HEMATOLOGICAL VARIABLES AS PREDICTORS OF BOVINE RESPIRATORY DISEASE IN NEWLY RECEIVED CATTLE FED IN CONFINEMENT

By:

Lauren Rose Fontenot

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ABSTRACT

Bovine respiratory disease (BRD) is the most common and costly health disorder in the US cattle feeding industry. Although antimicrobial metaphylaxis has been proven to be effective in reducing BRD morbidity rate and improving performance, today's beef consumers are demanding a reduction or cessation of antimicrobial use in food animals. This study sought to elucidate if hematologic variables determined upon feedlot arrival were associated with risk for BRD. If accurate and timely classification of health risk is possible, antimicrobial metaphylaxis could be refined for those animals identified at risk for BRD development. Utilizing hematologic information of 502 steers and bulls with unknown previous health history, we categorized individuals for each complete blood count (CBC) variable in two ways: 2-level categorization determined by receiver operating characteristic (ROC), and 3-level categorization determined by simple quartile division. A reduced eosinophil status upon arrival, expressed as a percentage of total leukocytes or as concentration, was correlated with increased odds (OR>1.6; P<0.04; CI = 1.03 to 2.57) of BRD morbidity outcome for 2- and 3-level categorizations. Animals with reduced hemoglobin and/or increased red blood cells (RBC) were determined to be at lesser risk (OR<0.7; P<0.04; CI 0.42 to 0.98) to be treated once for BRD based on 2-level categorization. Those in the lowest quartile for relative neutrophils and

neutrophil:lymphocyte ratio (NLR) had increased risk (OR>1.9; *P*=0.02; CI = 1.05 to 3.70) of being treated once for BRD in the 3-level categorization. Likewise, decreased percentage of lymphocytes in peripheral blood was associated with decreased mortality risk (OR<0.2; *P*<0.04; CI = 0.13-0.95) in both categorization methods. In concert with low percentage lymphocytes, a low NLR was also associated with increased mortality risk (OR>5; *P*=0.03; CI = 1.47 to 24.03) in the 3-level model, and reduced neutrophil concentration was associated with increased mortality risk (OR>2; *P*=0.02; CI = 1.14 to 6.29) in the 2-level model. Because eosinopenia has been previously reported in multiple mammalian species to be predictive of poor health outcomes, this particular variable may hold promise for use in feedlot health management decisions upon arrival.

Key words: bovine respiratory disease, BRD, CBC, prediction, eosinopenia

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SIGNATURE PAGE

Approved:

_____

[Chairman, Thesis Committee] [Date]
Dr. John Richeson

_____

[Member, Thesis Committee] [Date]
Dr. Pablo Pinedo

_____

[Member, Thesis Committee] [Date]
Dr. Ty Lawrence

_____

[Head, Major Department] [Date]

_____

[Dean, College] [Date]

_____

[Dean, Graduate School] [Date]

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CHAPTER I

INTRODUCTION

Bovine respiratory disease (BRD) is estimated to account for 70% of total feedlot morbidity and 50% of total feedlot mortality (Smith, 1998; Aich et al., 2009) and is the most common and costly health disorder in the US cattle feeding industry (Vogel et al., 1994; Edwards, 1996; Burciaga-Robles et al., 2009). The term BRD describes a complex illness potentially caused by multiple pathogens including: bovine viral diarrhea virus, bovine herpesvirus-1, bovine respiratory syncytial virus, parainfluenza-3 virus, *M. Haemolytica, P. multicida, H. somnus*, and varieties of *Mycoplasma* (Richeson et al., 2013; Klima et al., 2014). Calves may carry any combination or all of the above bacterial pathogens naturally in the upper respiratory tract, but will become clinically ill when immunocompromised (Bagley, 1997). Chronic stress can cause immunosuppression, and the stressful processes of weaning, transport, and social realignment as a calf leaves the ranch of origin frequently present the opportunity for affliction. If transport time is longer than a few hours, associated fasting, dehydration, and fatigue are additional stressors. Feedlots will typically classify such calves as "high-risk" and administer an antimicrobial to the entire group of arrivals, known as "metaphylaxis", in an attempt to control the disease process. Any procedure that could diminish the impact of BRD in feedlot cattle

and/or reduce the use of antimicrobials would address consumer and regulatory concerns and have invaluable industry impact. Because current consumer pressure exists to decrease antimicrobial use in food animals, processes that decrease metaphylactic drug use without increasing morbidity or mortality or decreasing performance will be received positively by both producers and consumers. The current study was designed to determine if a relationship(s) exists between one or more complete blood count (CBC) variables and subsequent BRD events in commercial feedlot cattle. If such a relationship exists, and the variable could be rapidly, reliably and repeatedly identified, a chute-side blood test could be employed to identify individual animals at greater BRD risk. This would be helpful for managers' decision-making, as treatment history is rarely known on arriving calves (Richeson et al., 2013). The plurality of causes for BRD needs to be deliberated when studying a diagnostic method; one method may reliably detect viral causes but neglect to identify bacterial agents. It is widely accepted that low-stress handling and vaccines are the best disease prevention strategies available, but few or no reliable predictive techniques exist (Babcock et al., 2013). A reliable predictive test for BRD risk added to the arsenal would allow sick animals to be treated before they become clinically ill and would aid feedlot personnel in better managing the disease, thus improving animal welfare and ensuring judicious use of antimicrobials used to control or treat BRD.

CHAPTER II

LITERATURE REVIEW

Bovine Respiratory Disease

Health of feedlot cattle involves multidimensional challenges, with different illnesses presenting unique challenges throughout the feeding process. Bovine respiratory disease (BRD) is more prevalent than other health disorders and occurs primarily during the early stages of feeding, while metabolic and digestive disorders tend to occur as days on feed (DOF) increase (Smith, 1998). The majority of BRD cases occur within the first two to four weeks of the feeding period, likely due to the stress of the relocation process; some specific stressors include novel feedstuffs, environment changes, and social restructuring (Smith et al., 1998; Sanderson et al., 2008; Hay et al., 2016a). In an analysis of records from 18,112 calves, Snowder et al. (2006) reported on average, treatment occurred 26-d after arrival, and that BRD incidence was greatest at two weeks on feed. Disease incidence was prevalent until 110 DOF (Snowder et al., 2006).

