	2.8
Study setting		
Feedlot[4]	95	67.4
Research facility (with no specification)	36	25.5
Cow-calf[5]	6	4.3
Stocker operation[6]	2	1.4
Not reported	6	4.3

[1]N = number of publications.
[2]Percentage of publications.
[3]Outcome reported as overall morbidity during backgrounding and feedlot.
[4]Commercial feedlot = 60, research feedlot = 35.
[5]Commercial cow-calf = 5, research cow-calf = 1.
[6]Commercial stocker operations = 2.

Table 4.4. Frequency distribution of study designs and outcomes from a scoping review of risk factors for bovine respiratory disease morbidity.

Study characteristic	N[1]	%[2]
Study design		
Experimental	101	71.6
Controlled trial (101)		
Observational	40	28.4
Cohort (33)		
Case-control (6)		
Case report (1)		
Health outcomes		
BRD morbidity	141	100.0
Overall mortality	87	61.7
BRD mortality	56	39.7
Retreatment	59	41.8
Performance outcomes[3]		
Average daily gain	93	66.0
Dry matter intake	54	38.3
Gain to feed	48	34.0
Hot carcass weight	26	18.4
Days on feed	10	7.1

[1]N = number of publications.
[2]Percentage of publications.
[3]Performance and carcass characteristics outcomes

Table 4.5. Frequency distribution of bovine respiratory disease case definitions from a scoping review of risk factors for bovine respiratory disease morbidity.

Case definition	N[1]	%[2]
Clinical signs only	21	14.8
Clinical signs and/or rectal temperature	4	2.8
Clinical signs and rectal temperature	82	57.7
Clinical signs and rectal temperature, or severe clinical signs	6	4.2
Clinical signs, rectal temperature, and abnormal blood leukocyte differentials	1	0.7
Lung lesions	1	0.7
Feedlot definition	6	4.2
Reported by a veterinarian	2	1.4
Not reported	19	13.4

[1]N = number of publications.
[2]Percentage of publications.

Table 4.6. Frequency distribution of groups[1] of antibiotics reported on publications related to metaphylaxis from a scoping review of risk factors for bovine respiratory disease morbidity.

Metaphylactic drug	N[1]	% groups[2]
Negative control	26	26.8
Tilmicosin	25	25.8
Tulathromycin	11	11.3
Oxytetracycline	8	8.2
Ceftiofur	4	4.1
Gamithromycin	4	4.1
Chlortetracycline	3	3.1
Florfenicol	3	3.1
Nitric oxide	3	3.1
Tilmicosin & chlortetracycline	2	2.1
Ceftiofur & tulathromycin	1	1.0
Chlortetracycline & sulfonamide	1	1.0
Penicillin	1	1.0
Tildipirosin	1	1.0
Penicillin & oxytetracycline	1	1.0
Tylosin	1	1.0
Not reported[3]	2	2.1

[1]N = number of groups of animals that received that intervention. Because a publication may have more than one study, and a study has more than one randomization group, treatment arms were extracted as "groups." [2]Percentage of groups (N = 97).
[3]Both studies used a survey as a tool in which the authors asked if metaphylaxis was used.

Table 4.7. Frequency distribution of groups[1] of bacteria and virus agents on vaccine-related publications from a scoping review of risk factors for bovine respiratory disease morbidity.

Vaccine	N[1]	% groups[2]
Control	20	29.9
IBR, BRSV, BVD, PI3	13	19.4
BRSV	6	9.0
Mh	6	9.0
IBR	3	4.5
BVD	2	3.0
Hs	2	3.0
IBR, PI3	2	3.0
Mh, HS	2	3.0
BRSV, PI3, *M. bovis*, *M. dispar*	1	1.5
BVD, PI3	1	1.5
Coronavirus	1	1.5
Hs, Mh	1	1.5
IBR, BRSV, BVD, PI3, Mh	1	1.5
IBR, BRSV, BVD, PI3, Mh, Pm	1	1.5
IBR, BRSV, BVD, PI3, Mh, Pm, Hs	1	1.5
IBR, Mh	1	1.5
IBR, PI3, Mh	1	1.5
Mh, Hs, BRSV	1	1.5
Ps	1	1.5

[1]N = number of groups of animals that received that intervention. Because a publication may have more than one study, and a study has more than one randomization group, treatment arms were extracted as "groups."
[2]Percentage of groups (N = 67).
IBR = Infectious bovine rhinotracheitis, BRSV= Bovine respiratory syncytial virus, BVD = Bovine viral diarrhea (either type I or II), PI3 = Parainfluenza 3, Mh = *Mannheimia haemolytica*, Hs = *Histophilus somni*, Pm = *Pasteurella multocida*, Ps = *Pasteurella spp.*

Table 4.8. Frequency distribution of groups[1] of interventions on publications related to "dietary supplements" from a scoping review of risk factors for bovine respiratory disease morbidity.

Intervention	N[1]	% groups[2]
Feed components		
Creep feed	2	7.4
Concentrated separated by-product	2	7.4
Different types of corn gluten feed	1	3.7
Dried distillers with corn or wheat	1	3.7
Percentage of grain in the diet	1	3.7
Feeding timing		
Intended days on feed[3]	1	3.7
Weeks on feed	1	3.7
Use of vitamins		
Vitamin E	2	7.4
Vitamin A, D, E, and C	1	3.7
Vitamin A, D, and E	1	3.7
Feed additives		
Yeast culture added to the diet	2	7.4
Megasphaera elsdenii in diet	1	3.7
Morinda citrifolia extract	1	3.7
Glycerin concentration	1	3.7
Minerals added to the diet		
Cu, Se, Mn, Zc	4	14.8
Cu, Co, Mn, Zc	1	3.7
Cu, Mn, Zn	1	3.7
Cu, Zn	1	3.7
Different types of Zn	1	3.7
Chromium concentration	1	3.7

[1]N = number of groups of animals that received that intervention. Because a publication may have more than one study, and a study has more than one randomization group, treatment arms were extracted as "groups."
[2]Percentage of groups (N = 27).
[3]Days on feed could be an outcome or a risk factor.

Table 4.9. Frequency distribution of groups[1] of program's characteristics on publications related to preconditioning programs from a scoping review of risk factors for bovine respiratory disease morbidity.

Preconditioning	N[1]	% groups[2]
Not preconditing[3]	15	34.1
Weaning	7	15.9
Weaning, castration, bunk broke, vaccination	3	6.8
Vaccination	2	4.5
Weaning, bunk broke	2	4.5
Weaning, vaccination	2	4.5
Bunk broke	3	6.8
Castration	1	2.3
Castration, dehorning	1	2.3
Castration, dehorning, vaccination	1	2.3
Not preconditioning and exposed to BVD-PI animals[4]	1	2.3
Weaning, castration, bunk broke	1	2.3
Weaning, castration, bunk broke, dehorning, vaccination	1	2.3
Weaning, castration, dehorning, vaccination	1	2.3
Weaning, castration, vaccination	1	2.3
Weaning, castration, vaccination (with BVD PI)[4]	1	2.3
Dehorning	1	2.3

[1]N = number of groups of animals that received that intervention. Because a publication may have more than one study, and a study has more than one randomization group, treatment arms were extracted as "groups."

[2]Percentage of groups of animals (N = 44).

[3]Author defined as animals not preconditioned or control animals for a given intervention (e.g., castration and vaccination vs. not castrated and unvaccinated).

[4]Some animals persistently infected with bovine viral diarrhea virus (BVD-PI) were included in the group as part of the intervention.

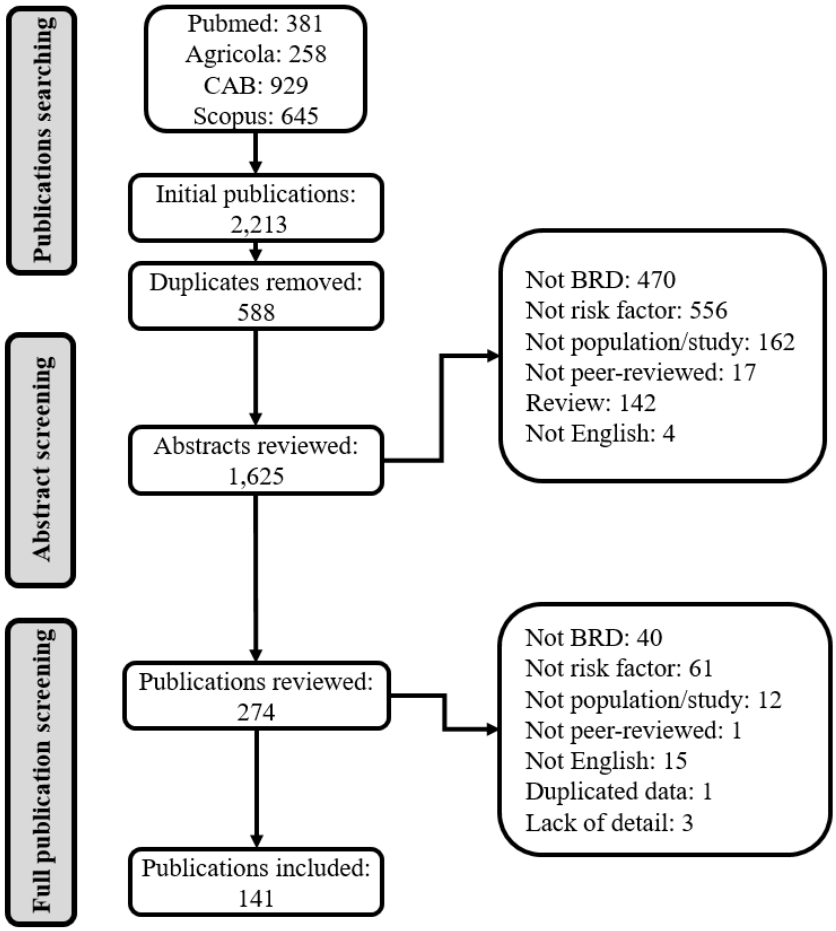

Figure 4.1. Flow chart of a scoping review of risk factors (RF) for bovine respiratory disease (BRD).

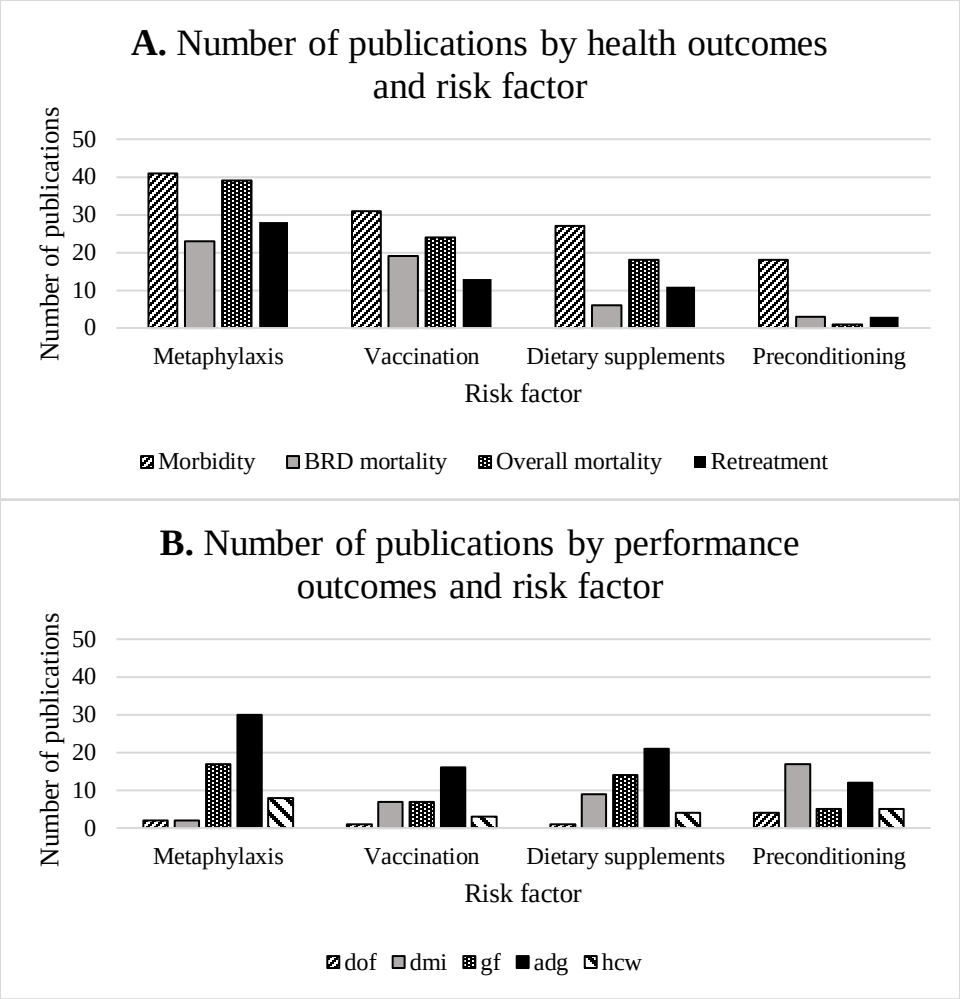

Figure 4.2. Number of publications by health and performance outcomes and bovine respiratory disease morbidity interventions from a scoping review.

Graphs depict number of publications for metaphylaxis, vaccination, dietary supplements, and preconditioning by: **A.** health-related outcomes, **B.** performance-related outcomes. dof = days on feed, dmi = dry matter intake, gf = gain to feed ratio or feed to gain ratio, adg = average daily gain, hcw = hot carcass weight.

Chapter 5 - **Type and timing of vaccination for the prevention of bovine respiratory disease morbidity in feedlot cattle: a network meta-analysis**

Manuscript prepared as per Preventive Veterinary Medicine guidelines.

Abstract

Although vaccination for the prevention of bovine respiratory disease (BRD) is widely used in the North American feedlot industry, evidence regarding their efficacy on reducing BRD morbidity is contradictory. The objective of the study was to evaluate the association between implementation of BRD vaccination programs administered before or upon feedlot arrival, with BRD morbidity in beef feedlot calves, using a network meta-analysis. Twenty-two publications, with 40 studies and 73 trial arms, regarding vaccination and preconditioning programs, were identified in a previous scoping review. Inclusion criteria were 1) beef cattle in North America or Australia, 2) comparison of two or more vaccination programs (or non-vaccination), and 3) naturally occurring BRD morbidity reported after feedlot arrival. Based on timing, trial arms (or vaccine groups for observational studies) were classified as "pre-feedlot" and "feedlot arrival" vaccination. Based on vaccination type, trial arms/groups were classified as "none," "viral," "bacterial," or "viral and bacterial." Arm-based Bayesian network meta-analysis was used to evaluate associations between vaccination programs and BRD morbidity. When compared to non-vaccination, the use of bacterial vaccination pre-feedlot arrival (odds ratio (OR) = 0.36; 95% CI: 0.11-0.88), viral and bacterial vaccination pre-feedlot arrival (OR = 0.18; 95% CI: 0.09-0.35), and viral and bacterial vaccination pre-feedlot arrival plus viral vaccination upon arrival (OR = 0.20; 95 % CI: 0.06-0.50) were associated with reduced BRD morbidity. When compared

to non-vaccination, the use of viral or bacterial vaccines upon feedlot arrival—alone or in combination—was not associated with reduced BRD morbidity. Likewise, compared to non-vaccination, giving only viral vaccines at pre-feedlot or only bacterial vaccines at both pre-feedlot and feedlot arrival were not found to be associated with reduced BRD morbidity. These results indicate that a reduction in BRD morbidity can be achieved when using pre-feedlot arrival vaccinations, which are commonly included in preconditioning programs. These results, however, do not support using vaccinations upon feedlot arrival, in contrast to standard feedlot practice. This research highlights the importance of timing and type of vaccination for the prevention of BRD morbidity and can help improve guidelines on BRD vaccination programs.

Introduction

Every year, bovine respiratory disease (BRD) poses a significant burden on animal welfare and feedlot production (United States Department of Agriculture, 2013a). Bovine respiratory disease costs are not limited to morbidity and mortality effects. Lower average daily gain, cost of antimicrobials, veterinary care, and operational expenses, among others, contribute to the reported impact of BRD of one billion USD annually (Griffin, 1997). Further, antimicrobial use is under increased scrutiny, motivating efforts to promote judicious antimicrobial use associated with BRD treatment and risk management.

Vaccines could aid in reducing BRD morbidity by improving animals' immunity and therefore help reduce BRD-related antimicrobial use in feedlots. The use of metaphylaxis, group medication of at-risk animals, is common, and concerns exist due to the possible increase of antimicrobial-resistant pathogens. For example, according to the National Animal Health Monitoring System (NAHMS), almost 60% of United States feedlots with more than 1,000 animals use metaphylaxis antimicrobials on at least some cattle upon arrival to the feedlot (United States Department of Agriculture, 2013a). Given these numbers, non-antimicrobial practices for the control of animal diseases were the subject of a recent scoping review and are increasingly being studied (Wisener et al., 2019). According to NAHMS, 85.1% to 96.6% of feedlots with a capacity of at least 1,000 animals vaccinate at least some cattle on arrival against bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR), parainfluenza type 3 (PI3), or bovine respiratory syncytial virus (BRSV). Bacterial vaccines, however, are not used as commonly as only two-thirds of feedlots use vaccines against *Histophilus somni* (Hs) and *Mannheimia haemolytica* (Mh) on some of their animals (United States Department of

Agriculture, 2013a).

In the United States, there are 36 different vaccines approved for BRD prevention (USDA: Center for Veterinary Biologics). Moreover, vaccines may be given in different stages of an animal's life cycle. For BRD prevention in beef cattle, vaccines can be given before weaning, at weaning, after weaning (but before feedlot shipment), upon feedlot arrival, or delayed after feedlot arrival. The combination of different vaccines available, the timing of administration, and the number of doses has led to substantial design variation in studies reporting research on BRD vaccines (Baruch, Chapter IV). According to Baruch (Chapter IV), there are 30 publications reporting research on vaccines for the prevention of BRD-related morbidity in beef cattle. Although there are a large number of publications, there are few comparable studies and little repeatability for specific vaccine interventions (Baruch, Chapter IV).

Some research synthesis studies describing the association between BRD-related vaccines and BRD morbidity are available (Snyder et al., 2019; Theurer et al., 2015). For example, Theurer et al. (2015) studied the effect of different types of vaccines on reducing BRD morbidity in beef and dairy cattle. Their study, however, did not differentiate by production phase or timing of administration (i.e., cow-calf, backgrounding, feedlot) and included natural and experimental disease. Snyder et al. (2019) studied the effect of delaying feedlot arrival vaccination programs instead of vaccinating upon feedlot arrival. Neither study, however, accounted for other characteristics of the study populations. Specifically, BRD vaccinations other than those randomized to treatment arms are commonly given to animals, either before the study or upon feedlot arrival. Failure to account for those other BRD vaccines may confound the

assessment of the vaccine effects if there are synergistic or antagonistic effects. Therefore, the totality of BRD vaccinations must be taken into consideration when conducting meta-analysis.

If a study reports comparing vaccine **A** vs. **B**, and a second study reports comparing vaccine **B** vs. **C**, we can know the relationship between **A**-**B** and **B**-**C** by direct evidence. Similarly, the relationship between **C**-**A** can be known by indirect evidence through **C**' association with **B**. Re-classifying trials based on all BRD-related vaccines used (randomized and not) results in a small number of replications for direct evidence comparisons, which makes traditional meta-analysis impractical. However, NMA methodology, which has been increasingly applied in veterinary medicine research (Abell et al., 2016; O'Connor et al., 2013), allows researchers to take advantage of both the direct and indirect evidence.

Addressing the knowledge gap in the association between vaccinations and BRD morbidity could advance the efforts on antimicrobial alternatives to reduce BRD morbidity. Therefore, the objective of this study was to evaluate the association between implementation of BRD vaccination programs, administered before or upon feedlot arrival, with BRD morbidity in beef feedlot calves, using a network meta-analysis.