Morbid animals have decreased profit through antimicrobial treatment cost, decreased performance, and increased labor; combined these may cost more than mortality loss from BRD (Bagley, 1997; Smith, 1998). Many studies (Bateman et al., 1990; Morck et al., 1993; Cernicchiaro et al., 2013) have reported a decrease in ADG in

clinically ill calves, though there are conflicting ideas of whether compensatory growth would close the performance gap between a sick animal and its healthy cohorts that are never diagnosed or treated. Cernicchiaro et al. (2013) analyzed the records of 212,867 cattle from a Midwestern feedlot and reported that as number of treatments for BRD increased, a corresponding decrease in net return, ADG, HCW, yield grade, and percent of animals grading choice or better was evident. However, other reports suggest that additional DOF for previously treated animals can allow equivalent finishing weight and carcass quality as healthy cohorts (Holland et al., 2010). Holland et al. (2010) sorted 360 heifers deemed high-risk into feedlot pens based on number of treatments during a 63-day preconditioning period. Using ultrasonography of the longissimus and visual appraisal of conditioning to determine appropriate harvest time, the authors reported heifers treated for BRD zero, one or two times during the preconditioning phase finished after 163 DOF. However, heifers that were treated three times required 19 more days to reach finished condition, and heifers labeled as chronically ill required 26 days longer than those treated twice or less (Holland et al., 2010). Allowing the treated animals longer days on feed resulted in no significant differences in carcass traits (Holland et al., 2010).

Effects of calf stress

Weaning, transport, commingling, dehydration, novel feedstuffs and other stressors may cause an animal to be at higher risk for developing disease than a similar calf with less stress (Griebel et al., 2014). Carroll and Forsberg (2007) defined "stressor" as a stimulus that results in activation of the hypothalamic-pituitary-adrenal axis and

nervous system to promote return to homeostasis. Prolonged or chronic stress has been shown to have a negative impact on immune function. Part of the innate immune system an animal has prior to acquired immunity is phagocytic cells including neutrophils and macrophages, and granulocytes including basophils and eosinophils (Carroll and Forsberg, 2007). The concentration of these hematological variables may be measured to monitor the physiologic impact of different stressors.

To evaluate the effect of stress on immunity, Biolatti et al. (2005) administered dexamethasone, a synthetic glucocorticoid, to veal calves then weighed the thymus after slaughter. The calves that received dexamethasone had smaller thymus glands than the control calves, which indicated impaired immune function. Weight gain in the calves administered dexamethasone was also less (Biolatti et al., 2005).

Using 40 Hereford-Angus cross heifers, Griebel et al. (2014) measured interferon-gamma (IFN-γ), tumor necrosis factor (TNF), and serum haptoglobin response to weaning stress, transportation stress and bovine herpesvirus-1(BHV-1) challenge. One group was abruptly weaned and transported to the research facility and had the greatest amount of viral shedding. The group that was gradually weaned but not acclimated to transport had greater IFN-γ in serum, indicative of stress and inflammation (Griebel et al., 2014). The combination of transport stress and abrupt weaning resulted in increased haptoglobin on day 3 post-BHV-1 challenge, though transportation stress alone did not. By the fifth day, serum haptoglobin concentration was similar to the control group. The authors concluded weaning stress caused a stronger inflammatory response than transportation stress, but this is largely dependent on the distance traveled (Griebel et al.,

2014). They also acknowledge the transient nature of haptoglobin makes it more suitable for diagnosis, not prediction, of disease.

Mackenzie et al. (1997) also investigated the effects of weaning and transport on calves via measurement of antibody response to a keyhole limpet hemocyanin (KLH) challenge. They observed that combined weaning and transport had the greatest impact on calf immunity, but calves weaned prior to transport also had elevated IgG. The concentrations for all immunoglobulins measured were greatest between days 20 and 30 after arrival at the calf-raising facility, suggesting that humoral immune response is greatest at that time (Mackenzie et al., 1997). The authors concluded the interaction between stressors and immunity needed further investigation.

Carroll and Forsberg (2007) reported innate immunity of an animal may be compromised in times of stress, dehydration, injury, and poor nutrition. Those authors also state that it may only be sustained stress that causes immunosuppression; short-term stress in fact stimulates immune cell production while prolonged stress reduces stimulation of effector cells. As the glucocorticoid receptors of the effector cells stay activated, they lose the ability to differentiate foreign bodies and will begin to target the animal's own tissue (Carroll and Forsberg, 2007). The normal action of glucocorticoids in the thymus gland is also influenced by prolonged stress; at elevated levels, immature thymocytes undergo apoptosis which in turn results in degeneration of the thymus (Carroll and Forsberg, 2007).

Roth et al. (1985) stated glucocorticoids have been shown to decrease lymphocyte counts and increase number of neutrophils in circulation, which may be useful in

diagnosis or prediction of disease. An increase in circulating neutrophils during stressful times may indicate they are not migrating to sites of infection efficiently. Impact of plasma cortisol on monocytes is unknown, but bacterial toxins and local damage activate monocytes, which secrete cytokines to begin the inflammatory processes so they may have a role in disease prediction (Roth, 1985; Petersen et al., 2004).

Stressors

For this paper, a stressor is identified as a phenomenon of human impact on cattle that results in the animal being stressed, versus risk factors which are inherent characteristics or events that man has no or limited control over. Regardless of its source, stress may make an animal more vulnerable to illness due to its impact on the immune system. This relationship was described by elevated concentrations of plasma cortisol in conjunction with transport, weaning, castration, and other stressful processing practices in beef cattle (Roth, 1985). Even synthetic glucocorticoids such as dexamethasone have been shown to cause mild malaise to progress into acute disease (Roth, 1985).

Transportation

Length of transit time may influence cattle health by increasing the severity of dehydration, fasting, fatigue, and inhalation of exhaust fumes and particulate matter as the event endures. Sanderson et al. (2008) reported increased morbidity in long-haul calves, in part due to decreased hay consumption for the first four days after feedlot arrival. Those authors postulated calves undergoing shorter transit times may be worth a premium due to lower morbidity risk. According to Sanderson et al. (2008), for every additional 160 km calves were transported, they had a 10% increase in BRD likelihood.

Cernicchiaro et al., (2012) reported cattle hauled further than 250 km had greater BRD risk, but countered the claim by Sanderson et al. (2008) of a linear risk association. Contrastingly, Cole et al. (1988) reported between treatments of calves hauled 12 versus 24 hours, the group transported the shorter distance experienced greater morbidity and mortality, though the ADG was similar between groups. The authors offered no reason for why the short-haul group had greater morbidity and mortality, but did state that the majority of illness occurred after day 5 (Cole et al. 1988).