Materials and methods

Scoping review and data extraction

The data used in this study was extracted from a scoping review (Baruch, Chapter IV). Briefly, electronic searches were conducted on Pubmed, Agricola, CAB, and Scopus databases on April 8th, 2017 based on key terms (Baruch, Chapter IV). Inclusion criteria for the scoping review were *Bos taurus* beef breeds or crossbreeds, any production stage (i.e., cow-calf, backgrounding, feedlot), sex, age, body weight, risk factor for BRD-related morbidity, study

design, publication year, country, and studies that reported BRD-related morbidity (Baruch, Chapter IV). However, challenge studies or studies with non-naturally occurring BRD were excluded as well as studies that used dairy animals, veal calves, or bulls (Baruch, Chapter IV).

Thirty and 17 publications regarding vaccination or preconditioning programs, respectively, were identified on the scoping review (Baruch, Chapter IV). Further inclusion criteria were used for the NMA. Studies had to: 1) include morbidity outcomes after feedlot arrival, 2) be conducted in North America or Australia, 3) have morbidity estimates to extract for each comparison group (vaccinated vs. non-vaccinated or other vaccines), and 4) have different vaccine pathogens on the comparison groups (e.g., not only comparing the same vaccine components from two different manufacturers). Studies that described comparisons between vaccinations upon feedlot arrival versus delayed vaccinations or between the numbers of doses used for each comparison group were not included.

Studies (experimental or observational) within publication (journal publication) were extracted separately. Data on BRD morbidity estimates, number of animals, BRD-related vaccines, and timing of each vaccine (prior to feedlot arrival or upon feedlot arrival) were extracted from each trial arm (trial arm for experimental studies or comparison group for observational studies). Other BRD-related vaccines given to all animals in the trial as a part of a routine health management procedure were also extracted. For example, if a study reported the effect of Mh vaccine vs. non-vaccination, but also vaccinated all animals with a 5-way viral vaccine (BVD I & II, IBR, PI3, and BRSV), then the study was assumed to compare a 5-way and Mh vaccines vs. a 5-way vaccine.

In addition, other risk factors for BRD morbidity that might act as confounding factors

between the association of vaccines and BRD morbidity were extracted from each trial arm. These potential confounding factors extracted as yes/no variables were: 1) animals purchased through a sale barn, 2) animals that received metaphylaxis, 3) animals acclimated to eating from a feed bunk (i.e., bunk broke) prior to feedlot arrival, 4) animals weaned at least 30 days prior to feedlot arrival, and 5) at least 95% of animals castrated 30 days prior to feedlot arrival.

Assumptions

If a study did not report any vaccination prior to feedlot arrival, it was assumed that the animals did not receive any BRD-related vaccination pre-arrival. Whenever possible, authors were contacted when the description of potential confounding factors was not clear. When there was no explanation of the animals' source of origin, it was assumed animals originated from sale barns. If there was no specific description of metaphylaxis or acclimation to eating from a feed bunk, it was assumed they were not applied/conducted. Animals were assumed to be weaned at least 30 days prior to feedlot arrival if they came directly from a ranch; otherwise, it was assumed that animals were weaned at shipment or less than 30 days prior to shipment. Animals were assumed to have been castrated 30 days prior if there was no reporting of castration at arrival.

Meta-analysis

A Bayesian NMA was conducted using the *pcnetmeta* package in R (Lin et al., 2017). The initial attempts of categorizing vaccines based on specific pathogens were not considered. Instead, trial arms within studies were classified based on the timing of vaccination administrated prior to feedlot arrival (pre-feedlot) and upon feedlot arrival. Further classifications were made by grouping vaccines into viral, bacterial, and viral and bacterial based on the pathogens present in the vaccines. **Table 1** depicts the classification of vaccines and the number of studies in each

trial arm. Trial arms with the same intervention for timing and class of vaccination within a study were collapsed into a trial arm. This collapsing lead to some studies having only one trial arm (single-trial arm studies). However, since the *pcnetmeta* package allows for the inclusion of studies with a single-trial arm, no studies were excluded.

Odds ratios (OR) and treatment rank probabilities were derived from each treatment's absolute risk using available features in the *pcnetmeta* package. Based on a better fit as per the deviance information criterium, the homogenous equal correlation model (hom_eqcor) was chosen over the heterogeneous equal correlation model (het_eqcor). The unstructured covariance matrix model was also fitted but did not converge, given the limited number of data points. The homogeneous equal correlation model assumes equal correlation and equal variance among treatment arms within a study (Lin et al., 2017). The following prior distributions were used for model fitting. 1) Vague N (0, 1000) priors were used for the mean absolute risk of a given treatment (which assumes absolute risk is between 0 and 1), 2) uniform (-0.125, 1) priors were used for the within-study dependence among treatments, and 3) uniform priors (0, 2) were used for the standard deviation of the mean absolute risk for a given treatment. By using a uniform (0, 2) prior for the standard deviation, it is assumed that if the true morbidity for a given treatment is 0.2, then the 95% confidence interval would be 0.003 to 0.87.

Model convergence was evaluated through visual appraisal of the trace plots and autocorrelation plots, the scale reduction factor (using a cut of 1.1; (Brooks and Gelman, 1998)), and the Heidelberger and Welch's convergence diagnostic (Heidelberger and Welch, 1983). The total number of burn-in iterations were determined by a combination of the visual appraisal of an initial run of 5,000 iterations and the Raftery and Lewis's diagnostics (Raftery and Lewis, 1992).

The final model was burned-in for 10,000 iterations and ran for 500,000 iterations while using a thinning of 20.

Sensitivity analysis

Sensitivity analysis was performed for the prior corresponding to the standard deviation of the absolute risk. Uniform priors (0, 1), (0, 5), and (0, 10) were fitted, and changes on the absolute risk of the outputs were monitored. However, given that no substantial differences were observed, the (0, 2) prior was used. Sensitivity analysis was also performed comparing a model that included the single-trial arm studies and a model with only studies that had at least one comparison group. However, when no substantial differences were found, the single-trial arm studies were also included. The assumption of no vaccination prior to feedlot arrival for those studies that reported unknown vaccination status or did not report any vaccination detail was tested. The same model, as described above, was run, but instead of making that vaccine assumption, it was assumed that those studies had used a viral vaccination before feedlot arrival. The original assumption was kept because of the results from the original model, and this new model presented no substantial differences.

Analysis of potential confounders

Frequency tables were computed depicting the distribution of the potential confounding factors (sale barn, metaphylaxis, bunk broke, weaning, and castration) between treatment arms. The distribution of these potential confounders by treatment arms was evaluated qualitatively without statistical tests.

In addition, an extra model was fitted using the same methodology as described above but dividing vaccination treatments by weaning and not weaning 30 days prior to feedlot arrival.

In order to assess the potential confounding effect of weaning, the results from the weaning model were compared to the original model.

Results

From the initial 47 publications identified in the scoping review (Baruch, Chapter IV), 27 were eliminated based on not meeting the inclusion criteria (**Table 2**). The network in this study included 22 publications, 20 from the scoping review, and two included from a hand search of the literature (**Table 1**). From the 22 publications, 41 studies comprising 73 trial arms were identified. The network plot is depicted in **Figure 1**, in which a non-vaccination group and eight vaccination programs are identified (**Table 1**). Thirteen studies represented a comparison of a non-vaccination group vs. a vaccination group, and 18 studies represented a vaccination-to-vaccination comparison. Ten studies were included as single-arm studies after collapsing treatment arms within studies with the same exposure treatment.

Odds ratios for all possible comparisons from the NMA are depicted in **Table 3**. Using viral and bacterial vaccinations pre-feedlot arrival with or without viral vaccination upon arrival decreased BRD morbidity when compared to non-vaccination (**Table 3**). The use of viral vaccines at arrival on animals that were already vaccinated with viral and bacterial vaccines pre-feedlot did not reduce their BRD morbidity (**Table 3**; VB_V vs. VB_C). This result suggests no additional reduction in BRD morbidity when adding a viral vaccine upon arrival (**Table 3**). The use of bacterial vaccines prior to arrival with no vaccination at arrival was associated with decreased BRD morbidity when compared to non-vaccination. Conversely, using any type of feedlot arrival vaccinations only, or only viral vaccinations pre-feedlot arrival was not associated with a reduction in BRD morbidity when compared to non-vaccination (**Table 3**).

Ranking probabilities for each treatment group are depicted in **Table 4**. The highest rankings corresponded to vaccination programs that used viral and bacterial vaccines pre-feedlot arrival. Specifically, between 79-88% of the time, the viral and bacterial programs pre-feedlot arrival, with or without viral vaccination at arrival ranked in the two best choices of treatment (**Table 4**).

The distribution of potential confounders varied between vaccination programs (**Table 5**). Calf groups that received viral and bacterial vaccination pre-feedlot arrival, without vaccination at arrival, were primarily sold directly to the feedlot, castrated, and weaned pre-feedlot arrival (**Table 5**). Calf groups that received bacterial vaccination prior to feedlot arrival, and no vaccine at feedlot arrival, were weaned, castrated, and sold through a sale barn, but were not acclimated to eating from a feed bunk nor received metaphylaxis. One-third and almost one-half of the groups that received viral, or viral and bacterial, vaccinations upon feedlot arrival, respectively, received metaphylaxis upon arrival and were castrated prior to feedlot arrival (**Table 5**). In contrast, the non-vaccination group included primarily animals that were not acclimated to eating from a feed bunk, did not receive metaphylaxis, were not weaned or castrated prior to feedlot arrival and were primarily sold through a sale barn (**Table 5**).

Although assumptions were made for weaning status when no other information was available, only 5% of groups were assumed to have weaned animals because of direct shipment from a ranch (**Table 6**). In contrast, 44% of groups were assumed to have non-weaned animals because they came from a sale barn, and no other information was available (**Table 6**).

Viral vaccination at arrival (C_V), viral and bacterial vaccination at arrival (C_VB), and non-vaccination were the only programs to have non-weaned and weaned calf groups (**Table 5**). Therefore, the potential confounding effect of weaning on the association of vaccination

programs and BRD was only evaluated between these programs (**Table 7**). Weaned animals receiving either of these two arrival vaccination programs (C_V, C_VB) were not associated with reduced BRD morbidity when compared to non-vaccination on weaned or non-weaned animals (**Table 7**). However, when compared to non-vaccination without weaning, these vaccination programs' point estimated OR were above one when animals were not weaned and vaccinated, and below one when animals were weaned and vaccinated; indicating a potential point estimate reduction when vaccination is used on weaned animals as opposed to not weaned animals.

Discussion

This study elucidated how timing and type of BRD vaccination programs were associated with BRD morbidity after feedlot arrival. When compared to non-vaccination, the odds of BRD morbidity were lower when vaccination programs were implemented prior to feedlot arrival. Hence vaccination programs prior to feedlot arrival could contribute to preventing BRD morbidity, while reducing the use of antimicrobials. However, the literature provides little evidence to support the use of vaccination programs upon feedlot arrival.

Viral and bacterial vaccinations prior to feedlot arrival, with and without viral vaccination at feedlot arrival, were associated with reduced BRD morbidity when compared to non-vaccination. This association supports the producers' belief that preconditioning programs (which include vaccination) are useful to reduce BRD morbidity (United States Department of Agriculture, 2013b). The association between vaccination programs with reduced morbidity could be attributed to the animals' development of an immune response to the BRD pathogen(s) in the time between vaccination and feedlot arrival. Animals may be stressed while being

handled upon feedlot arrival; stress generates cortisol, which in turn, lowers the animal's immunity (Edwards, 2010). By vaccinating animals prior to feedlot arrival, producers might allow enough time for vaccine immunity to develop before handling (Blecha et al., 1984; Edwards, 2010). In addition, vaccinated animals go through a handling process prior to feedlot arrival, which might make them less susceptible to stress. Although the potential stress reduction of pre-feedlot animal handling was not addressed in this study, perhaps not all the observed benefit of vaccination is related to the vaccine, but rather a complex interaction of several risk factors (i.e., animal handling, castration, weaning, vaccination).

Similarly, no additional reduction in BRD morbidity was found for viral vaccination upon feedlot arrival combined with viral and bacterial vaccinations prior to feedlot arrival. This effect might also be attributed to the cortisol produced by animal handling upon arrival (Edwards, 2010), or simply due to no additional benefit of including another vaccination. This result should be studied further to identify if vaccination upon feedlot arrival is a beneficial addition to preconditioning programs.

While administering bacterial only vaccinations prior to feedlot arrival was associated with reduced BRD morbidity when compared to non-vaccination, viral only vaccinations prior to feedlot arrival were not. Bacterial only vaccinations prior to feedlot arrival were not significantly different from viral and bacterial prior to feedlot arrival. Similarly, bacterial and viral and bacterial vaccinations prior to feedlot arrival were associated with reduced BRD morbidity when compared to viral only pre-arrival vaccination. These findings suggest that bacterial rather than viral pre-arrival vaccinations may be more beneficial. Given that publications reporting research on bacterial vaccines are older than the ones on viral vaccines (Baruch, Chapter IV), future

research should study the efficacy of bacterial vaccines.

Viral, bacterial, or viral and bacterial, vaccinations upon feedlot arrival were not associated with reduced BRD morbidity compared to any other program or non-vaccination. This result could be due to the time required to produce vaccination immunity and the timing of early BRD morbidity in the feedlot. According to Edwards (Edwards, 2010), animals require between 14 to 21 days to develop immunity through vaccination against BRD agents. However, depending on cattle characteristics, as high as 80 to 90% of BRD cases in feedlots would occur by day 21 (Babcock et al., 2010). This timing indicates that most BRD cases would occur before animals can develop immunity to vaccines given on arrival. Likewise, others (O'Connor et al., 2019), found no association between feedlot arrival vaccinations and BRD morbidity. Despite this information, more than 90% of feedlots with more than 1,000 animals use arrival vaccination programs on at least some of their animals (United States Department of Agriculture, 2013a). Given the widespread use of arrival vaccinations, it seems warranted that more studies investigating vaccinations upon arrival compared to non-vaccination should be conducted.

Network treatment ranks for programs with viral and bacterial vaccines given prior to arrival were most commonly first or second. This result is not surprising given that these programs were associated with reduced BRD morbidity when compared to most programs in the network. Therefore, these treatment ranks support the odds ratios' estimates. However, these ranking probabilities do not necessarily relate to treatment success. For example, a treatment may be ranked better than others for a given model iteration, but both treatments may not be associated with decreased BRD morbidity (Hu et al., 2019).

The impact of the potential confounding factors on the association of vaccination

programs with BRD-related morbidity is uncertain The non-vaccination program had a frequency distribution of confounders that would indicate a high risk for BRD morbidity (most calf groups came from a sale barn without prior castration or prior weaning and did not receive metaphylaxis). The viral, and viral and bacterial, vaccines upon arrival groups (C_V, C_VB) had a low to moderate risk of BRD morbidity (common metaphylaxis and prior castration) but were not associated with reduced morbidity when compared to non-vaccination, even though their distribution of confounders would indicate a lower BRD morbidity risk than the non-vaccination groups. There are two explanations for these results. First, these potential confounders were not confounders in our data, and these vaccination programs do not reduce morbidity. Second, these vaccination programs do reduce BRD morbidity, but their distribution of confounders increased the animals' BRD risk, and therefore no reduction in BRD morbidity was observed. However, the second explanation would be inconsistent with common knowledge of the benefit of metaphylaxis, weaning, and castration.

Similarly, viral vaccinations prior to feedlot arrival (V_C) had a lower risk distribution of potential confounders (calf groups always castrated and weaned prior to feedlot arrival), than the non-vaccination calf groups. However, the viral vaccination pre-arrival program (V_C) was not associated with reduced morbidity when compared to non-vaccination. Although a similar distribution of confounders to those of viral vaccination prior (V_C) was found on bacterial and viral and bacterial vaccinations prior (B_C, VB_C, VB_V), the latter programs were associated with reduced BRD morbidity when compared to non-vaccination. The distribution effect of these potential confounders on the association of vaccination programs and BRD morbidity must be studied further. Given the lack of treatment replication by each confounder's level, further research is needed in this area to conduct a quantitative analysis of these results.

When dividing arrival vaccination programs by weaning status, results were similar to those obtained through the original model (all estimated credible intervals overlapped one). Interestingly, OR point estimates comparing non-weaned vaccinated calves to non-vaccinated calves were greater than one, but point estimates comparing weaned vaccinated calves to non-vaccinated calves were less than one. While the 95% credible intervals for both groups overlapped one, the switch in direction of the association is concerning and suggests that confounding might be present in the association of vaccination and weaning with morbidity. This result could support the contention that when weaning is performed prior to feedlot arrival, animals may respond better to vaccination due to a separation of the stressful events that animals suffer when arriving at a feedlot. Therefore, it seems warranted that further research should address the question of whether weaning has an impact on the association of vaccination programs and BRD morbidity.

A large number of vaccination programs were identified due to considering other vaccines than the randomized treatments (for studies that were randomized trials) to define a vaccination program (data not shown). Considering all vaccines administered is a more accurate description of the vaccine regimen than only accounting for those that are randomized. However, large numbers of unique programs generates non-connected networks, which prevents the possibility of conducting NMA (O'Connor et al., 2019). To address this problem, we classified vaccines as viral or bacterial, which limits the ability of this study to assess the impact of specific vaccines on BRD morbidity. In order to improve network connectivity, future research could be designed to include at least one treatment arm (or negative control) that has been studied previously. This repetition of treatment arms would allow for future treatment networks to be connected, enhancing the applicability of research synthesis methods.

Assumptions regarding pre-feedlot arrival vaccination status had to be made due to lack of reporting. If these assumptions are incorrect, they could bias our study results by making inferences on treatments that, in reality, incorporated other vaccinations. However, when tested, this assumption did not influence our results; which supports our assumptions as likely correct. This finding can be explained by the characteristics of the animals in those studies where the assumption was made. Pre-arrival vaccination is recognized as a positive attribute of cattle and potentially associated with a higher market value. Therefore, sellers of cattle with prior vaccinations would generally disclose vaccination information, while cattle with no disclosed history are likely unvaccinated. Lack of knowledge of prior vaccination may represent a "real world" situation of limited knowledge.

No parameters related to the costs of vaccination or other operational procedures were included in this study, which means that even though some vaccinations prior to feedlot arrival were associated with reduced BRD morbidity when compared to non-vaccination, there is no estimation of the economic costs and benefits. However, these results contribute to the study of antimicrobial alternatives for BRD prevention, an area of increasing demand given the increased number of regulations for antimicrobial use in livestock production (Wisener et al., 2019).

In conclusion, when compared to non-vaccination, the combination of viral and bacterial vaccination prior to feedlot arrival presents the best scenario to reduce feedlot BRD morbidity. In addition, there is limited evidence to support the use of vaccinations upon feedlot arrival. The timing and type of vaccination are of particular importance in the United States, where most large feedlots vaccinate cattle upon arrival. Given the importance of BRD to the feedlot industry and the increasing pressure by governments and the public to promote the judicious use of

antimicrobials and antimicrobial alternatives, further research into specific vaccination programs is warranted.

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Table 5.1. Classification of vaccines used in a network meta-analysis evaluating the association of pre-feedlot arrival and feedlot arrival vaccinations with bovine respiratory disease morbidity in feedlot cattle.

Code[1]	N[2]	Pre-Feedlot	Feedlot arrival	References
C_C	13	None	None	(Duff et al., 2000; Martin et al., 1984; O'Connor et al., 2001; Richeson et al., 2015; Woods et al., 1972, 1973b)
C_V	17	None	Viral only	(Bechtol and Jones, 1996; Bryant et al., 2008; Donkersgoed et al., 1993, 1990; Duff et al., 2000; Frank et al., 2002; Macgregor et al., 2003; Morter et al., 1982; O'Connor et al., 2001; Richeson et al., 2015; Seeger et al., 2008)
C_B	1	None	Bacterial	(O'Connor et al., 2001)
C_VB	24	None	Viral and bacterial	(Bechtol and Jones, 1996; Bryant et al., 2008; Donkersgoed et al., 1993, 1990; Frank et al., 2002; Harland et al., 1992; Macgregor et al., 2003; Malcolm-Callis and Galyean, 1994; Morter et al., 1982; Morter and Amstutz, 1986; O'Connor et al., 2001; Richeson et al., 2012; Schunicht et al., 2003; Step et al., 2008; Wildman et al., 2008)
V_C	5	Viral only	None	(Martin et al., 1984; Woods et al., 1972, 1973b)
B_C	2	Bacterial	None	(Bailey et al., 2016a; Martin et al., 1984)
B_B	1	Bacterial	Bacterial	(Martin et al., 1984)
VB_C	7	Viral and bacterial	None	(Bailey et al., 2016a; Donkersgoed et al., 1990; Richeson et al., 2012; Step et al., 2008; Woods et al., 1972, 1973b)
VB _V	3	Viral and bacterial	Viral only	(Bailey et al., 2016a; Donkersgoed et al., 1990; Seeger et al., 2008)

[1]Letters on the left side of the underscore correspond to pre-feedlot arrival vaccination, and letters on the right side of the underscore correspond to feedlot arrival vaccinations. V = viral, B = bacterial, VB = viral and bacterial, and C = non-vaccination.
[2]Number of studies.

Table 5.2. Description of reasons for the elimination of publications identified in a previous scoping review of the literature to be used in a network meta-analysis evaluating the association of pre-feedlot arrival and feedlot arrival vaccinations with bovine respiratory disease morbidity in feedlot cattle.

Elimination reason	Count	Reference
Not enough details to extract morbidity estimates or vaccines used	5	(Bingham et al., 2000; Bohlencler, 1984; K E Hay et al., 2016; K. E. Hay et al., 2016; Macartney et al., 2003)
No vaccine intervention[1]	6	(Bailey et al., 2016b, 2015a, 2015b; Loe et al., 2002; Loerch and Fluharty, 2000; Wieringa et al., 1976)
Historical control	1	(Lopez et al., 1984)
Difference between vaccine timing (feedlot arrival vs. delayed)	6	(Lippolis et al., 2016; Richeson et al., 2009, 2008; Rogers et al., 2016; Step et al., 2009; White et al., 2008)
BRD morbidity reported before feedlot arrival	4	(Donkersgoed et al., 1994; Hanzlicek et al., 2013; Ratcliff et al., 2014; Woods et al., 1973a)
Same vaccine pathogens, different brand	2	(Hanzlicek et al., 2010; Wells, 2015)
Not applicable country	2	(Howard et al., 1987; Stilwell et al., 2008)
Coronavirus vaccine	1	(Plummer et al., 2004)
Total	27	

[1]No vaccine interventions were used in these publications.

Table 5.3. Odds ratios (bottom of the table) and 95% credible intervals (top of the table) of all possible comparisons obtained from a network meta-analysis evaluating the association of pre-feedlot arrival and feedlot arrival vaccinations with bovine respiratory disease morbidity in feedlot cattle.

Code[1]	C_C	C_V	C_B	C_VB	V_C	B_C	B_B	VB_C	VB_V
C_C	-	(0.76, 1.92)	(0.13, 2.41)	(0.65, 1.70)	(0.72, 2.11)	**(0.11, 0.88)**	(0.19, 2.27)	**(0.09, 0.35)**	**(0.06, 0.50)**
C_V	1.22	-	(0.11, 2.02)	(0.64, 1.18)	(0.53, 1.97)	**(0.09, 0.75)**	(0.15, 2.00)	**(0.08, 0.28)**	**(0.05, 0.38)**
C_B	0.64	0.53	-	(0.43, 7.92)	(0.46, 9.93)	(0.10, 3.22)	(0.17, 7.73)	(0.07, 1.48)	(0.06, 1.67)
C_VB	1.05	0.87	1.64	-	(0.60, 2.31)	**(0.11, 0.86)**	(0.17, 2.34)	**(0.09, 0.32)**	**(0.06, 0.44)**
V_C	1.22	1.00	1.91	1.16	-	**(0.08, 0.81)**	(0.14, 2.06)	**(0.06, 0.34)**	**(0.04, 0.46)**
B_C	**0.36**	**0.29**	0.55	**0.34**	**0.29**	-	(0.41, 10.10)	(0.19, 1.65)	(0.15, 1.98)
B_B	0.70	0.57	1.09	0.66	0.57	1.96	-	(0.07, 1.09)	(0.05, 1.32)
VB_C	**0.18**	**0.15**	0.29	**0.17**	**0.15**	0.52	0.26	-	(0.34, 2.70)
VB_V	**0.20**	**0.16**	0.31	**0.19**	**0.16**	0.56	0.28	1.08	-

[1]Letters on the left side of the underscore correspond to pre-feedlot arrival vaccination, and letters on the right side of the underscore correspond to feedlot arrival vaccinations. V = viral, B = bacterial, VB = viral and bacterial, and C = non-vaccination.
Example: The odds ratio of comparing viral vaccinations upon feedlot arrival (C_V) to non-vaccination (C_C) is 1.22, with a credible interval of 0.76 to 1.92.
Bold numbers represent significant associations.

Table 5.4. Treatment rank probabilities from a network meta-analysis evaluating the associations between vaccination programs and bovine respiratory disease morbidity in feedlot cattle.

Code[1]	Rank1	Rank2	Rank3	Rank4	Rank5	Rank6	Rank7	Rank8	Rank9
C_C	0.00	0.00	0.00	0.04	**0.21**	**0.35**	**0.23**	0.13	0.03
C_V	0.00	0.00	0.00	0.01	0.04	0.11	**0.21**	**0.33**	**0.31**
C_B	0.04	0.05	0.14	0.29	0.19	0.06	0.05	0.06	0.12
C_VB	0.00	0.00	0.00	0.04	0.16	**0.27**	**0.27**	**0.20**	0.06
V_C	0.00	0.00	0.00	0.02	0.08	0.14	0.18	0.21	0.37
B_C	0.07	0.13	**0.46**	**0.25**	0.07	0.01	0.00	0.00	0.00
B_B	0.02	0.03	0.12	**0.29**	**0.24**	0.07	0.06	0.06	0.12
VB_C	**0.48**	**0.40**	0.11	0.01	0.00	0.00	0.00	0.00	0.00
VB_V	**0.40**	**0.39**	0.17	0.04	0.00	0.00	0.00	0.00	0.00

[1]Letters on the left side of the underscore correspond to pre-feedlot arrival vaccination, and letters on the right side of the underscore correspond to at feedlot arrival vaccinations. V = viral, B = bacterial, VB = viral and bacterial, and C = non-vaccination.
Lower rankings represent vaccination treatments that were ranked as having lower bovine respiratory disease morbidity than higher rankings.

Table 5.5. Distribution of treatment arms by potential confounding factors from a network meta-analysis evaluating the associations between vaccination programs and bovine respiratory disease morbidity in feedlot cattle.

Code[1]	Metaphylaxis		Prior feed[2]		Sale barn		Prior castration		Prior weaning	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
C_C	1	12	3	10	12	1	6	7	3	10
C_V	6	11	0	17	16	1	16	1	3	14
C_B	0	1	0	1	1	0	1	0	0	1
C_VB	10	14	0	24	23	1	20	4	4	20
V_C	0	5	1	4	5	0	5	0	5	0
B_C	0	2	0	2	2	0	2	0	2	0
B_B	0	1	0	1	1	0	1	0	1	0
VB_C	0	7	2	5	3	4	7	0	7	0
VB _V	0	3	0	3	2	1	3	0	3	0

[1]Letters on the left side of the underscore correspond to pre-feedlot arrival vaccination, and letters on the right side of the underscore correspond to at feedlot arrival vaccinations. V = viral, B = bacterial, VB = viral and bacterial, and C = non-vaccination.
[2]Prior feed = animals acclimated to eating from a feed bunk (i.e., bunk broke) prior to feedlot arrival.

Table 5.6. Distribution of treatment arms by assumptions related to weaning from a network meta-analysis evaluating the associations between vaccination programs and bovine respiratory disease morbidity in feedlot cattle.

Code[1]	Weaned		Not weaned		Total
	Reported	Assumed[2]	Reported	Assumed[3]	
C_C	3	0	7	3	13
C_V	1	2	2	12	17
C_B	0	0	1	0	1
C_VB	2	2	3	17	24
V_C	5	0	0	0	5
B_C	2	0	0	0	2
B_B	1	0	0	0	1
VB_C	7	0	0	0	7
VB _V	3	0	0	0	3

[1]Letters on the left side of the underscore correspond to pre-feedlot arrival vaccination, and letters on the right side of the underscore correspond to at feedlot arrival vaccinations. V = viral, B = bacterial, VB = viral and bacterial, and C = non-vaccination.
[2]Assumed to have been weaned due to animals coming directly from the ranch.
[3]Assumed to have not been weaned due to animals coming from a sale barn and not providing any other health information.

Table 5.7. Odds ratios (OR) for vaccination programs by weaning combinations obtained from a network meta-analysis evaluating the association of pre-feedlot arrival and feedlot arrival vaccinations with bovine respiratory disease morbidity in feedlot cattle.

Vaccination*weaning	Vaccination*weaning	OR (95% CI)
C_V	**C_C**	
Not weaned	Not weaned	1.21 (0.76, 2.05)
Weaned	Not weaned	0.60 (0.21, 1.67)
C_V	**C_C**	
Not weaned	Weaned	1.50 (0.63, 3.64)
Weaned	Weaned	0.72 (0.21, 2.43)
C_VB	**C_C**	
Not weaned	Not weaned	1.06 (0.62, 1.78)
Weaned	Not weaned	0.68 (0.28, 1.57)
C_VB	**C_C**	
Not weaned	Weaned	1.26 (0.53, 3.11)
Weaned	Weaned	0.81 (0.27, 2.44)

Letters on the left side of the underscore correspond to pre-feedlot arrival vaccination, and letters on the right side of the underscore correspond to at feedlot arrival vaccinations. V = viral, VB = viral and bacterial, and C = non-vaccination.

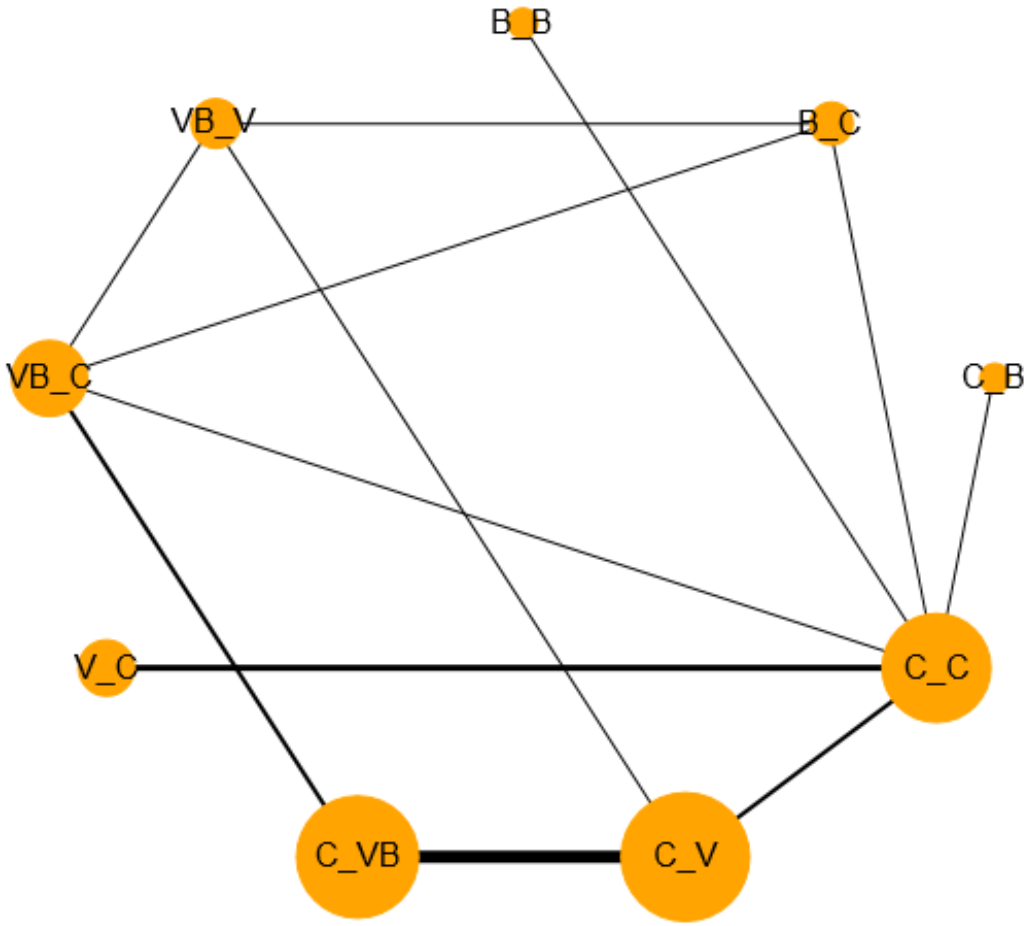

Figure 5.1. The network of treatment arms used in a network meta-analysis that evaluated the associations between vaccination programs and bovine respiratory disease morbidity in feedlot cattle.

Letters on the left side of the underscore correspond to pre-feedlot arrival vaccination, and letters on the right side of the underscore correspond to at feedlot arrival vaccinations. V = viral, B = bacterial, VB = viral and bacterial, and C = non-vaccination. Node size is relative to the number of trial arms for that intervention, and arrow width is relative to the number of direct comparisons

Chapter 6 - **Risk assessment of risk factors for bovine respiratory disease morbidity in feedlot cattle**

Manuscript prepared as per Preventive Veterinary Medicine guidelines.

Abstract

Multiple risk factors may be present and interact with each other in the epidemiology of bovine respiratory disease (BRD). These risk factor characteristics make the study of production system approaches for disease control through randomized control trials cumbersome and economically challenging. Through disease modeling methodologies, morbidity risk scenarios can be used to define risk categories and generate specific research hypotheses. Therefore, the objective of this study was to estimate BRD morbidity risk distributions in cohorts of feedlot cattle based on cohort-specific characteristics using a Monte-Carlo simulation model to estimate morbidity risks, optimal interventions, and develop research hypotheses. Data from a prior scoping review and meta-analyses of metaphylaxis and vaccine interventions were used to parameterize a Monte-Carlo simulation model, and to obtain BRD morbidity risk distributions. Body weight (4 levels), cohort size (2 levels), weaning and vaccination programs (12 levels), and metaphylaxis (9 levels) were modeled. Model baseline morbidity corresponded to animals in groups of <150 animals, weighing less than 600 lb., unweaned prior to feedlot arrival, and that received no BRD-related vaccinations or metaphylaxis. For cattle groups weighing less than 600 lb. and purchased in cohorts of <150, the five single interventions administered at feedlot arrival with the lowest median morbidity estimates were all related to metaphylaxis. However, within the same weight and cohort size categories, cattle given florfenicol, oxytetracycline, ceftiofur, danofloxacin, enrofloxacin, presented similar morbidity distributions as weaned cattle receiving viral vaccinations upon feedlot arrival but no metaphylaxis. These results would indicate that when

less effective antimicrobials are being used, non-antimicrobial interventions may be equally useful. Alternatively, animals of less than 600 lb. in small cohorts receiving the most effective antimicrobials had similar BRD morbidity distributions as weaned cattle with increased body weight and cohort size receiving viral vaccinations upon feedlot arrival. Similarly, while keeping body weight and cohort size constant, if cattle arrive at feedlot operations having already received viral and bacterial vaccines, median morbidity estimates would be lower than those obtained by only using any metaphylaxis antimicrobial. Our results provide BRD morbidity estimates for scenarios with common cattle characteristics and disease interventions and enable hypothesis generation for further randomized controlled trials. Given the relevance of BRD to the feedlot industry, and variability in cattle characteristics and potential intervention strategies, field validation of these results is warranted.

Introduction

Most beef cattle in the United States finish their production cycle at large feedlot operations with more than 8,000 animals (United States Department of Agriculture, 2013a). In these operations, the most common and costly disease is bovine respiratory disease (BRD), a multifactorial disease complex that decreases animal welfare and economic returns (Griffin, 1997). Given BRD's complexity, numerous researchers have studied associated risk factors (Baruch Chapter IV), and several interventions are associated with reductions in BRD morbidity risk. Conversely, due to a large number of combinations of risk factors related to cattle characteristics and disease interventions, and despite large research efforts towards its control, it is unclear how to best classify BRD risk consistently and accurately upon arrival to feedlot operations (Amrine et al., 2014; Babcock et al., 2013).

Groups of animals that are purchased together and managed together after arrival to the feedlot are commonly defined as cohorts (Cernicchiaro et al., 2012b). Several small cohorts may be mixed in the same pen to achieve a desired number of animals per pen, which increases animal mixing and BRD risk. Therefore, cohort size may be a proxy for animal mixing. Feedlot operations must classify these cohorts based on their expected BRD risk to provide targeted interventions. Disease interventions like vaccination and metaphylaxis are commonly implemented at the cohort level to manage BRD risk in large feedlots. For example, according to the National Animal Health Monitoring System (NAHMS), of feedlots with more than 1,000 animals, 96% vaccinate, and between 45 to 93% administer metaphylaxis to at least some cattle upon feedlot arrival (United States Department of Agriculture, 2013a).

Recent meta-analyses have contributed to understanding the association of metaphylaxis and BRD morbidity in feedlot cattle (O'Connor et al., 2019a) and the type and timing of

vaccination and BRD morbidity (Baruch Chapter IV and V). Knowledge of the combined effect of these two common disease prevention interventions for BRD morbidity in feedlot cattle could help feedlot producers better target interventions to the specific cohorts of cattle purchased.

Given the complexity of combinations of cattle characteristics and disease interventions, large randomized controlled trials that evaluate multiple disease interventions across cattle characteristics are expensive and impractical. However, modeling disease risk through Monte-Carlo simulations has the advantage of allowing researchers to model combinations of disease interventions when data are available (Dodd et al., 2011; Smith et al., 2010). Although there is a recent risk assessment study in dairy cattle (Maier et al., 2020), no Monte-Carlo simulation model has evaluated the effect of feedlot cattle characteristics and disease interventions to estimate BRD morbidity risk and optimal interventions.

Therefore, the objective of this study was to estimate BRD morbidity risk in cohorts of feedlot cattle based on cohort-specific characteristics using a Monte-Carlo simulation model to estimate morbidity risks, optimal interventions, and develop research hypotheses. For this purpose, data from a prior scoping review and meta-analyses of metaphylaxis and vaccine interventions were used to construct a Monte-Carlo model to simulate cattle characteristics and disease interventions stochastically.

Materials and methods

Conceptual model and data development

A conceptual model was developed using two cohort characteristics (cohort size and group level body weight) and three disease interventions (metaphylaxis, vaccination, and weaning) (**Figure 1**). A quantitative risk estimation model was developed using published data to

establish a baseline morbidity category and simulate BRD morbidity risk for alternate risk disease scenarios (**Figure 1**).

Baseline morbidity, the referent category for all comparisons, was estimated from published studies reporting BRD morbidity in feedlot cattle. The following inclusion criteria were used, 1) cattle were unvaccinated against BRD pathogens ever, 2) cattle were unweaned 30 days before feedlot shipment, 3) cattle were castrated before feedlot arrival, 4) cattle weighed less than 600 lb. upon feedlot arrival, 5) cattle were in cohorts of less than 150 animals upon feedlot arrival, and 6) cattle did not receive metaphylaxis. This baseline distribution was fitted from publications identified in a scoping review of BRD risk factors (Baruch, Chapter IV). From this scoping review, risk categories defined in that publication as "vaccination," "preconditioning," "metaphylaxis," and "dietary supplements" were explored to obtain morbidity estimates from groups of cattle that met the inclusion criteria (**Table 1**). Morbidity data were extracted, weighted by the inverse standard error of morbidity, resampled to account for the precision of each study (giving a higher weight to those studies with larger precision), and a baseline morbidity distribution was fitted using the resampled data and the fit distribution tool in @Risk (Palisade Company, LLC) (**Table 1**). The fitted *beta (1.839, 3.079)* distribution was chosen based on the best fit using AIC.