Comingling

Cattle are herd animals that have an established hierarchy within the herd. Comingling calves results in social disruption, along with exposure of naïve calves to novel pathogens. Nose-to-nose contact transmits pathogens in nasal secretions of infected calves, and aerosolized agents can be transmitted by coughing (Bryson, 1985). Sanderson et al. (2008) stated that cattle in pens commingled from multiple sources were twice as likely to become morbid as pens originating from a single source. Step et al. (2008) tested for origin (ranch vs. auction market) and commingling effects in 500 calves; they reported no difference in ADG, but calves originating from auction and the treatment of ranch-origin calves commingled with auction calves experienced greater morbidity and mortality than calves of ranch-origin that remained isolated (Step et al., 2008). In the same study, animals that were weaned on the shipping truck as opposed to pre-weaned on the ranch experienced greater illness. O'Connor et al. (2005) also reported increased BRD risk in commingled feedlot pens, with commingled pens being 3 times more likely to develop BRD compared to pens comprised of single source cattle. Interestingly, that

study reported single-source pens with a PI positive calf to have the lowest morbidity rate and the commingled pens with a PI calf to have the highest. Therefore, the authors concluded the factor with the most risk associated for BRD in their study was commingling, and that though all cattle in the study were likely exposed to BVDV via employees walking pens, the increased stress in commingled pens contributed to immunosuppression and thus greater vulnerability to clinical BVD (O'Connor et al., 2005.)

Predisposing factors

Some external stimuli have also been correlated to BRD risk such as season of arrival at the feedlot, ambient temperature extremes and temperature fluctuation accompanied by precipitation, and length of transport. Internal factors that have been investigated include sex, age, weight, and breed.

Season

There are mixed findings on the impact of season on BRD incidence in feedlot calves. Hay et al. (2016a) observed that cattle enrolled in spring were 1.6 times more likely to become sick than cattle inducted in winter, and 2.6 times more likely than those inducted in summer. Spring usually involves the most drastic temperature changes between nighttime lows and daytime highs which is physiologically more demanding on the animal than a consistent high or low temperature. Among cohorts that had been hauled similarly long distances, Cernicchiaro et al. (2012) reported greater morbidity in the summer than other seasons. Babcock et al. (2013) observed greatest morbidity in autumn, though the authors attributed this to the greater influx of calves in fall

overwhelming feedlot personnel. Clearly more research is required to elucidate the true impact of season on cattle health.

Sex

Association with sex effect may be confounded because most feedlots feed greater numbers of steers, but several investigations have revealed that steers have greater incidence of respiratory disease than heifers (Snowder et al., 2006; Cernicchiaro et al., 2012; Hay et al., 2016a). Those studies did not specify whether the steers arrived already castrated or were castrated on arrival. Sanderson et al. (2008) reported no difference in disease susceptibility between heifers and steers, but pens with mixed gender were at higher risk for morbidity. Richeson et al., (2013) compared male calves that arrived to the feedlot intact versus those previously castrated and reported that steers had decreased odds of BRD. Although castration may be further indicative of preconditioning protocols including vaccination, weaning and bunk training are also known to reduce the risk of BRD in the feedlot.

Age and Weight

Younger cattle are more likely to have less exposure to pathogens and thus less acquired immunity compared to older, heavier cattle. Hay et al. (2016b) used number of erupted incisors as an estimate of age and reported no correlation to morbidity risk but reported cattle heavier than 480 kg were almost half as likely to develop BRD as calves lighter than 400kg. A 30% reduction in risk was estimated for every 100kg increase in entry weight (Hay et al., 2016a). Sanderson et al. (2008) reported morbidity of calves heavier than 318kg to be less than lighter calves. Between groups of cattle that had been

transported similar distance, Cernicchiaro et al. (2012) reported less disease risk in heavier calves.

Breed

Different breeds may have different innate responses to disease challenge. Hereford cattle were reported to be twice as likely to develop BRD as Angus, and *bos indicus* breeds and their crosses were half as likely to become ill compared to *bos taurus* (Hay et al., 2016a). Snowder et al. (2006) also reported Angus to have lower disease risk, along with Charolais, Gelbvieh and several crossbreeds developed at the research center (MARC I, II, and III). The same authors reported Simmental and Limousin were at greater risk. Snowder et al. (2006) did not discover evidence of hybrid vigor of the crossbreeds providing resistance to BRD.

Pathogens of BRD

Several viral and bacterial agents can cause BRD. However, in many species including cattle, a viral respiratory infection precedes bacterial pneumonia, and co-infection results in a phenomenon known as viral-bacterial synergy (Cusack et al., 2003). Bovine respiratory disease appears to depend on bacterial-viral synergy. Hay et al. (2016c) measured increases in serum titers for two respiratory viruses upon arrival at Australian feedlots and reported that if an animal only had a seroincrease for one virus, BRD risk increased minimally. However, if the animal had a seroincrease for multiple viruses, BRD risk increased significantly, providing further evidence of the multifaceted nature of this disease complex. Ten of the 14 feedlots used in that analysis gave a modified-live BHV-1 vaccine (Rhinogard, Zoetis) upon arrival, so vaccination and

presence of BVDV-1 PI calves were used as covariates in their analyses (Hay et al., 2016c).

Viruses

Bovine herpesvirus-1, (BHV-1) responsible for causing the disease infectious bovine rhinotracheitis, destroys the epithelium of the nasopharynx and trachea (Cusack et al., 2003). First using its envelope glycoproteins to fuse to the cell membrane, the virus then undergoes DNA replication in the host cell nucleus (Ellis, 2009). The host cell's protein production is ceased via BHV-1 signaling, resulting in cell necrosis. The animal incidentally inhales the degrading tissues, resulting frequently in inhalation pneumonia (Cusack et al., 2003). Necrosis of epithelial cells is what causes the notable tracheal and pulmonary lesions characteristic of infectious bovine rhinotracheitis (Ellis, 2009). This virus appears to have increased in severity and prevalence over the past decades, because Bryson (1985) reported that BHV-1 was not a principal agent in BRD. Ellis (2009) stated that a principal reason a significant BHV-1 infection can be so detrimental is that the virus is capable in replicating in all cell types, not just respiratory tissues.

Parainfluenza-3 virus (PI-3V) differs from BHV-1 in that it focuses its invasion in the lower respiratory tract, though it can also replicate in the upper respiratory tract. Initial lung inflammation progresses to tissue destruction and lung defense mechanisms are rendered less effective as PI-3V attacks the alveolar macrophages (Bryson, 1985).