Data related to modeling the effect of cohort size and body weight were obtained from studies reported from the same scoping review (Baruch, Chapter IV) (**Table 2**). To account for the variability of those study' results for the effect of cohort size and body weight, the minimum value of the confidence intervals of all measures of association was extracted as the minimum of a pert distribution, the average of all point estimates was the most likely, and the highest value was the maximum (**Table 2**).

Model parameters related to metaphylaxis were obtained from a recent meta-analysis (O'Connor et al., 2019a), and modeled using a pert distribution. For these distributions, the minimum was equal to the 2.5 percentile of the 95% credible interval of the reported risk ratio, the mode was the 50 percentile, and the max was the 97.5 percentile (**Table 3**). In order to limit the number of metaphylaxis scenarios modeled, some distributions were combined for those antimicrobials with similar associations with BRD morbidity (O'Connor et al., 2019a) (**Table 3**). Therefore, florfenicol, oxytetracycline, and ceftiofur were combined into "FOC," and danofloxacin and enrofloxacin were combined into "DE." Averages of the minimum, mode, and maximum of each distribution were used to fit the new distributions for these combined antibiotics. Vaccination and weaning program measures of association with BRD morbidity were obtained from a meta-analysis (Baruch, Chapter V). Likewise, for these distributions, the minimum was equal to the 2.5 percentile of the 95% credible interval of reported odds ratio (OR), whereas the mode was the 50 percentile, and the max was the 97.5 percentile (**Table 3**).

Model simulation

The model was run for 10,000 iterations. The baseline morbidity and the scenario with the lowest expected morbidity were tested for convergence by monitoring the change estimated in morbidity risk at 1,000 iteration intervals. When the morbidity percentage point change was less than 1%, the model was assumed to have converged.

The baseline distribution described above (*beta (1.839, 3.079)*) was modified by cohort size, body weight, weaning, vaccination, and metaphylaxis for a 100 animal group using **Eq 1.** For each step on the conceptual model (**Figure 1**), the morbidity distribution was obtained using the OR's distributions (**Tables 2 and 3**), **Equation 1**, and the baseline morbidity (*beta (1.839, 3.079)*).

Equation 1.

$$Morbidity = 100 - \left(\frac{N_{baseline\ healthy} * 100}{(OR_{intervention} * N_{baseline\ sick}) + N_{baseline\ healthy}}\right)$$

Where *Morbidity* is the morbidity for a given step in the conceptual model (**Figure 1**). $N_{baseline\ healthy}$ is the number of healthy animals out of 100 in the prior step of the conceptual model (**Figure 1**). $N_{baseline\ sick}$ is the number of BRD morbid animals out of 100 in the prior step of the conceptual model (**Figure 1**). $OR_{intervention}$ is the OR of a given intervention or cattle characteristic (**Table 2 and 3**).

Input distributions, described in **Tables 2 and 3**, were included in the model as additive effects. However, when metaphylaxis and vaccination were used together, they were adjusted to account for their likely non-additive effects and the sampling correlation between their distributions. The need to account for these non-additive effects and the potential correlation between their distributions is due to the fact that animals that would respond positively to a vaccination program also would likely respond positively to metaphylaxis, making the additive effect of both interventions less than a simple addition of both interventions. This way of including inputs means that for **Equation 1**, OR's of all cattle characteristics and disease interventions are as described in **Tables 2 and 3**. However, the ORs of metaphylaxis and vaccination combined are modified by a sampling correlation and a dependency distribution described as follows.

The sampling correlation accounts for the fact that animals that would respond positively to metaphylaxis would likely respond positively to vaccination. This correlation, therefore, creates correlated samples between the natural logarithm of the odds ratio (lnOR) of metaphylaxis and vaccination. The data to fit this correlation were obtained from studies in which groups of animals were randomized to receive only metaphylaxis or only vaccination.

Thus, using these studies, we measured the correlation between BRD morbidity on similar cattle populations receiving these two interventions. Three studies were identified from the previous scoping review and a conference proceeding, and morbidity estimates were extracted from each group (**Table 4**). A simple Pearson correlation was calculated between the morbidity estimates reported for the metaphylaxis without vaccination and vaccination without metaphylaxis groups reported in **Table 4**.

The dependency distribution is described in **Table 4 and Supplementary Figure 1**. The dependency distribution, to adjust the non-additive effect of metaphylaxis and vaccination combined, was obtained using the data from the meta-analysis of vaccination interventions (Baruch Chapter V). For this purpose, trial arms were classified by vaccination and metaphylaxis status as: 1) no vaccine nor metaphylaxis, 2) vaccine only, 3) metaphylaxis only, and 4) vaccine and metaphylaxis. A network meta-analysis model was run as described previously (Baruch, Chapter V). The dependency distribution was estimated by calculating the difference between the additive ln OR of 2 and 3 compared to 1, versus the ln OR of 4 compared to 1. For those differences, the fit distribution tool in @Risk was used (Palisade Company, LLC), and estimated based on the best fit using AIC.

Finally, ORs of vaccine and metaphylaxis (adjusted and combined) were incorporated in **Equation 1** by sampling the ln OR of each intervention with the described correlation, added together, and multiplied by the dependency distribution.

Median and 95% prediction intervals for each step of the conceptual model were recorded and tabulated for comparison. Cumulative descending probability curves were generated for specific scenarios based on the possible utility of the results. For these specific scenarios, two comparisons were made. First, the best four metaphylaxis interventions and the

best two vaccine interventions upon feedlot arrival were compared for cattle arriving weighing less than 600 lb. and in cohorts of less than 150 animals. These scenarios evaluate feedlot arrival interventions for cattle within the same body weight and cohort size category. Second, the best vaccination programs before feedlot arrival (VB_V_W) and upon feedlot arrival (C_V_W), the best two metaphylaxis interventions (TUL, GAM), and combinations of body weight (<600 lb., 600-700 lb.) and cohort size (<150, >150) were compared. Lastly, based on the most recent national survey of feedlot production (United States Department of Agriculture, 2013a), the probability of exceeding the national median pen-level morbidity estimate of 17% for feedlots of more than 8,000 animals was recorded in order to compare specific scenarios.

Sensitivity analysis

A local sensitivity analysis was conducted to estimate changes in morbidity and the percentage change in the median morbidity risk when changing specific input parameters. The original model (model described above) was repeated while changing one input parameter at a time. The morbidity estimates and the percentage change in the median from the affected scenarios were recorded and compared to the original model. The following inputs were changed in individual models: 1) the dependency between the association of metaphylaxis and vaccination on BRD morbidity was set to the minimum of the distribution, 2) the dependency between the association of metaphylaxis and vaccination on BRD morbidity was set to the maximum of the distribution, 3) the sampling correlation between metaphylaxis and vaccination was set to -0.5, 4) the sampling correlation between metaphylaxis and vaccination was set to 0.0, and 5) the association between weaning and morbidity was modified by body weight categories; decreasing the OR by 10% in each body weight category increase.

A global sensitivity analysis was conducted by estimating the Spearman Rank correlation between input parameter distributions and model morbidity outputs for specific scenarios. The specific scenarios selected were chosen based upon variations of cohort size, weaning, vaccination, and metaphylaxis on cattle arriving at the feedlot weighing less than 600 lb. The following scenarios were chosen: 1) weaned animals in cohorts of <150 that received tulathromycin and viral vaccines upon arrival, 2) unweaned animals in cohorts of <150 that received tulathromycin and viral vaccines upon arrival, 3) weaned animals in cohorts of >150 that received tulathromycin and viral vaccines upon arrival, 4) weaned animals in cohorts of <150 that received viral and bacterial vaccines before feedlot arrival, 5) weaned animals in cohorts of <150 that received viral and bacterial vaccines before feedlot arrival and tulathromycin upon arrival, 6) weaned animals in cohorts of >150 that received viral and bacterial vaccines before feedlot arrival, and 7) weaned animals in cohorts of >150 that received viral and bacterial vaccines before feedlot arrival and tulathromycin upon arrival. Tulathromycin metaphylaxis, viral vaccinations upon arrival, and viral and bacterial vaccinations before feedlot arrival were chosen due to being the most effective interventions to reduce BRD morbidity (among arrival and before arrival interventions, respectively).

Results

BRD morbidity estimates

The mean baseline BRD morbidity was 37.4%, with a median of 35.5% and an interquartile range 21.8–51.6%. Although the prediction intervals overlapped broadly, as body weight and cohort size increased, median BRD morbidity decreased (**Table 5 and 6**). With this decrease, prediction intervals narrowed, indicating a reduction in BRD morbidity variability and decreased probability of high morbidity outcomes (**Tables 5 and 6**). Although metaphylaxis with

FOC and DE antimicrobials did reduce BRD morbidity when compared to baseline, tulathromycin, gamithromycin, tildipirosin, and tilmicosin, respectively, were the most effective antimicrobials across all body weight and cohort categories (**Table 5 and 6**). The 95% prediction intervals for morbidity following metaphylactic treatment of <600 lb. calves with FOC or DE were similar to those of 700-800 lb. and >800 lb. calves that did not receive metaphylaxis (**Table 5**). Similarly, unweaned, unvaccinated calves, in cohorts of >150 had similar morbidity estimates to those unweaned, unvaccinated, <150 calves groups that received DE and FOC.

For each body weight category, weaning alone without vaccination (C_C_W) had a minimal effect on estimated median BRD morbidity compared to not weaning (C_C_NW); the prediction intervals overlapped almost completely (**Table 5 and 6**). Viral vaccinations used on unweaned animals upon feedlot arrival (C_V_NW) increased median morbidity estimates by 2-6 percentage points when compared to unweaned, unvaccinated animals (C_C_NW) (**Table 5 and 6**). However, when weaned animals were given viral, or viral and bacterial, vaccinations at arrival (C_V_W, C_VB_W), the median morbidity estimate was reduced by 7-8 percentage points when compared to unweaned, unvaccinated cattle (**Table 5 and 6**). Animals that were weaned and vaccinated with viral, or viral and bacterial, vaccines upon feedlot arrival (C_V_W, C_VB_W) had similar morbidity estimates as unweaned, unvaccinated calves that received metaphylaxis with FOC and DE (**Tables 5 and 6**). Further, within the same weight category, using those same vaccinations (C_V_W, C_VB_W) on cohorts of >150 animals presented medians with 5-7 percentage points less than on cohorts of <150 animal given FOC and DE (**Table 6**).

Weaned cattle receiving a viral vaccination before feedlot arrival (V_C_W) had 6-percentage points higher morbidity than unweaned, unvaccinated cattle (**Table 5 and 6**). However, weaned cattle that received combinations of viral and bacterial vaccines before feedlot arrival (VB_C_W, VB_V_W) had the lowest morbidity estimates of all vaccine scenarios (**Table 5 and 6**). Additionally, weaned cattle receiving viral and bacterial vaccinations before feedlot arrival (VB_C_W, VB_V_W) had a lower median estimated morbidity than any metaphylaxis antimicrobial applied to unvaccinated, unweaned cattle (**Table 5 and 6**).

Combinations of metaphylaxis and vaccinations for cohort size and body weight categories are depicted in **Table 7**. Given the number of possible risk group combinations, and that in the United States only 5% of cattle weighing >700 lbs are given metaphylaxis (United States Department of Agriculture, 2013a), we focused on cattle weighing <600 or 600–700 lb., and the use of tilmicosin, gamithromycin, and tulathromycin with vaccinations (**Table 7**). Because the tildipirosin estimate was based on a single study, tilmicosin was included in **Table 7**, despite tildipirosin having a slightly lower median morbidity. Similarly, bacterial vaccines upon feedlot arrival were also excluded in order to limit the number of potential interactions due to their estimates being based on a single study (**Table 7**). However, all possible interactions are available upon request to the authors.

While keeping body weight and cohort size constant, combinations of feedlot arrival vaccines and metaphylaxis presented similar median morbidities to those of animals receiving viral and bacterial vaccines before feedlot arrival (VB_C_W, VB_V_W) (**Table 7**). The lowest morbidity estimates, however, were obtained when combining viral and bacterial vaccines before feedlot arrival (VB_C_W, VB_V_W) with tulathromycin (**Table 7**). As cohort size or body

weight increases, similar median morbidities to those of unvaccinated weaned animals receiving tulathromycin were observed for tilmicosin and viral vaccinations (**Table 7**). Similarly, a 45% reduction in median BRD morbidity was observed when comparing weaned calves receiving tilmicosin and a viral vaccination on arrival in cohorts of >150 to weaned calves receiving tilmicosin and no vaccination on arrival in cohorts of <150 (**Table 7**). These variations indicate that different combinations of vaccinations, metaphylaxis, cohort size, and body weight can result in similar BRD morbidities.

BRD cumulative probability estimates

Depicted in **Figure 2** are the descending cumulative probability curves of estimated morbidity risk for combinations of upon feedlot arrival interventions. Unweaned cattle receiving viral, viral and bacterial, or no vaccination upon arrival (C_V_NW, C_VB_NW, C_C_NW) had greater than an 80% probability of exceeding the median pen-level morbidity of 17% (United States Department of Agriculture, 2013a) (**Figure 2**). When weaned animals received viral or viral and bacterial vaccinations upon arrival, those probabilities decreased to 69% and 73%, respectively (**Figure 2**). In contrast, unweaned animals receiving tilmicosin, tildipirosin, gamithromycin, or tulathromycin had 57%, 51%, 45%, and 32% probability of exceeding 17% morbidity, respectively (**Figure 2**). The best combination of upon arrival interventions was achieved by giving viral vaccinations and tulathromycin to weaned animals, in which case, the probability of exceeding 17% morbidity was 27% (**Figure 2**).

Depicted in **Figure 3** are the decreasing cumulative probabilities for combinations of before and upon feedlot arrival interventions for cohort size and body weight combinations. The lowest probabilities of exceeding 17% morbidity were obtained on weaned animals receiving viral and bacterial vaccines before arrival with viral vaccines and metaphylaxis upon feedlot

arrival (5% and 8% for tulathromycin and gamithromycin, respectively) (**Figure 3**). However, a similar probability (9%) was obtained when the same vaccines but no metaphylaxis were used in large cohorts sizes (>150) and animals weighing 600-700 lb. (**Figure 3**). Similarly, when not using metaphylaxis in animals weighing <600 lb., the lowest probability (14%) of exceeding 17% morbidity was obtained when viral and bacterial vaccines before arrival with viral vaccines on arrival were used on weaned animals in large cohorts (VB_V_W_>150) (**Figure 3**).

Sensitivity analyses

The dependency distribution for the combined effect of metaphylaxis and vaccination on BRD morbidity had the highest impact on the scenario sensitivity analysis (**Table 8**). Although the percentage changes on the dependency distribution were as large as 38%, the base morbidity estimates were low; thus, small changes result in large percentage changes (**Table 8**). The sampling correlation between metaphylaxis and vaccination had little impact on BRD morbidity. The assumption of the non-interaction effect between weaning and body weight had a progressively higher impact as body weight categories increased (**Table 8**). Similar to the dependency distribution, the effect of weaning was large in percentage change, from a low baseline morbidity estimates (**Table 8**). During the global sensitivity analysis, results were consistent with the local analysis. The correlation between model outputs and inputs was highest for the input parameters related to the dependency distribution and the baseline morbidity (results not shown).

Discussion

This study is the first to present quantitative estimations of BRD morbidity risk distributions based upon cattle characteristics and disease interventions in feedlot cattle, which could aid in targeting disease control efforts to specific risk categories. Defining morbidity risk

categories for incoming cattle and the potential impact of disease interventions is challenging due to the complex interactions of BRD risk factors. As antimicrobial use faces increased scrutiny and non-antimicrobial approaches for disease control become increasingly important (Wisener et al., 2019), feedlot operators are faced with decision-making challenges each time they purchase cattle. An understanding of the full distribution of risk for incoming lots of cattle based on known characteristics is necessary for making decisions regarding optimal animal health management, performance, and judicious antibiotic use.

The baseline morbidity estimate (C_C_NW) derived from the three publications (Martin et al., 1984; Woods et al., 1972; Woods et al., 1973) was higher than the United States national feedlot survey estimates (United States Department of Agriculture, 2013a). However, the national estimates likely represent groups of cattle with heterogeneous BRD morbidity risks (combinations of vaccines, metaphylaxis, weight, cohort size) and, therefore, a lower and right-skewed distribution would be expected. The group we defined as baseline would not commonly be encountered in commercial feedlot operations as these groups would likely receive metaphylaxis and vaccination. However, using this group as the base-line allows us to estimate the value of metaphylaxis and vaccination in these cattle. Further, the model outputs can be used to compare any defined group to another group of interest.

Increasing a cohort's average body weight reduced the median morbidity estimate and narrowed the prediction interval. This result is not surprising given that body weight is commonly associated with BRD risk and used as a proxy for age. As age increases, animals can cope better with feedlot arrival stress due to a stronger immunological system (Taylor et al., 2010). Similarly, as cohort size increases, as represented in our model, there is less mixing of animals before and upon feedlot arrival, which reduces BRD morbidity risk by reducing animal

commingling from different sources (Hay et al., 2016). The purchase of heavier cattle in larger cohorts could reduce BRD morbidity and create more homogenous morbidity distributions than when <600 lb. cattle in small cohorts are purchased.

Most scenarios using metaphylaxis interventions resulted in a relatively low morbidity risk when compared to baseline morbidity, which is consistent with expected metaphylaxis efficacy (O'Connor et al., 2019a). Although antimicrobial use faces increased scrutiny, the five feedlot arrival interventions with the lowest median morbidity estimates for unweaned, unvaccinated cattle were all related to metaphylaxis. This result indicates that metaphylaxis is valuable in the reduction of BRD morbidity when used as a single intervention (in unweaned and unvaccinated cattle). However, scenarios related to unweaned cattle receiving FOC and DE presented similar median morbidity to weaned animals receiving viral vaccinations upon feedlot arrival. Among feedlot operations larger than 8,000 animals that use metaphylaxis, 20% use FOC or DE as a metaphylaxis antimicrobial (United States Department of Agriculture, 2019). Therefore, randomized control trials could study the relevance of these antimicrobials (florfenicol, oxytetracycline, ceftiofur, danofloxacin, enrofloxacin) when arrival vaccination alternatives may perform similarly in some cattle populations.

Weaning alone, without vaccination, presented slightly lower but similar median morbidity risk to unweaned, unvaccinated cattle. This finding is surprising given the prior belief that feedlot producers have on the importance of weaning (United States Department of Agriculture, 2013b). However, when almost all feedlots use viral vaccines upon feedlot arrival (United States Department of Agriculture, 2013a), the producers' observed benefit of weaning may be given by the fact that unweaned animals given a viral vaccine presented higher BRD morbidity than weaned animals receiving viral vaccinations (**Table 5 and 6**). These results

would indicate that feedlot producers' perceived benefit might be due to the potential interaction of weaning and vaccination, rather than weaning alone. This potential interaction may be different for bacterial vaccines. Bacterial vaccines on unweaned animals performed similarly to viral vaccines on weaned animals, indicating that vaccination programs could be modified based upon weaning status. This finding should be studied further through randomized control trials before making recommendations on vaccination programs based on weaning status. Weaning status, however, may be unknown on most cohorts, given that only 42.5% of cow-calf operators provide buyers with previous management information (United States Department of Agriculture, 2020, 2013b). Thus, implementing optimal decisions for individual cohorts may not be realistic until more complete information is provided to feedlot producers.

Viral and bacterial vaccinations before feedlot arrival (VB_V_W, VB_C_W) presented the lowest BRD morbidity distributions as compared to all other vaccination or metaphylaxis alone scenarios (**Tables 5 and 6**). Although weaned, vaccinated cattle cost more, these interventions (VB_V_W, VB_C_W) present optimal (low) morbidity risk scenarios if purchasing cattle of less than 600 lbs. in small cohorts. Heavier cattle in larger cohorts also had low BRD morbidity distributions when they received viral and bacterial vaccinations before arrival. For example, while keeping cohort weight constant, the model suggested a 30% reduction in median BRD morbidity when using these vaccination programs in larger cohorts (VB_V_W, VB_C_W in cohorts >150), compared to the best (more efficacious) arrival combination of vaccines and metaphylaxis in small cohorts (C_V_W_TUL in cohorts <150) (**Table 7**). Therefore, we could potentially reduce antimicrobial use and obtain lower median morbidity estimates by substituting metaphylaxis for purchase of larger cohort size, increased body weight, and before feedlot arrival vaccinations. These types of hypotheses are normally not tested through randomized control

trials; however, there is potential in studying how combinations of cohort size, body weight, and vaccinations would be associated with BRD morbidity as an alternative to metaphylaxis.