Bovine respiratory syncytial virus (BRSV) is similar to BHV-1 and PI-3V in its invasion of pulmonary cilia and alveolar macrophages to impair cellular immunity, ultimately allowing infection of bacteria (Baker et al., 1997). Though it is incapable of

the latency BHV-1 possesses, BRSV remains present in nasal secretions for almost three weeks after initial exposure, providing ample opportunity for transmission to other animals (Bryson, 1985) and may persist in the lymphoid tissue for months (Ellis, 2009). Baker et al. (1997) reported the incubation period of BRSV to be less than one week. Neither PI-3V nor BRSV alone usually cause severe respiratory illness, but severe illness is usually seen when both act together and in the autumn season (Bryson, 1985). Bovine respiratory syncytial virus replicates in the cell cytoplasm after binding to the host cell membrane with glycosaminoglycans, then nucleocapsids bud out of the host cell membrane to infect other cells (Ellis, 2009). According to Ellis (2009) BHV-1 possesses anti-apoptotic pathways during early infection so as to promote its lifespan, but later host cells undergo apoptosis to form tracheal and pulmonary lesions.

Bovine viral diarrhea virus (BVDV) was once debated whether or not it is a primary agent in the BRD pathway because of its different etiology, but it is frequently found in nasal secretions of clinically ill calves (Bryson, 1985). Rather than directly attacking the respiratory system, BVDV handicaps the lymphatic system, leading to immunosuppression and providing opportunity for the other respiratory pathogens. The virus impedes lymph function, antibody production, and proper leukocyte function (Roth et al., 1981). Although diarrhea is commonly seen in cattle infected with BVDV, it was not observed in any of the subjects in the study conducted by Roth et al. (1981). Shahriar et al. (2002) observed lesions in the tunica intima and tunica media of arteries in cattle infected with BVDV, and suggested such vascular lesions could be used as a diagnostic indicator of that virus.

Mycoplasma and bacteria

Mycoplasma have a similar role as viruses in the BRD pathogenesis in that they cause mild to moderate primary damage, then the bacterial species later invade to cause severe pulmonary destruction. In clinically ill calves, mycoplasma species such as *M. dispar, M. bovis,* and *M. bovirhinis* are frequently recovered from the respiratory tract (Bryson, 1985). Mycoplasmas differ from the other pathogen types in that they are not ubiquitous, and are only transmitted by contact with afflicted animals (Suleman et al., 2016). Due to the lack of a cell wall, many mycoplasmas are inherently antimicrobial resistant, which is why many chronically ill cattle are presumed to be at least coinfected with mycoplasma. Mycoplasmas are difficult for the host immune system to mediate due to the variance in surface proteins they possess (Suleman et al., 2016). These pathogens can cause lung lesions on their own, but are more responsible for inflammation and thickening of pulmonary tissue (Bryson, 1985). Suleman et al. (2016) also demonstrated that *M. bovis* interferes with alveolar macrophages' normal apoptosis, resulting in a greater-than-normal concentration of pro-inflammatory cytokines. This increased presence of cytokines resulted in lung lesions. A study in western Canada observed frequent BVDV-mycoplasma coinfection in cattle that experienced chronic bronchopneumonia, and a subset with fibrino-necrotizing polyarthritis (Shahriar et al., 2002). Those researchers specifically selected cattle out of chronic feedlot pens that had received "extensive" antimicrobial treatment, and reported that over 90% of the cattle studied were positive for *M. bovis* (Shahriar et al., 2002). Another Canadian study, Haines et al. (2001) tested lung, synovial fluid, and cardiac issue in 49 cattle from a

chronic pen at a feedlot that had been treated with a variety of antimicrobials and suffered from severe arthritis for presence of *M. bovis, H. somnus, M. haemolytica*, and BVDV. *Mycoplasma bovis* was present in 71% of lung samples, and 45% of joint synovium; BVDV was present in 41% of samples, with 39% of samples having BVDV-mycoplasma coinfection. *Mannheimia haemolytica* was present in 22% of samples and only from the lung; 14% of lung samples were positive for *H. somnus* (Haines et al., 2001).

While many bacterial species may take advantage of an opportunity presented by viral respiratory infection, the two most commonly found in BRD cases are *Mannheimia haemolytica* (previously known as *Pasteurella haemolytica*) and *Pasteurella multocida.* Gram negative bacteria such as these have lipopolysaccharide endotoxin in the cell wall, which results in vascular permeability, inflammation and associated edema, and deposition of fibrin in the pulmonary tissue (Cusack et al., 2003). Lipopolysaccharides are an example of pathogen-associated molecular patterns that identify invading pathogens to leukocytes and dendritic cells (Carroll and Forsberg, 2007). Many bacterial pathogens secrete proteases to break down the respiratory mucus lining, making invasion of the underlying tissue with their toxic cell components easier (Corbeil and Gogolewski, 1985). The endotoxin in the cell wall of *Haemophilus somnus*, a BRD-associated bacteria found more frequently in cattle from northern U.S. states and Canada, causes similar damage, but it also results in inflammation of the vasculature and necrosis (Corbeil and Gogolewski, 1985). *M. haemolytica* destroys phagocytes with a leukotoxin secreted early in its life cycle. The leukotoxin is specific to ruminant leukocytes and disrupts

phagocytosis and kills resident macrophages. Batra et al. (2016) report that neutrophils are most susceptible to leukotoxin, and that of the offense mechanisms *M. haemolytica* has, leukotoxin is most virulent and therefore may be the key to vaccination against this pathogen. All of these bacteria take advantage of the inflammatory response of the lung by inhibiting neutrophil function then destroying the macrophages that arrive to relieve the overwhelmed neutrophils (Corbeil and Gogolewski, 1985). As the phagocytes are destroyed, their oxyradical contents are released causing further damage to surrounding cells. *H. somnus* also releases exotoxins that invade endothelium, macrophages, and neutrophils. The inflammation in the tissue thus cascades on itself, resulting in more damage than if the bacteria only attacked pulmonary epithelium in an uncomplicated manner (Corbeil and Gogolewski, 1985).

In a Finnish study consisting of 84 calves clinically ill with BRD, Nikunen et al. (2007) reported *P. multocida* to be the most common bacterium present, while not observing *M. haemolytica* in any of the nasal lavages. Contrastingly, Klima et al. (2014) collected lung tissue samples from calves from Alberta, Texas, and Nebraska that succumbed to BRD and reported 91% were positive for *Mannheimia* spp. and 69% were positive for BVDV. In the Finnish study, the authors investigated links between acute phase proteins (APPs), clinical signs, and lavage flora. They found that calves with *P. multocida* displayed more severe signs of respiratory distress and had the greatest concentrations of APPs. Despite some of the calves having viral agents present, the researchers did not observe any links with viruses and clinical symptoms or APPs. The authors concluded that viruses have a secondary role in BRD by allowing bacteria to

invade, and that *P. multocida* has a strong pathogenic role when no other bacteria species are noted (Nikunen et al., 2007).

Vaccination against BRD pathogens

Determining efficacy of vaccination depends heavily on the circumstances of the host. Hay et al. (2016c) reported that cattle that were seropositive for two respiratory viruses at feedlot arrival were at reduced risk for BRD, and cattle that were seronegative for respiratory viruses upon arrival were at greater risk for BRD. These results indicate that pre-existing antibody, whether from vaccination or natural exposure, prior to marketing is beneficial for reduction in subsequent BRD morbidity. Immunity through vaccination relies on development of immune memory cells and antibody, which take several days to be produced. Vaccinations must be administered prior to pathogen exposure in order to be effective. Effective disease prevention is an industry priority as it is much more cost effective than treating a sick calf; Stegner et al. (2013) reported the 2010 average for BRD treatment costs per head was $92.00. Despite knowing that vaccination prior to exposure is important, 99.2% of US feedlots with capacities over 8000 head reported giving a respiratory vaccine at arrival (USDA Feedlot 2011, Part I).

Hay et al. (2016b) reported results from a survey following 10,721 cattle. They calculated that animals vaccinated 14 days prior to feedlot induction with inactivated BVDV vaccine (Pestigard; Zoetis) tended to be at reduced risk for BRD. The same study showed animals given an inactivated *M. haemolytica* vaccine (Bovilis MH; Cooper's Animal Health) were at reduced risk for BRD. Vaccinations may be administered intranasally or parenterally, with intranasal vaccines stimulating mucosal immunity

through IgA and parenteral stimulating systemic IgG. Mahan et al. (2016) tested efficacy of an intranasal modified-live vaccine (INFORCE 3, Zoetis) in neonatal dairy calves that were either colostrum deprived and kept seronegative for BHV-1, or were fed colostrum and were seropositive. One group of colostrum-deprived, seronegative calves received a bovine herpesvirus-1 (BHV-1) challenge intranasally 28-days post-vaccination, the second colostrum-deprived group was challenged 193-days post-vaccination, and the third colostrum-fed group received the challenge 105-d post-vaccination when their serum titers dropped below 4. Calves in experiments one and two were colostrum deprived, and experiment three calves were given one dose of colostrum with antibodies against BHV-1. In all three experiments, vaccinated calves had reduced rectal temperature, morbidity rates, duration of infection, and shed less BHV-1 in nasal secretions after the viral challenge was administered. The authors concluded that intranasal vaccination was effective in neonatal calves both in the presence of maternal antibodies and without (Mahan et al., 2016).

Theurer et al. (2015) completed a meta-analysis to elucidate vaccine efficacy against BRD causative agents. They reported animals vaccinated with modified-live BHV-1, inactivated BHV-1, modified-live BVDV, inactivated BVDV, or inactivated BRSV were at lower risk than controls in those studies. Animals vaccinated with modified-live BRSV and/or modified-live PI3 were not different from controls for BRD risk. Comparing 5-way and 3-way vaccines showed no differences in morbidity risk with either type and controls, but those cattle were vaccinated three days after arrival at the

feed lot (Theurer et al., 2015). This analysis further provides more evidence for the idea that vaccine timing is crucial and must be done at least a week before disease challenge.

In a review of 25 vaccination studies, Martin (1983) concluded that in a feedlot setting, respiratory vaccines were not clearly effective in reducing morbidity and increasing animal performance. Evidence of live BVDV vaccines causing an increase in morbidity caused Martin (1983) to elucidate that live-pathogen vaccines in immunocompromised calves actually has a negative effect on animal health. That author further acknowledges that with the extensive claims of vaccine efficacy, data supporting their utility should not be so scant (Martin, 1983). If live-attenuated vaccines are detrimental to stressed-calf health, the alternative may lie in either use of killed vaccines upon arrival or most effectively vaccinating with either live-attenuated or killed antigens at least several weeks prior to shipping and weaning to allow the animal time to develop protective antibodies prior to natural exposure. Because calf origin is frequently unknown, opportunity to vaccinate is limited to when a group is compiled at the auction barn or upon feedlot arrival, both being inadequate timeframes for proper immune response (Frank et al., 2002). Frank et al. (2002) studied 121 steers from Tennessee and 84 steers from New Mexico. Prior to transit, every other steer coming through the chute in the Tennessee group was vaccinated with *M. haemolytica* bacterin-toxoid. Also prior to transit, every other steer entering the chute in the New Mexico group were vaccinated with modified-live *M. haemolytica* serotype 1. Once both groups arrived at the feedlot, 30 of the Tennessee-origin and 21 of the New Mexico-origin vaccinated steers were administered florfenicol, with equal number of non-vaccinates administered florfenicol.

No differences in morbidity were observed between vaccinated and non-vaccinated cattle, but cattle treated with florfenicol had significantly less morbidity (Frank et al., 2002). Van Donkersgoed (1992) reported in a meta-analysis of 10 studies that vaccination has been largely ineffective, which is why so many feedlots have adopted antimicrobial metaphylaxis.

Fell et al. (1998) compared ADG and morbidity in Australian calves either paddock-weaned or yard-weaned six months prior to feedlot arrival, and calves either unvaccinated or vaccinated 77-13 days prior to arrival. Vaccination prior to feedlot arrival did improve ADG, but not morbidity. That study reported morbidity as the percentage of calves pulled from their pen, and the authors acknowledged that some pulls classified as morbid may not have been clinical, but were just experiencing lethargy as an adverse effect of vaccination (Fell et al., 1998). The treatment group that was weaned and vaccinated prior to arrival earned 18 Australian dollars more compared to the control group, so there appears to be merit in pre-conditioning (Fell et al., 1998).