When comparing feedlot arrival interventions, combinations of metaphylaxis and vaccination were the interventions with the lowest BRD morbidity across all body weight and cohort size categories. These results are consistent with the industry expected efficacy and estimates of vaccinations and metaphylaxis use in feedlot cattle (United States Department of Agriculture, 2013a). The benefit of metaphylaxis depended on the vaccination program. For example, the most efficacious antimicrobials (i.e., tulathromycin, tilmicosin, gamythromycin, tildipirosin) when used in combination with arrival viral vaccines (C_V_W) resulted in 40-60% reduction in median expected morbidity compared to arrival viral vaccination (C_V_W) alone (**Table 7**). Alternatively, these combinations of vaccine and metaphylaxis resulted in a 10-20% reduction in median expected morbidity when compared to metaphylaxis alone (**Table 7**). These results indicate that there might be a limited additional value in incorporating viral vaccination upon arrival when already using the most efficacious metaphylaxis antimicrobials.

When comparing all possible combinations of interventions (before and after feedlot arrival), cattle that received metaphylaxis and before feedlot vaccinations (VB_V_W, VB_C_W, B_C_W) presented the lowest morbidities of all scenarios. However, the administration of metaphylaxis to low-risk cattle did not result in large absolute decrease in morbidity risk and would not be common in feedlot operations. For example, only 5% of cattle weighing >700 lbs. (expected low BRD risk) receive metaphylaxis (United States Department of Agriculture, 2013a), indicating that these results' may have limited applicability to the feedlot industry.

Cumulative probabilities were generated because different producers may be interested in evaluating their risk of exceeding different morbidity thresholds. We evaluated the median pen-

level United States BRD morbidity (17%) as reported by NAHMS, but other values can be estimated through the curves in **Figures 2 and 3**. For unweaned, unvaccinated cattle in small cohorts and <600 lb. the most efficacious metaphylaxis antimicrobials decreased the probability of exceeding the reported national median morbidity to 32-57%; however, these risks may still seem relatively high for a risk-averse individual. Feedlot producers could reduce the probability of exceeding this threshold by purchasing cattle that had received before arrival vaccinations or by modifying purchases as to body weight and cohort size (**Figure 3**). These results support the use of preconditioning programs to lower BRD morbidity risk.

Sensitivity analyses allow researchers to identify the impacts that input distributions have on model output, which is perhaps of particular importance for those inputs in which little information is known. The additive effect between metaphylaxis and vaccination, which was defined by the dependency distribution, presented a large proportional impact on morbidity risk estimates. However, the absolute value changes were relatively small, and the prediction intervals broadly overlapped (**Table 8**). While additional data would resolve some uncertainty, the sensitivity analysis results support the overall validity of the estimates used. The sampling correlation in the distribution of metaphylaxis and vaccination did not impact the model output substantially, which was reflected by small percentage-wise and percentage point changes (**Table 8**). The value for this correlation used in the original model (0.51 correlation) seems reasonable given that it would be expected that animals that respond well to one BRD intervention would respond similarly well to another. However, given that this information was obtained from only three studies, further information in this area is warranted.

The main limitations of this study correspond to the lack of data for some input parameters. Specifically, baseline morbidity was estimated based on three publications which

were conducted over four decades ago (Martin et al., 1984; Woods et al., 1973, 1972). This limitation means that if animals' BRD morbidity risk has changed over time, morbidity estimates might be biased. However, we assessed this issue by evaluating those publications critically, and although residual bias may be present, the distributions were deemed acceptable based on cattle characteristics and reported BRD morbidity. This lack of data was due to our strict inclusion criteria for the baseline distribution. As most feedlot operations use some type of metaphylaxis or vaccination, most research trials vaccinate all cattle to represent an "industry standard" situation, which in turn compromises the availability of data for baseline morbidity. Given the few studies that were used to fit cohort size and body weight input distributions, additional targeted trials are needed. While other publications from studies conducted in Australia were available on this subject (Hay et al., 2016, 2014), cattle characteristics and animal handling were not similar enough with the North American cattle industry to use in this model.

The lack of data on the dependency distribution for the combined effect of metaphylaxis and vaccination on BRD morbidity could be mainly attributed to the fact that most studies that report the use of metaphylaxis also reported the use of vaccination due to the industry-standard assumption mentioned above. However, the sensitivity analysis suggests this distribution is not overly influential in our results. A full additive effect was assumed for combinations of cohort size and body weight with vaccination or metaphylaxis. This assumption may be incorrect, but bias was limited by obtaining data on cohort size and body weight through publications in which models estimated direct effects rather than total effects. The sex of cattle cohorts could not be assessed through this study, as the available data was confounded with vaccination and metaphylaxis. The lack of assessment of sex effects means that this residual variability of steer, heifer, and mixed cohorts is present on the morbidity risk estimates. Similarly, details regarding

specific vaccination programs, beyond timing and viral vs. bacterial, were also unavailable for this study. This lack of data comes from having few studies evaluating the same vaccination protocol, which has been pointed out by Baruch (Baruch, Chapters IV and V) and O'Connor et al. (O'Connor et al., 2019b).

Another limitation of our study is related to whether increasing cohort size and body weight would impact the BRD morbidity risk in other sections of the beef production chain (cow-calf, backgrounding). The United States industry is comprised of many small cow-calf operations (United States Department of Agriculture, 2020), and at some point, many cattle go through some type of mixing at low weight categories. Therefore, research questions should address whether mixing at a younger age in a lower animal-density setting (i.e., cow-calf, backgrounding) has less of an impact on BRD morbidity than mixing at an older age in higher animal-density settings (i.e., feedlot). Understanding this relationship could potentially reduce antimicrobial use in feedlot operations without increasing it in other parts of the beef production chain.

While some of these scenarios may present overlapping BRD morbidity risk distributions, decision making in feedlot operations has to factor in economic costs and returns. Therefore, intervention, animal handling, and animal purchasing costs, as well as costs of treatment, animal death, and loss of performance, should be balanced in the decision making process. These types of methodologies are common in agricultural economics and veterinary medicine (Dennis et al., 2018; Smith et al., 2014; Woodill et al., 2017), and should be taken into consideration before implementing management changes. However, this study provides important information related to the full distribution of risk probabilities as well as providing

hypothesis generation for future randomized control trials. Moreover, model validation through commercially available data is needed, given the relevance of BRD to the feedlot industry.

In conclusion, these results aid in defining full risk distributions based on cattle characteristics and management interventions, which has been a limited area of research. We found that when using arrival interventions, groups of weaned cattle vaccinated with viral vaccinations presented similar BRD morbidity risk distributions to those cattle administered FOC and DE antimicrobials. The addition of metaphylaxis to on arrival vaccination further decreased median morbidity risk and decreased the probability of exceeding the national median pen-level BRD morbidity estimate (17%). Similarly, when interventions before feedlot arrival are used, morbidity estimates are the lowest when using viral and bacterial vaccinations with or without metaphylaxis, suggesting pre-feedlot arrival management could be an alternative to metaphylaxis in some situations. Feedlot producers and veterinarians could use these results when defining risk categories and interventions to use upon arrival cattle, while BRD researchers could identify potential areas of research in which more information is needed.

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Table 6.1. Input data for a baseline bovine respiratory disease (BRD) morbidity distribution parameter from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of BRD morbidity in feedlot cattle.

N total	Morbidity	SE	Weight	Reference
32	0.84	0.06	0.09	(Woods et al., 1972)
30	0.60	0.09	0.06	(Woods et al., 1972)
17	0.76	0.10	0.06	(Woods et al., 1972)
405	0.23	0.02	0.28	(Woods et al., 1973)
267	0.30	0.03	0.21	(Woods et al., 1973)
274	0.20	0.02	0.24	(Woods et al., 1973)
36	0.36	0.08	0.07	(Martin et al., 1984)

N total = number of animals in the study, Morbidity = BRD morbidity, SE = standard error of BRD morbidity, Weight = sampling weight assigned for resampling of studies. Weight was calculated as the inverse of the SE.

Table 6.2. Body weight and cohort size input distributions from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of bovine respiratory disease morbidity in feedlot cattle.

Input	Data points	Distribution[1]
Weight (lb.)		**Odds ratio**
<600 (Ref)	NA	Ref
600 - 700	0.52 (**0.25** - 1.01)(Sanderson et al., 2008)	Pert(min = 0.25, mode = 0.79, max = 1.35)[1]
	0.70 (0.69 - 0.93)(Cernicchiaro et al., 2012a)	
	1.08 (0.86 - **1.35**)(Cernicchiaro et al., 2012b)	
	0.84 (0.79 - 0.88)(Cernicchiaro et al., 2012c)	
700 - 800	0.79 (0.59 - 0.81)(Cernicchiaro et al., 2012a)	Pert(min = 0.56, mode = 0.69, max = 0.86)[1]
	0.69 (**0.56** - **0.86**)(Cernicchiaro et al., 2012b)	
	0.60 (0.56 - 0.64)(Cernicchiaro et al., 2012c)	
>800	0.55 (0.44 - **0.69**)(Cernicchiaro et al., 2012b)	Pert(min = 0.36, mode = 0.47, max = 0.69)[1]
	0.39 (**0.36** - 0.43)(Cernicchiaro et al., 2012c)	
Cohort size		**Odds ratio**
<150 (Ref)	NA	Ref
>151	0.64 (0.61 - 0.66)(Cernicchiaro et al., 2012c)	Pert(min = 0.42, mode = 0.65, max = 1.02)[1]
	0.94 (0.87 - **1.02**)(Cernicchiaro et al., 2012a)	
	0.63 (0.59 - 0.67)(Cernicchiaro et al., 2012b)	
	0.43 (**0.42** - 0.45)(Cernicchiaro et al., 2012c)	
	0.73 (0.67 - 0.80)(Cernicchiaro et al., 2012a)	
	0.51 (0.48 - 0.54)(Cernicchiaro et al., 2012b)	

[1]The min is the lowest of all CIs, the max is the highest of all CIs, and the mode is the average of the point estimates. Bold values indicate the minimum and the maximum of the fitted distribution.

Table 6.3. Vaccination, weaning, and metaphylaxis input distributions from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of bovine respiratory disease morbidity in feedlot cattle.

Intervention	**Odds ratio[2]**
Vac and weaning[1]	
C_C_NW (Ref)	Ref
C_C_W	Pert(min = 0.34, mode = 0.84, max = 2.00)
C_V_W	Pert(min = 0.21, mode = 0.60, max = 1.67)
C_V_NW	Pert(min = 0.75, mode = 1.26, max = 2.00)
C_B_NW	Pert(min = 0.13, mode = 0.64, max = 2.41)
C_VB_W	Pert(min = 0.27, mode = 0.68, max = 1.57)
C_VB_NW	Pert(min = 0.62, mode = 1.06, max = 1.78)
V_C_W	Pert(min = 0.72, mode = 1.22, max = 2.11)
B_C_W	Pert(min = 0.11, mode = 0.36, max = 0.88)
B_B_W	Pert(min = 0.19, mode = 0.70, max = 2.27)
VB_C_W	Pert(min = 0.09, mode = 0.16, max = 0.35)
VB_V_W	Pert(min = 0.06, mode = 0.20, max = 0.50)
Metaphylaxis	
No metaphylaxis (Ref)	Ref
Tilmicosin (TIL)	Pert(min = 0.29, mode = 0.43, max = 0.74)
Gamithromycin (GAM)	Pert(min = 0.19, mode = 0.31, max = 0.63)
Tulathromycin (TUL)	Pert(min = 0.12, mode = 0.21, max = 0.49)
Flor, Oxy, Ceft (FOC)	Pert(min = 0.36, mode = 0.58, max = 0.89)[3]
Tildipirosin (TILD)	Pert(min = 0.16, mode = 0.36, max = 0.85)
Dano, Enro (DE)	Pert(min = 0.30, mode = 0.63, max = 1.22)[3]

Vac = vaccination, V = viral, B = bacterial, C = no vaccination, W = weaned, NW = unweaned, Flor = Florfenicol, Oxy = Oxytetracycline, Ceft = Ceftiofur, Dano = Danofloxacin, Enro = Enrofloxacin.

[1]Letters to the left of the first underscore are vaccinations pre-feedlot arrival, and letters to the right of the first underscore are vaccinations upon feedlot arrival. Letters to the right of the second underscore represent weaning.

[2]Min = 2.5 percentile, Mode = median, Max = 97.5 percentile.

[3]The min, mode, and max of these distributions were calculated by averaging the inputs for each antimicrobial in that group.

Table 6.4. Sampling correlation and dependency distribution for the combination of metaphylaxis and vaccination used on a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of bovine respiratory disease morbidity in feedlot cattle.

Input	**Data**		**Distribution**	**Reference**
Dependency[2]	NA	NA	Gamma(shape=17.44, scale =0.03) + (0.42)	Baruch Chapter V
Correlation[1]	Meta no vac	Vac no meta	Correlation = 0.51	
	0.47	0.50		(Frank et al., 2002)
	0.44	0.56		(Frank et al., 2002)
	0.21	0.49		(Munoz et al., 2019)

NA = Not applicable, Meta no vac = metaphylaxis without vaccination morbidity estimates, Vac no meta = vaccination without metaphylaxis morbidity estimates.

[2]Metaphylaxis and vaccination's Ln(OR) is added and multiplied by this distribution to obtain their combined OR. The gamma distribution is depicted in **Supplementary material Figure 1**.

[1]The sampling correlation between the iterations of the OR distribution of metaphylaxis and vaccination interventions.

Table 6.5. Bovine respiratory disease (BRD) median morbidities and 95% predictive intervals for cattle in cohorts of <150 animals by body weight and disease interventions (metaphylaxis and vaccination) from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of BRD morbidity in feedlot cattle.

	Body weight			
Intervention	**<600 (baseline)**	**600 - 700**	**700 - 800**	**>800**
C_C_NW	35.49 (8.03 – 72.71)	29.74 (5.88 – 68.33)	27.64 (5.74 – 64.88)	21.19 (3.94 – 56.49)
Metaphylaxis[1]				
Tilmicosin	19.86 (3.72 – 54.92)	15.87 (2.69 – 49.99)	14.71 (2.56 – 46.04)	10.79 (1.81 – 37.54)
Gamithromycin	15.53 (2.67 – 48.34)	12.07 (1.91 – 43.49)	11.35 (1.85 – 39.74)	8.19 (1.30 – 31.24)
Tulathromycin	11.34 (1.88 – 40.01)	8.75 (1.35 – 34.86)	8.18 (1.32 – 31.80)	5.83 (0.91 – 24.67)
FOC	24.28 (4.72 – 61.54)	19.55 (3.48 – 56.37)	18.26 (3.32 – 52.84)	13.57 (2.31 – 43.86)
Tildipirosin	17.73 (2.94 – 52.62)	13.90 (2.22 – 47.85)	12.98 (2.06 – 43.79)	9.43 (1.42 – 35.49)
DE	26.44 (5.03 – 65.06)	21.16 (3.67 – 60.25)	19.91 (3.58 – 56.59)	14.86 (2.48 – 48.09)
Vaccination[2]				
C_C_W	33.22 (6.68 – 72.11)	27.47 (4.94 – 68.73)	25.50 (4.67 – 64.66)	19.36 (3.28 – 56.16)
C_V_W	26.63 (4.77 – 66.46)	21.40 (3.48 – 61.60)	20.07 (3.36 – 57.85)	14.96 (2.35 – 49.20)
C_V_NW	41.36 (9.68 – 77.79)	33.58 (7.27 – 73.97)	32.93 (6.94 – 71.04)	25.63 (4.88 – 62.68)
C_B_NW	28.22 (4.63 – 70.01)	22.83 (3.48 – 65.55)	21.42 (3.25 – 62.08)	16.00 (2.27 – 53.52)
C_VB_W	28.48 (5.57 – 68.22)	23.07 (4.16 – 63.04)	21.66 (3.90 – 59.84)	16.26 (2.75 – 51.01)
C_VB_NW	37.39 (8.29 – 74.73)	30.92 (6.21 – 70.66)	29.30 (5.98 – 67.46)	22.40 (4.14 – 58.91)
V_C_W	41.40 (9.77 – 78.10)	34.79 (7.22 – 74.27)	32.91 (6.96 – 71.31)	25.44 (4.83 – 63.52)
B_C_W	16.81 (2.82 – 52.25)	13.35 (2.06 – 46.82)	12.29 (1.94 – 43.36)	8.90 (1.38 – 34.62)
B_B_W	30.33 (5.49 – 71.38)	24.75 (4.05 – 67.02)	23.22 (3.81 – 63.61)	17.45 (2.67 – 55.09)
VB_C_W	9.67 (1.47 – 36.67)	7.55 (1.07 – 32.40)	6.88 (1.02 – 28.72)	4.93 (0.71 – 22.07)
VB_V_W	8.91 (1.42 – 33.13)	6.88 (1.02 – 29.03)	6.39 (0.98 – 25.51)	4.52 (0.69 – 19.40)

[1]Metaphylaxis applied to unvaccinated unweaned cattle (C_C_NW cattle); FOC = florfenicol, oxytetracycline, ceftiofur. DE = danofloxacin, enrofloxacin.

[2]Vaccination categories outcomes with no metaphylaxis; Letters to the left of the first underscore are vaccinations pre-feedlot arrival, and letters to the right of the first underscore are vaccinations upon feedlot arrival. Letters to the right of the second underscore represent weaning. V = viral, B = bacterial, C = no vaccination, W = weaned, NW = unweaned.

Table 6.6. Bovine respiratory disease (BRD) median morbidities and 95% predictive intervals for cattle in cohorts of >150 animals by body weight and disease interventions (metaphylaxis and vaccination) from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of BRD morbidity in feedlot cattle.

	Body weight			
Intervention	**<600 (baseline)**	**600 - 700**	**700 - 800**	**>800**
C_C_NW	26.86 (5.36 – 64.17)	21.75 (3.92 – 59.62)	20.29 (3.76 – 55.44)	15.11 (2.60 – 46.64)
Metaphylaxis[1]				
Tilmicosin	14.20 (2.46 – 45.24)	11.17 (1.76 – 40.58)	10.31 (1.71 – 36.57)	7.45 (1.19 – 28.98)
Gamithromycin	10.94 (1.77 – 38.59)	8.47 (1.28 – 34.15)	7.87 (1.22 – 30.42)	5.64 (0.86 – 23.46)
Tulathromycin	7.79 (1.24 – 30.83)	6.02 (0.87 – 26.59)	5.56 (0.86 – 23.71)	3.96 (0.60 – 17.80)
FOC	17.77 (3.14 – 52.31)	13.97 (2.25 – 46.79)	13.01 (2.18 – 43.03)	9.51 (1.51 – 34.64)
Tildipirosin	12.46 (1.98 – 43.07)	9.74 (1.46 – 38.47)	8.97 (1.35 – 34.49)	6.48 (0.95 – 27.09)
DE	19.27 (3.8 – 55.42)	15.13 (2.48 – 50.27)	14.19 (2.36 – 46.60)	10.40 (1.64 – 37.95)
Vaccination[2]				
C_C_W	24.83 (4.37 – 63.97)	20.00 (3.35 – 59.34)	18.67 (3.14 – 55.05)	13.77 (2.18 – 46.78)
C_V_W	19.43 (3.14 – 57.70)	15.26 (2.32 – 52.19)	14.37 (2.18 – 48.67)	10.54 (1.53 – 40.04)
C_V_NW	32.02 (6.50 – 70.11)	26.12 (4.80 – 65.66)	24.58 (4.58 – 62.19)	18.53 (3.13 – 54.46)
C_B_NW	21.03 (3.07 – 61.66)	16.41 (2.28 – 56.14)	15.49 (2.18 – 52.67)	11.33 (1.54 – 43.81)
C_VB_W	21.05 (3.65 – 59.04)	16.66 (2.74 – 53.37)	15.64 (2.56 – 50.36)	11.39 (1.78 – 41.32)
C_VB_NW	28.35 (5.55 – 66.79)	23.20 (4.09 – 62.41)	21.50 (3.93 – 58.30)	16.13 (2.74 – 49.77)
V_C_W	32.04 (6.57 – 70.90)	26.19 (4.75 – 66.22)	24.57 (4.60 – 63.33)	18.43 (3.19 – 54.38)
B_C_W	11.83 (1.87 – 42.40)	9.33 (1.35 – 37.41)	8.58 (1.30 – 34.10)	6.12 (0.89 – 26.35)
B_B_W	22.52 (3.61 – 62.88)	17.95 (2.67 – 57.61)	16.78 (2.25 – 54.34)	12.36 (1.76 – 45.31)
VB_C_W	6.62 (0.95 – 28.12)	5.13 (0.70 – 24.15)	4.72 (0.67 – 21.46)	3.35 (0.46 – 16.26)
VB_V_W	6.11 (0.94 – 25.36)	4.69 (0.66 – 21.64)	4.31 (0.65 – 19.25)	3.05 (0.45 – 14.15)

[1]Metaphylaxis applied to unvaccinated unweaned cattle (C_C_NW cattle); FOC = florfenicol, oxytetracycline, ceftiofur. DE = danofloxacin, enrofloxacin.