Control and treatment of BRD

Current protocol in many feedlots entails labeling arriving groups of calves according to their perceived health risk and administering antimicrobial metaphylaxis to those deemed high-risk to control an anticipated BRD outbreak. Depending on the drug administered, a moratorium is implemented lasting approximately 3 to 14 days after arrival, a time known as the post-metaphylactic interval (PMI) when upon expiration feedlot personnel may begin pulling and treating calves based on qualitative observations of clinical signs. The most common routes of administration for antimicrobials intended

to treat or control (i.e. metaphylaxis) BRD are parenteral or orally through the feed. Some efficacy challenges with medicated feed is that morbid animals are frequently anorectic and do not consume enough feed to achieve the intended dose, and controlling dosage for the more aggressive eaters in the pen is a similar concern (Ives and Richeson, 2015). Some feed-through antimicrobials labeled for control and/or treatment of BRD include: tilmicosin (by Veterinary Feed Directive only), chlortetracycline-sulfamethazine, chlortetracycline, and oxytetracycline (Ives and Richeson, 2015). The FDA-approved injectable antimicrobials for control of BRD are: danofloxacin, enrofloxacin, tulathromycin, ceftiofur, tilmicosin, florfenicol, tildipirosin, and gamithromycin (Ives and Richeson, 2015). The PMI differs based on the pharmacokinetics of each drug in its target tissue; for example, ceftiofur has a recommended seven day PMI and is injected into the base of the ear, while tulathromycin has a PMI recommendation of 14 days and is injected subcutaneously in the neck (Step et al., 2007).

Ives and Richeson (2015) observed that a longer PMI allowed feedlot personnel to focus on helping the new arrivals adapt to their new environment and diet instead of the often overwhelming task of BRD identification. Along those lines, the improved gain and health performance observed for cattle metaphylactically treated with tulathromycin compared to those treated with tilmicosin could in part be due to lower stress attributed to reduced handling (Lofgreen, 1983; Stegner et al., 2013). Handling is reduced for animals treated with antimicrobials with longer PMI's, because the pharmaceutical is still active in the tissue negating the need to re-treat. The USDA Feedlot 2011 Part IV report stated 57.6% of feedlots use tilmicosin for BRD control at arrival, 45.3% use tulathromycin,

and 39.7% use ceftiofur, making those the three most commonly used antimicrobials for metaphylaxis.

Illustrating the efficacy of metaphylaxis, Amrine et al. (2014) used 33 crossbred heifers assigned to tildipirosin, tulathromycin, or saline metaphylactic treatment 10 days prior to challenge with *M. haemolytica*. Calves were observed daily before and after the challenge until euthanasia three days post-challenge. Ninety-two percent of heifers' lungs in the tildipirosin group had less than 10% lesions; 50% of the tulathromycin treated heifers had less than 10%; none of the control heifers had less than 10%, and 66% had more than 15% lesions. The treated groups had less than 8% probability of abnormal respiration, while the control group had 22% probability. Upon culture of pulmonary tissue, 100% of the tulathromycin and control calves had *M. haemolytica* compared to 25% of the tildipirosin group. The authors concluded this was the first study of its kind to measure the efficacy of metaphylaxis by treating the animals 10 days before a disease challenge resembling a natural pen outbreak in the feedlot. They further concluded these results indicate that metaphylaxis was beneficial for calf health (Amrine et al., 2014).

Tennant et al. (2014) also reported metaphylaxis to be beneficial for ADG, finished weight, decreased morbidity and mortality and overall financial returns, but all treatment groups had similar incidence of lung lesions. Those authors suggested the primary reason medicated cattle were more profitable was due to the decreased ADG and increased mortality of the non-medicated group. In the study conducted by Tennant et al. (2014), some of the cattle that presented lung lesions at slaughter were never identified as clinically ill; this could be mediated by predictive technology at feedlot arrival. Results

from Lofgreen (1983) also confirm metaphylactic administration of oxytetracycline and sulfadimethoxine was effective in reducing treatment days by 90% as compared to control calves, and resulted in greater ADG. Frank et al. (2002) observed a reduction in respiratory disease occurrence in calves prophylactically treated with florfenicol. Finally, in an extensive meta-analysis, Van Donkersgoed (1992) concluded mass medication reduced BRD morbidity and mortality, and increased ADG and feed efficiency. Contrastingly, Bagley (1997) claimed metaphylaxis can delay individualized treatment, hindering optimum response, as animals experiencing more severe illness are not separated from their pen mates for closer care. However, a point of Ives and Richeson (2015) was that during a longer PMI, animals that are experiencing more severe illness or that are submissive and not able to get feed can be moved to another pen with other docile animals. Preferably this pen would be near so as to avoid unnecessary handling stress, as discussed earlier.

After the PMI has passed, feedlot personnel will begin to visually appraise cattle for symptoms of disease. Typical signs of BRD-affected cattle include nasal and ocular discharge, lethargy, labored breathing, decreased appetite or anorexia, and sometimes coughing. Once the apparently sick animal is pulled, a rectal temperature ≥ 104°F (40°C) is a common threshold necessitating antimicrobial intervention (Bagley, 1997). Drugs with shorter post-treatment intervals (PTIs; tilmicosin, 3d; florfenicol, 3d) are typically used for follow-up treatments after metaphylaxis (Step et al., 2007). If the animal is not treated early in the disease process, prognosis can be poor; Bagley (1997) stated if the

disease state progresses to greater than 50% of lung tissue being damaged prior to drug administration, frequent relapse and even death can be expected.

Public and producer perception of antimicrobial use

The global animal agriculture industry uses more antimicrobials than any other sector, making responsible stewardship paramount to sustainability (Klima et al., 2014). As aforementioned, feedlots will administer antimicrobials metaphylactically to calves that have been deemed at increased risk for development of the disease; around 75% of high-capacity feedlots in the US utilize metaphylaxis at arrival (USDA Feedlot 2011, Part I; Klima et al., 2014). Metaphylaxis is more commonly administered (92.6%) at high-capacity feedlots to cattle less than 700 lbs. as smaller cattle are seen as higher risk for BRD development (USDA Feedlot 2011, Part IV). Feedlots with capacities under 8000 have less usage, with 40% using metaphylaxis control on arrival (USDA Feedlot 2011, Part I). Some countries, such as the Netherlands, have made extensive efforts to decrease agricultural antibiotic use. Speksnijder et al. (2015) stated the Dutch government ordered a 70% antimicrobial use reduction by 2015 as compared to level of use in 2009. In a survey of Dutch veterinarians' opinions on antimicrobial use reduction, Speksnijder et al. (2015) reported that as years of experience increased, practitioners felt less pressured by clients to prescribe drugs, but were also less optimistic about the industry's ability to decrease use. The authors also reported that the species perceived to be least able to reduce antimicrobial use in was cattle. Most responding veterinarians marked that improvement in feed and housing would allow for antimicrobial use reduction, but that

producers are hesitant due to perceived increases in labor and cost (Speksnijder et al., 2015).

Any solution for non-judicious use of antimicrobials will require the cooperation of all farm consultants so that producers do not receive conflicting advice from their counsellors (Speksnijder et al., 2015). Many producers have a consulting veterinarian who makes regular visits to the production facility. Conventional producers responding to a survey conducted by Habing et al. (2016) reported only 42% possessed a treatment protocol developed by their veterinarian for their operation, of which only 66% outlined when animals should be treated for a particular disease. This problem may be highlighted in circumstances as those described by Tennant et al. (2014) where 59% of carcasses with lung lesions were never pulled for treatment, evidencing a lack of ability to readily identify BRD in the production setting. Despite reliance upon antimicrobial treatment, the organic producers' surveyed had fewer sick and dying calves from calf diarrhea, with garlic and other herbal supplements being the most common treatment of choice. A glaring difference between organic and conventional producers is that organic operations tend to be much smaller, allowing for more attention to individual animals, and this is the likely cause of lower morbidity in those operations. In regards to opinions of whether agricultural antimicrobial use factors in resistant infections in humans, 49% of organic raisers agreed, compared to 7% of conventional producers (Habing et al., 2016). Even if conventional producers do not feel current levels of antimicrobial use are extreme, they would likely respond positively to a solution that could decrease drug use and thus cost, without sacrificing animal health. The problem of antimicrobial resistance is not a

theoretical one. Observing trends from 1994-2002, Welsh et al. (2004) reported susceptibility of *M. haemolytica* to erythromycin, florfenicol, spectinomycin, and tilmicosin had significantly decreased, with *P. multicida* also being resistant to those drugs and tetracycline and sulfamethoxazole. Klima et al. (2014) observed 72% of *M. haemolytica* isolates and 50% of *P. multicida* possessed genes linked to antibiotic resistant, and of those 30% of the *M. haemolytica* were resistant to more than seven classes of antimicrobials. Forty-five percent of other isolates were resistant to at least three antimicrobial drugs. The samples were collected from calves in Texas, Nebraska, and Alberta, but all of the antimicrobial resistant samples originated from calves located in Texas and Nebraska (Klima et al., 2014).

Complete blood count variables as predictors of BRD

Neutrophils

The first leukocyte to arrive at sites of injury or infection, neutrophils are polymorphonuclear cells that make up the largest percentage of immune cells (Abbas et al., 2007; Drescher and Bai, 2012). Neutrophils are produced in bone marrow and are filled with granules comprised of lysozyme, collagenase, defensins, and other such enzymes (Abbas et al., 2007). Those enzymes do not stain at high or low pH, which allows differentiation between these and other granulocytes like eosinophils and basophils (Abbas et al., 2007). In a healthy subject, these cells will live for about 6 hours (Abbas et al., 2007), but cytokines will extend their life during infections to help fight pathogens (Drescher and Bai, 2012). The primary neutrophil-associated defense mechanism is phagocytosis, but neutrophils also produce toxic reactive oxygen species

and extracellular traps made of chromatin and peptides that bind microorganisms (Drescher and Bai, 2012). Proteins released by the traps or from granules help activate dendritic cells, which initiates the progression of innate immunity into adaptive immunity (Drescher and Bai, 2012). Like other leukocytes, neutrophils can be devastating to self-cells if not carefully regulated (Drescher and Bai, 2012). Active infection causes elevated neutrophil production, and upon histological examination, a greater percentage of immature neutrophils will be observed (Abbas et al., 2007). Though blood concentration of neutrophils will decrease with an infection as those cells make their way into tissue, stress can cause inhibition of migration through the endothelium; Cole et al. (1988) reported significant increases in blood concentration of neutrophils in animals transported 12 and 24 hours. This phenomenon is caused by cortisol decreasing affinity of L-selectin on the neutrophil with its receptor on the endothelium, which decreases neutrophil rolling and in turn decreases translocation to tissue. Burton et al. (1995) observed changes in L-selectin and CD18 (another adhesion molecule) with administration of cortisol, dexamethasone, or placebo to dairy cows. They observed a dramatic decrease in L-selectin within 8 hours of dexamethasone administration that persisted for 5 days, meanwhile the cows experienced neutrophilia. Cortisol caused the same trends, just to a lesser degree. This interference with translocation is beneficial in decreasing neutrophil-associated inflammation, but is deleterious to infection mediation (Burton et al., 1995).

Monocytes

A monocyte is an immature immune cell produced in bone marrow that circulates in blood until activated by other immune cells. Once activated, they travel across the

endothelium into the tissue, maturing to become a macrophage (Abbas et al., 2007). Macrophages are phagocytic cells similar to neutrophils, with enzyme-filled granules to assist in microbe digestion (Abbas et al., 2007). Some macrophages reside in certain tissues, and are named for their particular location; alveolar macrophages are in pulmonary tissue, Kupffer cells are in the liver, microglial cells are in the central nervous system (Abbas et al., 2007). Macrophages are capable of replicating at infection sites, allowing them to be effective against invading pathogens much longer than neutrophils (Abbas et al., 2007).

Lymphocytes

Lymphocytes include natural killer cells, B cells, and T cells. Natural killer cells are part of innate immunity and have a primary role in viral infections, while T and B cells are components of adaptive immunity. The adaptive cells provide memory immunity in different ways; B lymphocytes produce antibodies specific to a particular antigen encountered, while T cells terminate cells infected with pathogens after recognizing cell-surface proteins unique to pathogens (Abbas et al., 2007). Elevated lymphocyte counts come several days after initial infection as adaptive immune cells are produced in response to antigen presentation by innate leukocytes or dendritic cells, and comprise approximately 90% of circulating blood lymphocytes (Abbas et al., 2007).