[2]Vaccination categories outcomes with no metaphylaxis; Letters to the left of the first underscore are vaccinations pre-feedlot arrival, and letters to the right of the first underscore are vaccinations upon feedlot arrival. Letters to the right of the second underscore represent weaning. V = viral, B = bacterial, C = no vaccination, W = weaned, NW = unweaned.

Table 6.7. Bovine respiratory disease (BRD) median morbidities and 95% predictive intervals from combinations of metaphylaxis, vaccinations, body weight, and cohort size obtained from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of BRD morbidity in feedlot cattle

	Body weight, cohort size, and metaphylaxis			
Vac-Wean Status[1]	**<600 (<150 N)**			
	No meta	**TIL**	**GAM**	**TUL**
C_C_NW	35.49 (8.03 – 72.71)	19.86 (3.72 – 54.92)	15.53 (2.67 – 48.34)	11.34 (1.88 – 40.01)
C_C_W	33.22 (6.68 – 72.11)	18.30 (3.09 – 54.86)	14.22 (2.28 – 47.93)	10.33 (1.54 – 39.08)
C_V_W	26.63 (4.77 – 66.46)	15.48 (2.25 – 51.70)	12.03 (1.80 – 46.25)	9.07 (1.26 – 39.03)
C_V_NW	41.36 (9.68 – 77.79)	25.63 (4.92 – 64.66)	21.01 (3.79 – 59.32)	16.45 (2.61 – 52.07)
C_VB_W	28.48 (5.57 – 68.22)	16.60 (2.61 – 52.84)	13.10 (2.00 – 47.54)	9.97 (1.44 – 39.40)
C_VB_NW	37.39 (8.29 – 74.73)	23.10 (4.11 – 61.57)	18.47 (3.08 – 55.14)	14.13 (2.31 – 48.17)
V_C_W	41.40 (9.77 – 78.10)	25.72 (4.85 – 64.60)	21.03 (3.63 – 59.42)	16.48 (2.63 – 53.25)
VB_C_W	9.67 (1.47 – 36.67)	4.65 (0.68 – 20.90)	3.59 (0.52 – 17.18)	2.67 (0.37 – 13.33)
VB_V_W	8.91 (1.42 – 33.13)	5.02 (0.68 – 23.85)	3.94 (0.52 – 19.18)	2.90 (0.37 – 15.88)
	600 – 700 (<150 N)			
	No meta	**TIL**	**GAM**	**TUL**
C_C_NW	29.74 (5.88 – 68.33)	15.87 (2.69 – 49.99)	12.07 (1.91 – 43.49)	8.75 (1.35 – 34.86)
C_C_W	27.47 (4.94 – 68.73)	14.58 (2.25 – 50.37)	11.01 (1.61 – 42.73)	7.93 (1.11 – 34.76)
C_V_W	21.40 (3.48 – 61.60)	12.17 (1.68 – 46.69)	9.38 (1.29 – 40.76)	7.06 (0.90 – 34.18)
C_V_NW	33.58 (7.27 – 73.97)	20.82 (3.58 – 59.46)	16.79 (2.66 – 54.14)	12.89 (1.94 – 46.89)
C_VB_W	23.07 (4.16 – 63.04)	12.95 (1.96 – 48.16)	10.30 (1.49 – 41.79)	7.76 (1.05 – 34.60)
C_VB_NW	30.92 (6.21 – 70.66)	18.26 (3.04 – 56.79)	14.60 (2.24 – 50.00)	11.17 (1.69 – 42.66)
V_C_W	34.79 (7.22 – 74.27)	20.74 (3.60 – 59.48)	16.88 (2.70 – 54.31)	12.99 (1.90 – 47.82)
VB_C_W	7.55 (1.07 – 32.40)	3.54 (0.50 – 17.44)	2.75 (0.37 – 14.57)	2.02 (0.27 – 11.24)
VB_V_W	6.88 (1.02 – 29.03)	3.85 (0.49 – 20.02)	2.99 (0.38 – 16.22)	2.21 (0.26 – 13.12)
	<600 (>150 N)			
	No meta	**TIL**	**GAM**	**TUL**
C_C_NW	26.86 (5.36 – 64.17)	14.20 (2.46 – 45.24)	10.94 (1.77 – 38.59)	7.79 (1.24 – 30.83)
C_C_W	24.83 (4.37 – 63.97)	12.86 (1.97 – 45.07)	9.86 (1.45 – 38.49)	7.06 (1.03 – 30.26)
C_V_W	19.43 (3.14 – 57.70)	10.83 (1.52 – 42.34)	8.45 (1.15 – 36.38)	6.25 (0.81 – 30.33)
C_V_NW	32.02 (6.50 – 70.11)	18.69 (3.24 – 55.26)	15.11 (2.47 – 50.03)	11.60 (1.73 – 42.13)
C_VB_W	21.05 (3.65 – 59.04)	11.64 (1.70 – 43.11)	9.15 (1.29 – 37.84)	6.84 (0.94 – 30.44)
C_VB_NW	28.35 (5.55 – 66.79)	16.66 (2.76 – 52.13)	13.21 (2.01 – 45.32)	9.86 (1.48 – 38.44)
V_C_W	32.04 (6.57 – 70.90)	18.79 (3.17 – 55.35)	15.14 (2.38 – 50.00)	11.65 (1.70 – 42.99)
VB_C_W	6.62 (0.95 – 28.12)	3.15 (0.45 – 15.24)	2.43 (0.33 – 12.27)	1.80 (0.24 – 9.38)
VB_V_W	6.11 (0.94 – 25.36)	3.39 (0.44 – 17.38)	2.64 (0.34 – 13.84)	1.94 (0.24 – 11.22)
	600 – 700 (>150 N)			
	No meta	**TIL**	**GAM**	**TUL**
C_C_NW	21.75 (3.92 – 59.62)	11.17 (1.76 – 40.58)	8.47 (1.28 – 34.15)	6.02 (0.87 – 26.59)
C_C_W	20.00 (3.35 – 59.34)	10.08 (1.44 – 40.18)	7.60 (1.07 – 33.30)	5.45 (0.73 – 26.09)
C_V_W	15.26 (2.32 – 52.19)	8.46 (1.09 – 37.34)	6.50 (0.85 – 32.03)	4.79 (0.58 – 25.98)
C_V_NW	26.12 (4.80 – 65.66)	14.88 (2.34 – 49.66)	11.82 (1.79 – 44.78)	8.95 (1.26 – 36.97)
C_VB_W	16.66 (2.74 – 53.37)	8.99 (1.28 – 38.33)	7.02 (0.96 – 32.82)	5.28 (0.68 – 26.11)
C_VB_NW	23.20 (4.09 – 62.41)	12.99 (1.99 – 47.09)	10.36 (1.47 – 40.47)	7.69 (1.11 – 33.46)
V_C_W	26.19 (4.75 – 66.22)	14.96 (2.39 – 50.21)	11.84 (1.76 – 44.10)	9.01 (1.21 – 38.29)
VB_C_W	5.13 (0.70 – 24.15)	2.39 (0.32 – 12.68)	1.83 (0.24 – 10.31)	1.35 (0.17 – 7.91)
VB_V_W	4.69 (0.66 – 21.64)	2.59 (0.32 – 14.68)	2.00 (0.25 – 11.51)	1.47 (0.18 – 9.33)

No Meta = no metaphylaxis. Vac-Wean Status = Vaccines and weaning.

[1]Letters on the left of the first underscore represent before feedlot arrival vaccinations, letters on the right of the first underscore represent upon feedlot arrival vaccinations, and letters to the right of the second underscore represent weaning status. C = no vaccination, V = viral, B = bacterial, W = weaned, NW = unweaned. Til = tilmicosin, Gam = gamithromycin, Tul = tulathromycin, TILD = tildipirosin, no_meta = no metaphylaxis.

Table 6.8. Sensitivity analysis results: inputs changed, base model, morbidity, and percentage point change among the different intervention/cattle characteristics affected by that input from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of bovine respiratory disease morbidity in feedlot cattle.

Input changed	Baseline model	Input set to	Base morb[1]	SA morb[1]	Change[2]
Dependency[3]	Gamma(shape=17.44, scale =0.03) + (0.42)	0.56 (min of the distribution)	8.45 (0.45 – 54.20)	10.88 (0.65 – 58.87)	28.76
		1.48 (max of the distribution)	8.45 (0.45 – 54.20)	5.17 (0.22 – 45.88)	-38.82
Correlation[3]	0.51	0.00 (no correlation)	8.45 (0.45 – 54.20)	8.44 (0.47 – 53.03)	-0.12
		-0.50 (opposite than the baseline model)	8.45 (0.45 – 54.20)	8.42 (0.50 – 52.00)	-0.36
Wean*<600 lb.[4]	pert(min = 0.33 mode = 0.83, max = 2.01)	pert(min = 0.34 mode = 0.84, max = 2.00)	NA	NA	NA
Wean*600-700 lb.[4]	pert(min = 0.33 mode = 0.83, max = 2.01)	pert(min = 0.31, mode = 0.76, max = 1.80)	12.65 (1.01 – 59.98)	11.49 (0.93 – 57.17)	-9.16
Wean*700-800 lb.[4]	pert(min = 0.33 mode = 0.83, max = 2.01)	pert(min = 0.28, mode = 0.68, max = 1.62)	11.71 (0.98– 56.05)	9.64 (0.78– 50.90)	-17.68
Wean*>800 lb.[4]	pert(min = 0.33 mode = 0.83, max = 2.01)	pert(min = 0.25, mode = 0.61, max = 1.46)	8.39 (0.67 – 47.22)	6.32 (0.48 – 39.24)	-24.67

[1]Median and credibility interval of bovine respiratory disease morbidity for the scenarios affected by this intervention. Base morb = morbidity in the baseline scenario, SA morb = morbidity in the sensitivity analysis scenario.
[2]Percentage change from the median baseline morbidity to median morbidity of the sensitivity analysis model.
[3]Dependency = additive of metaphylaxis and vaccination. Correlation = sampling correlation between vaccination and metaphylaxis.
[4]The OR of the association between weaning and morbidity is decreased by 10% for each body weight category increase.

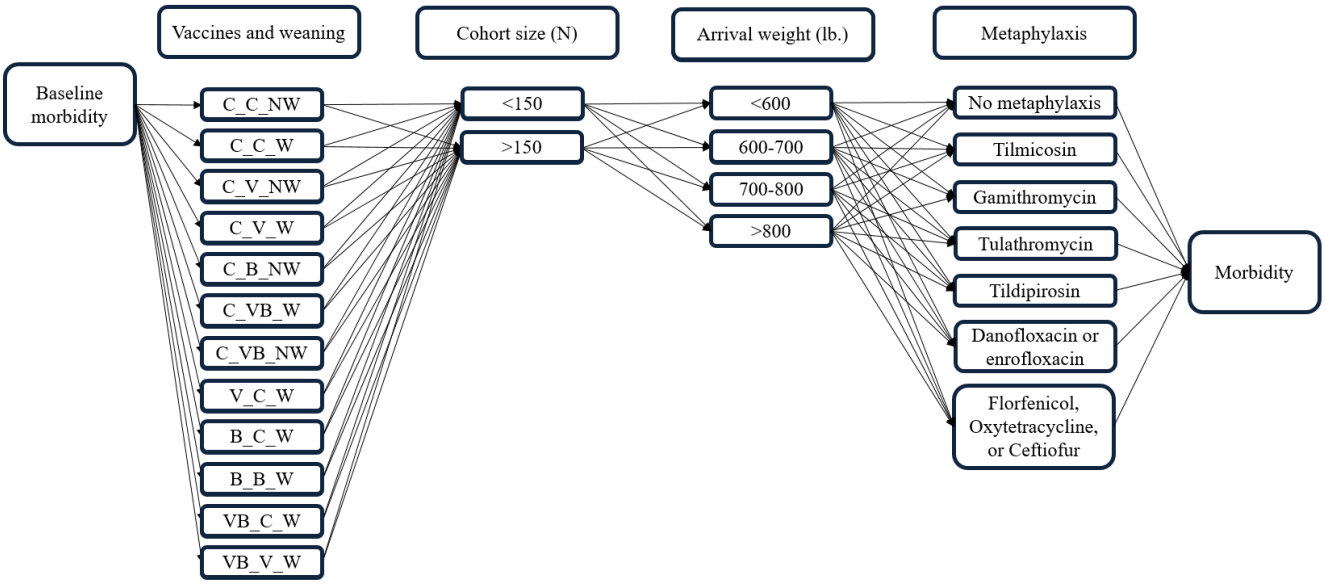

Figure 6.1. Conceptual model of a stochastic Monte-Carlo simulation model of bovine respiratory disease morbidity in feedlot cattle by cattle characteristics and disease interventions.

Arrows represent a pathway. Vaccines and weaning: letters on the left of the first underscore represent before feedlot arrival vaccinations, letters in the middle represent upon feedlot arrival vaccinations, and letters to the right of the second underscore represent weaning status. C = no vaccination, V = viral, B = bacterial, W = weaned, NW = unweaned.

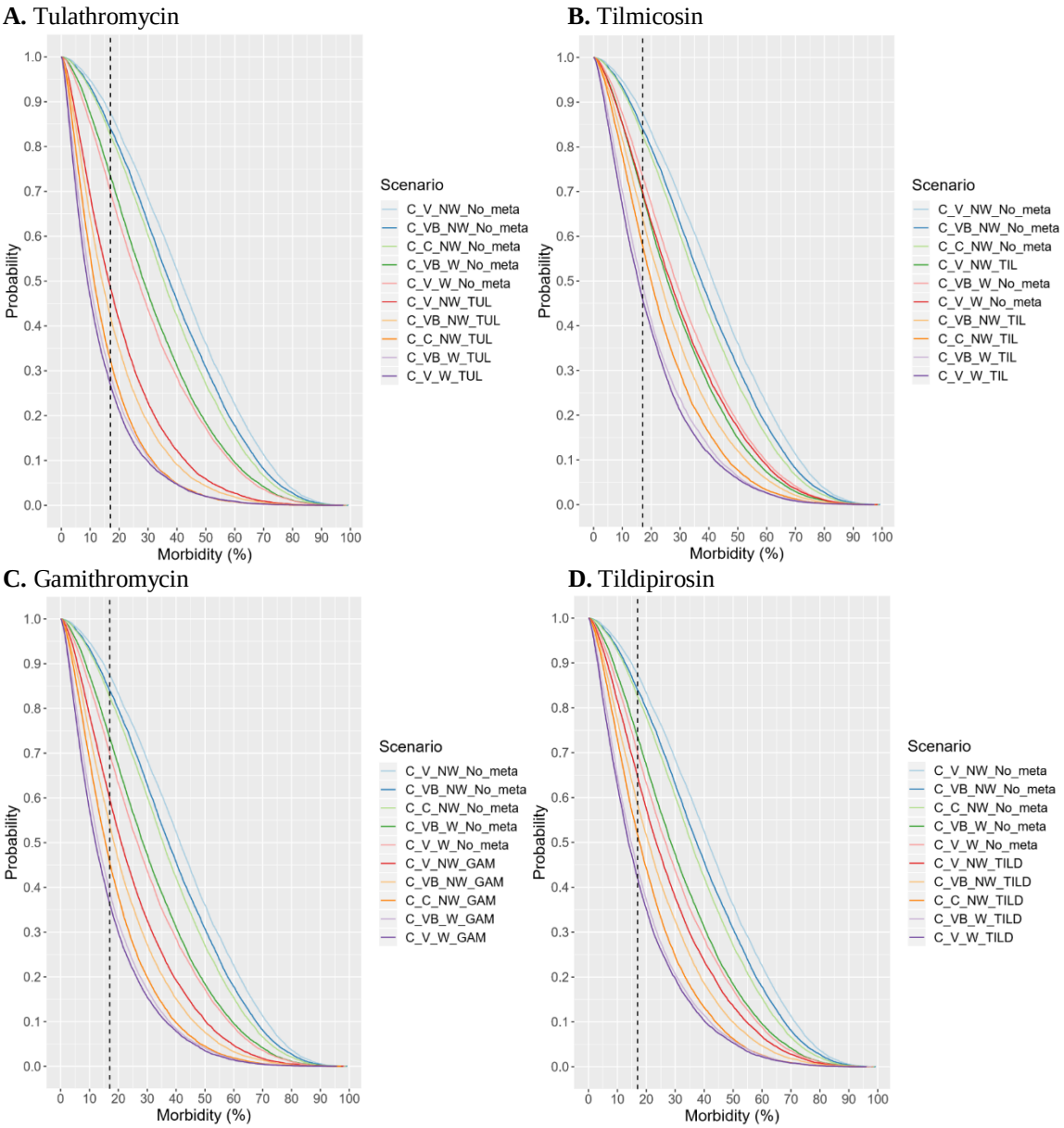

Figure 6.2. Upon feedlot arrival interventions. Descending cumulative probability curves for bovine respiratory disease (BRD) morbidity estimates for cattle less than 600 lb. and in cohorts of <150 animals.

The dashed vertical line represents 17% morbidity. **A.** Vaccination and weaning status compared to tulathromycin. **B.** Vaccination and weaning status compared to tilmicosin. **C.** Vaccination and weaning status compared to gamithromycin. **D.** Vaccination and weaning status compared to tildipirosin. Vaccines and weaning: letters on the left of the first underscore represent before feedlot arrival vaccinations, letters on the right of the first underscore represent upon feedlot arrival vaccinations, and letters to the right of the second underscore represent weaning status. C = no vaccination, V = viral, B = bacterial, W = weaned, NW = unweaned, Til = tilmicosin, Gam = gamithromycin, Tul = tulathromycin, TILD = tildipirosin, no_meta = no metaphylaxis. Curves' labels are arranged in decreasing probability order.

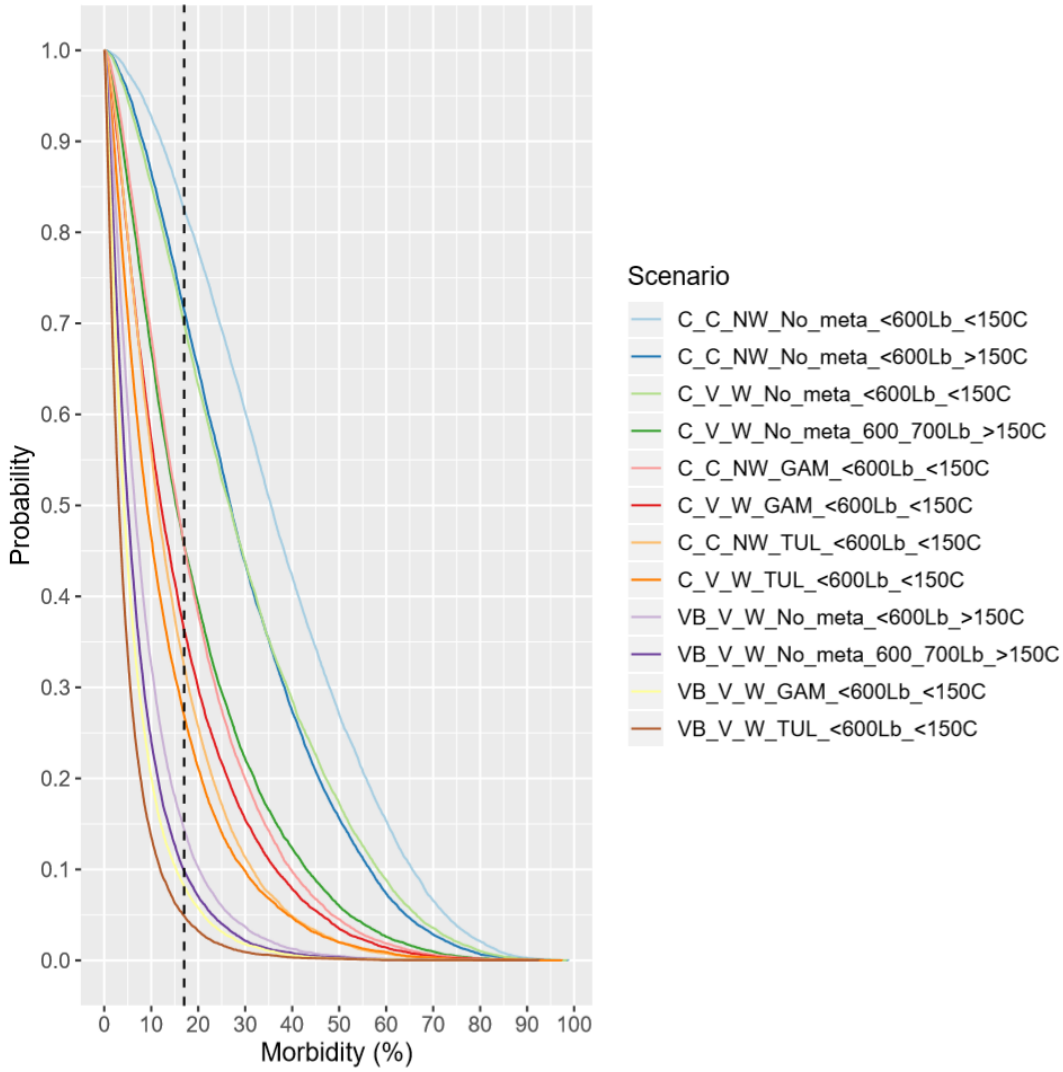

Figure 6.3. Combinations of pre and upon feedlot arrival interventions. Descending cumulative probability curves for bovine respiratory disease (BRD) morbidity estimates.

The dashed vertical line represents 17% morbidity. Vaccines and weaning: letters on the left of the first underscore represent before feedlot arrival vaccinations, letters on the right of the first underscore represent upon feedlot arrival vaccinations, and letters to the right of the second underscore represent weaning status. C = no vaccination, V = viral, B = bacterial, W = weaned, NW = unweaned, Gam = gamithromycin, Tul = tulathromycin, no_meta = no metaphylaxis. Body weight: <600 lb. or 600 – 700 lb. Cohort size: <150c = less than 150 animals, greater_150 = greater than 150 animals. Curves' labels are arranged in decreasing probability order.

Chapter 7 - **Conclusion**

The nature of the BRD complex is multifactorial, with multiple viral and bacterial agents involved in its epidemiology. Thus, we cannot solely rely on pathogen identification for the diagnosis of BRD, which often requires performing a necropsy for disease confirmation. Furthermore, disease presentation is non-specific, and there are no accurate antemortem diagnostic tests available. This disease presentation leaves feedlot operators with few diagnostic options, often relying on clinical signs and rectal temperatures to identify BRD cases (which are not accurate for BRD detection). Also, and because there is currently no gold standard test available for test validation, the study and development of diagnostic methods for BRD is complex and expensive.

We addressed the issue of BRD diagnostics in chapters two and three of this thesis by studying the performance of point-of-care diagnostic methods for BRD detection and evaluation of disease progression. Using an experimental challenge study, we reproduced a field case scenario of acute BRD and studied the performance of the following diagnostic methods in assessing BRD progression: clinical illness scores (CIS), rectal temperature, facial thermography, ultrasound, computer-aided stethoscopes, pulse oximetry, and leukocyte differentials.

While CIS provides important disease progression information, scoring is subjective and clinical signs for BRD are non-specific. Similarly, animals showing severe CIS may be less likely to recover due to large amounts of lung consolidation. Rectal temperature and facial thermography provide objective measurements but have low specificity. Thus, treatment regiments should not be established based only on body temperature, given the inability to differentiate between bacterial and viral infections, and because antimicrobials are not effective

against viral infections. Nonetheless, if remote temperature measurements can be implemented effectively, diagnostic sensitivity for asymptomatic cattle could be improved, and follow-up confirmatory tests could be used to enhance specificity.

Ultrasound, computer-aided stethoscopes, and pulse oximetry are objective means of assessing disease progression by helping to determine lung consolidation. Lung consolidation could be used as a proxy for bacterial infection and thus help with tailoring antimicrobial treatments. However, these diagnostic methods may be impractical as screening tools in feedlot operations, given that animals have to be brought to an animal handling facility. Due to their availability for use in the feedlot pen, clinical signs and measurements of facial temperature could be used as a screening tool and ultrasound, computer-aided stethoscope, or pulse oximetry measurements could complement the diagnosis by aiding in confirming respiratory involvement and disease severity (i.e., lung consolidation).

Leukocyte differentials, while not specific to BRD, could be useful to detect bacterial involvement through changes in cell concentrations over time and aid in the decision of using antimicrobial drugs. For example, lymphocytes, neutrophils, lymphocytes/neutrophils ratio, and fibrinogen could be used to assess disease progression as these cell types/results presented changes with respect to disease progression and bacterial inoculation. Except for a few assay differences (i.e., differentiation of band and segmented neutrophils, and assessment of plasma protein and fibrinogen by the laboratory leukocyte differential), the performance of both blood leukocyte differentials assays used was comparable in terms of magnitude and direction of association with disease progression. Although further assessment of the point-of-care leukocyte differential is warranted for test validation (with naturally occurring BRD), this challenge study

provided key data for the design of field validation studies, which could be an area of further research.

The applicability to field conditions is a critical factor in diagnostic methods for feedlot cattle. Sample collection and turnaround time are key aspects to field application of diagnostic methods, as treatment success decreases the longer an animal stays at the hospital facilities (Apley, 2006). Nevertheless, not many point-of-care diagnostic methods have been evaluated previously or comprehensively. Except for the laboratory leukocyte differentials studied in this thesis, all the other diagnostic methods report almost immediate results. Within these diagnostics, ultrasound presents the longest processing time, and even then, results can be obtained within minutes.

Other practical considerations for the applicability of these diagnostic methods are their cost and the level of training required for their use. Some producers may consider these diagnostic methods costly, but given the costs associated with BRD, the implications of improving diagnostic test characteristics are evident. An economic analysis of the advantages of using one or more combinations of these diagnostics comprises an area of further research. For example, CIS and rectal temperature could be compared to some combination(s) of these new diagnostic methods using a partial budget incorporating the costs (diagnostic test) and savings (reduction of un-necessary treatments, increased weight gains, reduced deaths) of each diagnostic scheme. As for the levels of training, these are different for each method, but most methods (except for ultrasound and laboratory leukocyte differentials) can be learned quickly, which improves their applicability. Alone or in combination, the diagnostic methods that have been described could be implemented to improve BRD case definition and early diagnosis.

While experimental challenge studies may not represent the typical onset of disease, given infection is achieved via inoculation of naïve calves with high pathogen doses, challenge studies are sometimes the only option available when there are no benchmark antemortem diagnostic tests available. Further research should be conducted to obtain estimates of test sensitivity and specificity and to validate these point-of-care diagnostics in cattle with naturally occurring BRD. Guidelines for the validation of diagnostic methods and to obtain estimates of test sensitivity and specificity are common in the literature (OIE, 2017).

In chapter four, we mapped the published research on risk factors for BRD morbidity using a scoping review. We retrieved 141 publications and verified that some risk factors were more commonly identified than others. Metaphylaxis, vaccination, dietary supplements, and preconditioning programs were studied extensively. Although the reason why more publications focused on these risk factors than others remains unclear, it is probably related to the potential market value of these interventions for BRD prevention, the availability of funding, effectiveness (perceived or real), and widespread use of some of these interventions (United States Department of Agriculture, 2013; O'Connor *et al.*, 2019).

Conversely, limited data were found in areas of research related to animal management, which is likely due to limited funding availability. Risk factors related to transportation, BVD-PI, and animal management (mixing, exposure to a sale barn, cohort size, feedlot-arrival body weight) were rarely published. Given the known association between animal stress and BRD, these areas could be considered research priorities. Mapping the existing literature on risk factors for BRD morbidity allows researchers to direct efforts towards those areas where little information is available, optimizing research efforts aiming at reducing the burden of BRD to the beef cattle industry.

Vaccination programs for BRD prevention are commonly used on cattle arriving at feedlots, but their effect on BRD morbidity is unclear. Likewise, BRD risk definitions for incoming animals are difficult to determine, due to the lack of knowledge related to the impact and interaction of the different risk factors. This knowledge gap leads to difficulties in implementing targeted interventions for BRD control, compromising the planning of such interventions. We aimed at addressing these difficulties and knowledge gaps by conducting a network meta-analysis and a quantitative risk assessment (chapters five and six).

In chapter five, we described the application of a meta-analysis methodology to study the association of BRD vaccination programs (implemented before or upon feedlot arrival) and BRD morbidity. We found that there is little evidence to support the use of most BRD vaccination programs upon feedlot arrival. An explanation for these results is that vaccinated cattle may take up to 21 days to develop immunity, whereas most BRD cases in high-risk cohorts occur before that time frame (Babcock *et al.*, 2010; Edwards, 2010). In addition, cattle stressed and compromised following transportation and arrival may have a suboptimal immune response to vaccination. Compared to non-vaccination, we observed decreased odds of BRD morbidity when vaccination programs (viral and bacterial or bacterial alone) were used before feedlot arrival. These results indicate that the implementation of vaccination programs before feedlot arrival could contribute to the prevention of BRD morbidity, as animals have the time needed to develop immunity before exposure to feedlot pathogens. Viral vaccines alone, before or after feedlot arrival, were not associated with a reduction of BRD morbidity, when compared to other vaccination programs or no vaccination. However, given the limited number of studies, more research should be conducted due to the common use of viral vaccines.

The potential confounding effect of weaning on the association of feedlot arrival vaccines and BRD morbidity is unclear. More information is needed to make specific recommendations regarding vaccine types and timing for incoming cattle characteristics (weaning, castration, travel through sale barn, bunk broke, metaphylaxis). As it seems that bacterial rather than viral vaccines may be responsible for vaccine protection against BRD morbidity when compared to non-vaccination, further studies should aim at understanding this relationship.

In chapter six, we provide BRD morbidity risk estimations based upon cattle characteristics and common disease interventions using a Monte-Carlo simulation model. These risk estimations are key to the feedlot industry as current feedlot risk predictions are often inaccurate (Amrine, White and Larson, 2014). Optimization of resources through accurate disease strategy planning could be achieved by understanding the risk of incoming animals and the impact of common interventions.

In this risk assessment, we concluded that non-weaned cattle in small cohorts with group weights lower than 600 lb. had the highest morbidities when no interventions were implemented, or when viral vaccines were administered upon arrival. We also concluded that metaphylaxis was an effective control measure upon arrival. However, effects of metaphylaxis were dependent on the antibiotic used, with tilmicosin, gamithromycin, tulathromycin, and tildipirosin presenting the lowest morbidities among the metaphylaxis interventions. Weaned animals that received viral, or viral and bacterial, vaccines upon arrival had similar median morbidities to unweaned, unvaccinated animals receiving florfenicol, oxytetracycline, ceftiofur, danofloxacin, enrofloxacin, which means that alternatives to metaphylaxis could be investigated (e.g., management and vaccination practices).

Moreover, feedlot operators can lower the probabilities of exceeding a given morbidity cut point by targeting calves with viral and bacterial vaccinations pre-feedlot arrival than by using a metaphylactic antimicrobial. Although vaccine-metaphylaxis combinations are the most effective interventions to reduce BRD morbidity, in most scenarios, there was no extra reduction in BRD morbidity by administrating arrival vaccines. Therefore, it is unclear whether animals that will receive metaphylaxis also should be vaccinated upon arrival. Similar results to these combinations of vaccine-metaphylaxis can be obtained by increasing average weight and cohort size paired with vaccination, which could contribute to reducing the use of antimicrobials.

Although the results obtained through the risk assessment are useful in defining risk distributions and obtaining probabilities for exceeding morbidity cut points, economic analyses must be performed to evaluate the economic benefit of the different disease scenarios. Future areas of research should aim at answering research questions related to the comparison of different combinations of cohort size, body weight, vaccinations, and metaphylaxis. The results from this risk assessment may help feedlot producers and veterinarians define risk categories and then decide which interventions to use in cattle upon feedlot arrival. At the same time, these results point to potential research areas in which randomized control trials could be conducted. For example, combinations of cohort size, body weight, vaccinations, and metaphylaxis could be evaluated, which are hypotheses not normally tested through randomized control trials.

In conclusion, the diagnostic methods evaluated in the first chapters of this thesis comprise useful tools for assessing disease progression, which in turn may aid in improving BRD case definition and tailor the use of antimicrobials according to disease severity. Furthermore, scientific literature on the use of metaphylaxis, vaccines, preconditioning, and dietary supplements in feedlot cattle has been published extensively, suggesting that research

funding and real or perceived effectiveness of these interventions may be driving research questions on risk factors for BRD. Upon evaluation of vaccination programs, viral and bacterial vaccinations pre-feedlot arrival were the most effective vaccines at reducing BRD morbidity. As for the attribution of BRD risk categories to incoming cattle, different characteristics and associated risk factors (e.g., weaned vs. non-weaned, smaller vs. larger cohort sizes, and low vs. high body weight), as well as different combinations of interventions (e.g., different vaccination types and/or metaphylaxis) were considered, and results may contribute to the decision of which strategies to implement.

As we reflected on the previous chapters and on the applicability of the results we presented, we also considered other research questions that could point to future directions in research efforts. These efforts will hopefully advance our knowledge of BRD prevention and control strategies. Therefore, targeting issues that encompass the economic burden of disease and market demands for safe beef, while fostering a sensible use of antimicrobials and, ultimately, promoting the welfare of cattle and the protection of the environment.

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Appendix A - Supplementary material

Chapter 2 supplementary table 1. Dates and reasons for euthanasia of 30 calves inoculated with IBR and *Mannheimia haemolytica* during a 14 trial.

Days	Live	Euthanized	Died[1]	Remaining
0-5	30	0	0	30
6	30	5	0	25
6.5	25	0	0	25
7	25	5	0	20
7.5	20	0	0	20
8	20	0	0	20
9	20	5	0	15
10	14[2]	2[3]	1	12
11	12	7[4]	0	5
12	5	0	0	5
13	5	5	0	0

[1]Died of other reasons than euthanasia (i.e., bovine respiratory disease).
[2]One died during the night.
[3]Euthanized due to a clinical illness score (CIS) of 4.
[4]Five were scheduled to be euthanized by protocol; two had to be euthanized due to a CIS of 4.

Chapter 2 supplementary table 2: descriptive statistics of clinical measurements in calves inoculated with IBR and *Mannheimia haemolytica*, by study day.

	Study Day										
Variable, unit	0	1	2	4	6	6.5	7	7.5	9	11	13
Body weight, kg											
Mean	465.7	-	-	-	445.6	-	427.2	-	435.3	414.8	394.8
Median	473	-	-	-	454	-	424	-	441	410	380
SD	31.6	-	-	-	29.7	-	31.5	-	44.4	31.8	41.2
Range	400-516	-	-	-	384-490	-	370-478	-	346-518	364-476	354-462
Ultrasound, %[1]											
Mean	0.1	-	-	-	0.1	-	5.2	-	16.9	22.5	16.0
Median	0.0	-	-	-	0.0	-	5.0	-	16.3	23.8	17.5
SD	0.5	-	-	-	0.5	-	3.5	-	6.5	9.0	6.3
Range	0.0-2.5	-	-	-	0.0-2.5	-	0.0-12.5	-	7.5-30	7.5-35	10-25
Rectal temperature, °C											
Mean	39.1	38.9	40.3	40.9	40.6	41.7	40.7	41.2	39.8	40.1	39.7
Median	39.0	38.9	40.3	40.9	40.6	41.7	40.8	41.2	40.2	40.4	39.9
SD	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.3	1.3	0.8	1.1
Range	38.7-39.6	38.3-39.9	39.5-41.3	40.3-41.5	39.5-41.1	40.6-42.5	39.7-41.4	40.6-41.7	36.8-41.6	38.1-40.9	38-40.8
Facial thermography temperature, °C											
Mean	38.1	37.5	38.5	39.8	39.4	40.4	39.2	39.7	36.8	38.4	36.5
Median	38.2	37.6	38.4	39.7	39.4	40.4	39.1	39.8	37.5	38.8	36.7
SD	0.6	0.6	0.7	0.7	0.6	0.8	0.5	0.4	2.9	1.7	0.9
Range	36.4-38.8	35.6-38.3	37.1-39.9	37.8-40.4	38.2-40.5	38.7-41.6	38.2-40.3	39.1-40.3	26.6-39.2	34.0-40.7	35.0-37.1
Oxygen saturation, %[2]											
Mean	97.9	97.6	98.1	97.8	95.5	94.4	95.4	95.9	92.0	91.5	93.2
Median	98.0	98.0	99.0	98.0	96.0	95.0	95.0	96.0	94.5	93.0	95.0
SD	1.8	1.7	2.0	1.9	2.1	2.2	2.2	2.9	8.4	6.3	3.8

Range	93-100	93-100	94-100	95-100	93-100	90-100	92-99	90-100	63-99	82-100	87-96
Total Calves	30	30	30	30	30	25	25	20	20	12	5

[1]Ultrasound (%): mean percentage consolidation between animal's left and right side.
[2]Oxygen saturation measured by a pulse oximeter.

Chapter 3 supplementary table 1. Descriptive statistics of clinical pathology parameters reported by a laboratory-based assay in calves inoculated with BHV-1 on day zero and *Mannheimia haemolytica* on day 6, by study day.

		Study day										
		0	1	2	4	6	6.5	7	7.5	9	11	13
Cell type[1], unit	Ref lim[2]											
Leuko, k/µL	5.0-10.0											
Mean		12.1	12.2	10.8	10.4	9.7	9.5	12.1	9.4	9.6	7.1	5.9
Median		12.3	11.4	10.2	9.7	9.1	8.9	11.2	8.5	8.2	6.2	6.0
SD		2.5	3.0	2.5	2.5	2.8	3.7	5.4	3.6	3.5	3.6	2.5
Range		8.0-17.3	6.8-18.5	5.9-16.3	7.1-17.7	6.3-21.7	5.9-25.0	7.4-35.2	5.9-22.3	5.6-16.8	3.6-16.6	2.5-8.5
Lymph, k/µL	2.5-7.5											
Mean		5.7	4.9	4.8	5.0	5.7	2.8	3.6	3.3	2.8	2.8	3.3
Median		5.4	4.7	4.4	4.6	5.0	2.7	3.5	3.1	2.7	2.5	3.1
SD		1.3	1.4	1.7	1.5	2.1	1.1	1.0	1.0	1.2	1.3	1.9
Range		3.4-9	2.4-8.4	2.7-9.3	2.7-8.5	3.6-14.3	1.4-5.1	2.1-5.8	1.5-5.3	0.9-5.9	1.4-5.6	1.1-5.3
Seg neut, k/µL	1.0-5.0											
Mean		5.1	5.6	4.9	4.3	2.9	4.8	6.6	4.9	4.0	3.1	1.6
Median		5.4	5.1	5.0	4.1	2.8	4.1	5.3	3.7	3.2	1.7	2.1
SD		1.6	2.2	1.5	1.6	1.3	3.0	4.8	3.6	2.6	3.0	0.9
Range		2.3-8.0	2.0-11.7	2.2-9.2	1.5-8.7	1.1-6.7	1.3-17.2	2.8-27.1	1.7-17.6	0.4-9.1	0.8-10.9	0.1-2.4
Neut/lymph, k/µL	NA											
Mean		0.9	1.3	1.1	0.9	0.6	1.9	1.9	1.7	1.8	1.3	0.5
Median		0.9	1.0	0.9	0.9	0.5	1.8	1.6	1.3	1.6	0.7	0.5
SD		0.4	0.8	0.5	0.4	0.3	1.1	1.1	1.5	2.0	1.2	0.3
Range		0.4-1.7	0.5-4.9	0.5-2.2	0.3-1.9	0.2-1.3	0.5-4.1	0.7-5.5	0.4-6.5	0.1-9.1	0.3-3.5	0.1-0.7
Monocytes, k/µL	0.0-0.8											
Mean		1.2	1.5	0.9	1.1	1.0	1.5	1.2	0.9	0.9	0.4	0.6
Median		1.1	1.3	0.7	1.0	1.0	1.3	1.0	0.8	0.9	0.4	0.5
SD		0.6	0.8	0.4	0.5	0.5	0.6	0.6	0.4	0.4	0.2	0.3
Range		0.5-2.2	0.5-4.4	0.4-1.9	0.3-2.4	0.3-2.5	0.3-2.5	0.3-2.4	0.4-2	0.2-1.8	0.1-0.7	0.3-1

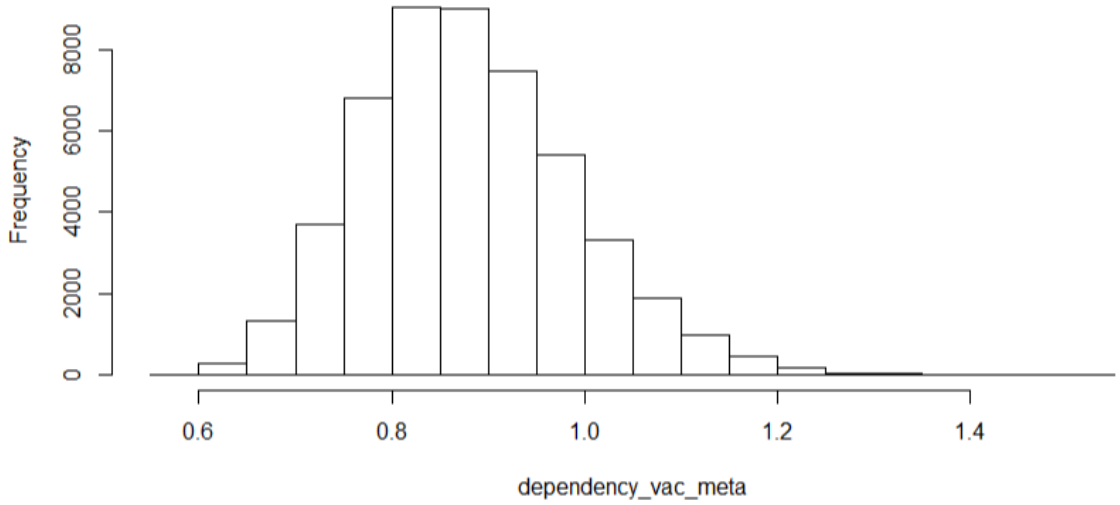
Hct (spun), %	26.0-42.0											
Mean		35.4	35.5	34.4	33.1	32.8	32.4	34.4	35	33.9	35.7	35.8
Median		35.5	35.0	34.0	33.0	33.0	32.0	34.0	35.0	33.0	34.5	36
SD		3.4	3.4	3.0	2.7	3.2	2.6	3.1	3.0	5.0	4.1	2.9
Range		30.0-46.0	30.0-44.0	30.0-43.0	27.0-41.0	28.0-39.0	28.0-37.0	29.0-42.0	29.0-41.0	26.0-46.0	30.0-43.0	31.0-38.0
Hct calculated, %	24.0-46.0											
Mean		32.3	32.2	31.1	29.9	29.7	28.7	30.6	31.4	31.7	32.2	33.8
Median		32.0	32.0	30.8	30.2	29.5	28.2	30.0	31.0	31.0	30.5	35.0
SD		3.3	2.9	2.7	2.4	2.9	2.2	2.5	2.6	4.9	4.0	2.9
Range		27.2-43.0	28.0-40.0	27.1-40.0	25.7-36.0	24.3-35.5	24.4-32.7	26.0-36.0	26.0-37.0	25.0-43.0	27.0-40.0	29.0-36.0
Hemoglobin, g/dL	8.0-15.0											
Mean		12.5	12.3	11.8	11.6	11.6	11.3	11.9	12.2	12.2	12.4	12.5
Median		12.6	12.3	11.8	11.7	11.6	11.2	11.7	12.1	12	11.8	12.9
SD		1.3	1.1	1.2	0.9	1.1	0.9	0.9	1.0	1.7	1.5	0.8
Range		10.3-16.6	10.4-15.3	7.8-14.9	9.9-14.2	9.5-13.9	9.6-13.1	10.1-13.9	9.9-14.5	10.0-16.2	10.5-15.1	11.2-13.2
Cell hemoglobin, g/dL	No ref											
Mean		12.4	12.2	11.8	11.4	11.4	11.0	11.6	12.0	12.0	12.3	12.7
Median		12.5	12.3	11.7	11.6	11.5	11.0	11.5	11.9	11.7	11.7	13.1
SD		1.2	1.1	1.0	0.9	1.1	0.8	1.0	1.0	1.7	1.5	0.9
Range		10.2-16.3	10.7-15.3	10.1-14.9	9.6-13.9	9.3-13.8	9.3-12.7	9.9-13.7	9.9-14.3	9.7-15.8	10.3-14.8	11.3-13.5
Fibrinogen, mg/dL	300-700											
Mean		423.3	460.0	443.3	453.3	553.3	560.0	604.0	570.0	755.0	825.0	860.0
Median		400.0	500.0	500.0	450.0	550.0	500.0	600.0	600.0	800.0	900.0	900.0
SD		85.8	67.5	133.1	163.4	104.2	125.8	176.7	213.0	114.6	196.0	167.3
Range		300-600	300-600	100-700	100-800	400-900	400-900	400-1200	100-1000	500-900	500-1000	700-1100
Plasma protein, g/dL	7.0-9.0											
Mean		7.6	7.4	7.5	7.4	7.2	7.0	6.9	6.9	7.2	7.3	7.7
Median		7.5	7.3	7.5	7.3	7.1	7.0	6.8	7.0	7.2	7.5	7.8
SD		0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.4	0.5	0.3
Range		7.1-8.5	6.9-8.3	7-8.2	6.5-8.1	6.6-8.1	6.4-7.7	6.1-7.8	6.2-7.9	6.3-7.8	6.2-7.9	7.2-8
Basophils, k/µL	0.0-0.2											

Mean		0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Median		0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SD		0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Range		0.0-0.3	0.0-0.3	0.0-0.3	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.0	0.0-0.1	0.0-0.0	0.0-0.1
Eosinophils, k/µL	0.0-1.6											
Mean		0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Median		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SD		0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Range		0.0-0.6	0.0-0.8	0.0-0.8	0.0-0.1	0.0-0.1	0.0-0.1	0.0-0.0	0.0-0.1	0.0-0.0	0.0-0.0	0-0
Band neut, k/µL	0.0-0.2											
Mean		0.0	0.0	0.0	0.0	0.0	0.4	0.7	0.3	1.1	0.6	0.2
Median		0.0	0.0	0.0	0.0	0.0	0.2	0.5	0.1	0.8	0.5	0.1
SD		0.0	0.0	0.0	0.0	0.0	0.5	0.7	0.4	1.0	0.5	0.2
Range		0.0-0.0	0.0-0.1	0.0-0.0	0.0-0.1	0.0-0.2	0.0-1.5	0.0-2.8	0.0-1.6	0.0-3.4	0.0-1.8	0.0-0.4
Total calves		30	30	30	30	30	25	25	20	20	12	5

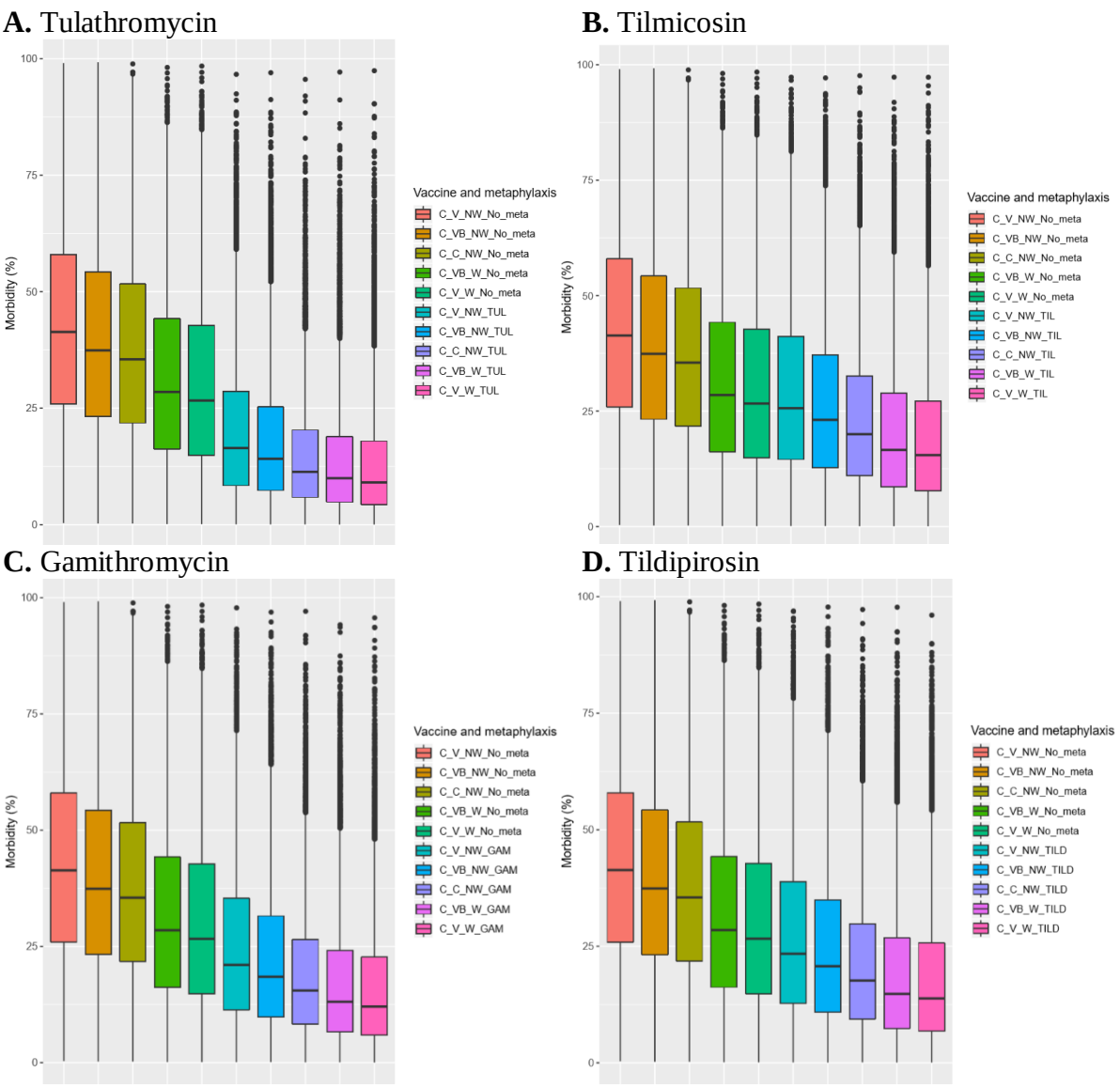
[1]Leuko=Leukocyte, Lymph=Lymphocyte, Seg neut=Segmented neutrophils, Neut/lymph=Segmented neutrophils/lymphocyte ratio, MCV=Mean corpuscular volume, MCH=Mean corpuscular hemoglobin, MCHC=Mean corpuscular hemoglobin concentration, Hct=Hematocrit, Band neut=Band neutrophils.
[2]Reference intervals, as provided by the Kansas State Veterinary Diagnostic Laboratory.

Chapter 3 supplementary table 2. Descriptive statistics of clinical pathology parameters reported by the point-of-care blood leukocyte differential assay in calves inoculated with BHV-1 on day zero and *Mannheimia haemolytica* on day 6, by study day.

	Study day										
	0	1	2	4	6	6.5	7	7.5	9	11	13
Variable, unit											
Leukocytes, k/µL											
Mean	14.4	14.7	13.3	12.2	11.0	11.0	14.5	11.1	11.4	8.3	7.6
Median	15.0	13.6	13.0	11.7	10.4	10.5	12.5	10.0	10.1	7.5	7.1
SD	2.9	3.7	3.1	2.9	3.1	4.5	6.8	4.4	4.2	3.9	3.6
Range	9.0-19.6	7.6-23.0	6.8-19.6	6.8-20.3	6.9-24.9	6.7-29.9	7.5-43.8	6.4-27.0	4.8-19.9	4.0-18.0	3.0-12.2
Lymphocytes, k/µL											
Mean	7.2	7.1	6.8	6.5	6.3	4.7	5.6	4.1	4.2	2.8	4.1
Median	6.8	7.0	6.5	6.3	5.9	4.4	5.2	4.0	3.8	2.1	3.5
SD	1.9	1.9	1.9	1.4	1.8	1.7	2.0	1.2	1.6	1.7	2.4
Range	4.5-12.0	3.1-11.8	3.4-11.1	3.7-10.3	4.3-14.2	2.8-10.8	3.4-13.5	2.4-6.3	1.6-7.2	1.5-7.4	1.3-7.7
Segmented neutrophils, k/µL											
Mean	6.8	7.2	5.9	5.0	4.0	5.6	8.1	6.4	6.7	5.1	3.2
Median	7.2	7.3	5.8	4.6	3.7	4.7	6.6	5.2	5.5	4.8	3.4
SD	1.6	2.4	2.0	1.6	1.4	2.9	5.0	3.9	2.9	2.9	1.3
Range	3.7-9.9	2.7-11.9	2.3-10.1	1.7-8.8	2.1-9.6	3.2-17.9	3.3-28.9	2.7-20.1	2.8-13.2	2.1-13.2	1.4-4.4
Total n calves	30	30	30	30	30	25	25	20	20	12	5

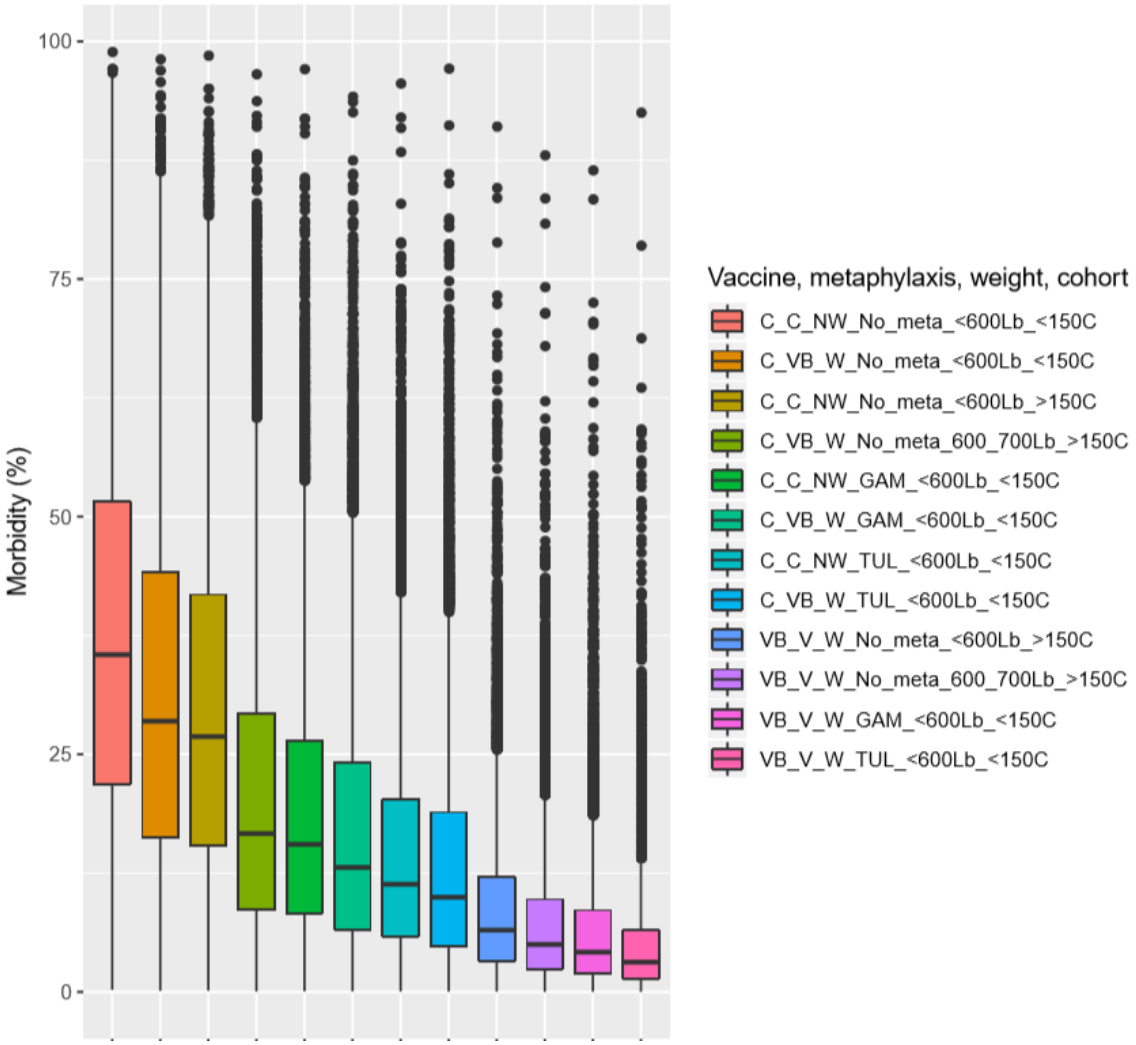


Chapter 6 supplementary material: Figure 1. Dependency distribution of the additive effect of metaphylaxis and vaccination on bovine respiratory disease morbidity from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of bovine respiratory disease morbidity in feedlot cattle.



Chapter 6 supplementary material: Figure 2. Bovine respiratory disease morbidity estimates for cattle less than 600 lb. and in cohorts of <150 animals compared by vaccination and weaning status.

A. Vaccination and weaning status compared to tulathromycin. **B.** Vaccination and weaning status compared to tilmicosin. **C.** Vaccination and weaning status compared to gamithromycin. **D.** Vaccination and weaning status compared to tildipirosin. Vaccines and weaning: letters on the left of the first underscore represent before feedlot arrival vaccinations, letters on the right of the first underscore represent upon feedlot arrival vaccinations, and letters to the right of the second underscore represent weaning status. C = no vaccination, V = viral, B = bacterial, W = weaned, NW = unweaned. Til = tilmicosin, Gam = gamithromycin, Tul = tulathromycin, TILD = tildipirosin, no_meta = no metaphylaxis.



Chapter 6 supplementary material: Figure 3. Bovine respiratory disease morbidity estimates from a Monte-Carlo simulation model of cattle characteristics and disease interventions for the prevention of bovine respiratory disease morbidity in feedlot cattle.

Vaccines and weaning: letters on the left of the first underscore represent before feedlot arrival vaccinations, letters on the right of the first underscore represent upon feedlot arrival vaccinations, and letters to the right of the second underscore represent weaning status. C = no vaccination, V = viral, B = bacterial, W = weaned, NW = unweaned. Gam = gamithromycin, Tul = tulathromycin, no_meta = no metaphylaxis. Body weight: <600 lb. or 600 – 700 lb. Cohort size: <150c = less than 150 animals, greater_150 = greater than 150 animals.