Red Blood Cells

Produced in bone marrow of adult mammals, red blood cells (RBC) function to transport oxygen and carbon dioxide between lungs and tissues. An elevated RBC count, or polycythemia, can be caused by an increase in cell concentration. As a mammal gets

dehydrated, the plasma component of the blood decreases causing concentration of red blood cells to increase (Lee and Arcasoy, 2015). Though the main objective of their study was not to determine how hematological variables predict BRD, Cole et al. (1988) reported a significant increase in RBC after 12-hour transport. This may indirectly indicate that marketing and transport caused cattle to become dehydrated. The elevated RBC count observed by Richeson et al. (2013) is indicative of dehydration, which alludes that those animals may have been in the marketing system longer or transported further, thus undergoing greater degrees of physiologic stress. Parker et al. (2003) observed elevated plasma cortisol having a diuretic effect in Merino wethers that contributed to dehydration; this implicates that during transport, cattle not only lose body water due to fluid restriction, but stress-associated diuresis may be antagonistic.

Eosinophils

Eosinophils are a granular leukocyte recently identified for their potential as a diagnostic marker. These cells are important in allergic pathways and are typically prevalent in association with mammals experiencing parasitic infection (Abbas et al., 2007; Acharya and Ackerman, 2014). One mechanism involves IgA and IgG antibodies that attract eosinophils to helminths, where the eosinophils then bind to the Fc portion of antibody and release granules containing major basic protein, destroying the helminth (Abbas et al., 2007). Part of the antimicrobial function of the eosinophil is its formation of extracellular DNA traps that kill pathogens with granular proteins (Acharya and Ackerman, 2014). The granules of eosinophils release toxic proteins that are very effective at cell termination, yet they accomplish this without specificity. The major basic

protein-1 (MBP-1) is a granular protein that has been implicated in pulmonary epithelial damage leading to asthma (Acharya and Ackerman, 2014). Low eosinophil counts lead to lower immune response, but excess concentrations can also be damaging.

An early study on how transportation length affects BRD incidence reported a significant decrease in eosinophils from calves transported for 24 hours, though the authors did not link hematological variables to disease incidence (Cole et al., 1988). Richeson et al. (2013) retrospectively monitored 591 steers and 588 bull calves from four experiments for 56 days following arrival to a research facility. The objective of the study was to determine if a relationship between one or more CBC variables and subsequent BRD morbidity could be determined. Overall, 55.8% of calves were diagnosed with BRD at least once, 32.7% were diagnosed twice, and 44.2% were never diagnosed with clinical BRD. Using multivariable logistic regression models, the authors discovered a relationship between initial BRD diagnosis and decreased eosinophil and increased RBC counts (shown below). The authors hypothesized the relationship between eosinopenia and BRD risk could be attributed to stressed calves having altered hematopoiesis from eosinophils being redirected from circulation to a source of inflammation. Furthermore, cortisol produced upon activation of the HPA axis can result in eosinopenia. Elevated cortisol has been linked to eosinopenia in parturient goats (Fonteque et al., 2013), humans (Ray and Kanagasabapathy, 1993), bulls (Gupta et al., 2007), and cats (Middleton et al., 1987). This does not exclusively occur in mammals; Wojtaszek et al. (2002) even observed this phenomenon in carp, with the fish developing eosinopenia and neutrophilia within 24 hours of intraperitoneal cortisol injection. Roth et al. (1981) also observed

decreased eosinophils three days after BVDV challenge, along with decreases in lymphocytes and neutrophils.

Odds of calves developing BRD with various concentrations of eosinophils and RBC. Adapted from: Richeson et al. (2013)

		BRD1			BRD 2	
Variable	**OR**	**95% CI**	***P* Value**	**OR**	**95% CI**	***P* value**
2- level categorization						
Eosinophils (x10^3 cells/ uL)			< 0.001			<0.001
< 0.054	1.98	1.31-2.89		2.04	1.41-2.97	
≥ 0.054	---	---		---	---	
RBCs (x10^6 cells/ uL)			0.058			0.03
< 10.0	0.75	0.49-1.01		0.69	0.49-0.97	
≥ 10.0	---	---		---	---	
3- level categorization						
Eosinophils (x10^3 cells/ uL)			0.002			<0.001
< 0.011	2.65	1.54-4.57		2.51	1.51-4.17	
0.011-0.108	1.55	0.99-2.41		1.71	1.07-2.73	
> 0.108	---	---		---	---	
RBCs (x10^6 cells/ uL)			0.002			0.007
< 9.46	0.62	0.36-1.05		0.63	0.41-0.98	
9.46-11.2	0.44	0.28-0.70		0.53	0.36-0.79	
> 11.2	---	---		---	---	

Only CBC variables with significant ($P \leq 0.05$) effects on determination of BRD at least once are included

In a study examining impact of short (12-hour) or long (24-hour) hauls, Cole et al. (1988) reported a positive linear relationship between transit time and WBC and neutrophils, and a negative linear relationship between transit time and RBC, lymphocyte, and eosinophil values. These variables may thus be able to indicate to feedlot managers the degree of

stress a calf might have undergone prior to arrival, assisting in drug-administration decisions.

Eosinopenia in humans has also been linked to elevated mortality risk. Abidi et al. (2011) compared arrival eosinophil data in ICU patients that died or survived after 7 days and found that of the 200 patients observed, those with eosinophil count less than $40/mm^3$ had a significantly greater chance of mortality. The consequence of abnormal eosinophil counts is different in different circumstances. For example, Mikalsen et al. (2014) discussed a study that reported elevated eosinophil count in infants with bronchiolitis was related to those children later developing asthma, bronchial hyper-responsiveness, and having lower FEV_1 (forced expiratory volume in first second). The authors were unable to conclude whether the correlation was due to direct damage caused by the eosinophils during infant bronchiolitis or just an indication of that child's pulmonary immune dysfunction. More research is needed across all species to determine the effects of eosinopenia and eosinophilia. An important point to make for this particular leukocyte is that it is present in very low quantities, relative to the other leukocytes such as neutrophils and lymphocytes, therefore machine sensitivity is of the utmost importance. In a letter to the editor, Amundsen et al (2015) summarize a study they carried out to test the accuracy of eosinophil counts on Advia 2120i and Sysmex XE-2100, explaining that the duplicated samples ran through each machine ended with different results, particularly in samples from patients with low eosinophil counts. To that note, using eosinopenia as a predictor of morbidity makes it of utmost importance to ensure calibration and sensitivity of the testing machine.

Aich et al. (2009) wanted to discover if serum analysis for protein, metabolite, and other elements contained biomarkers that could be predictive of BRD survival. In that study, 20 calves were infected with BHV-1 on day 0, serum was obtained for days 0-4, then on day 4 calves were challenged with *M. haemolytica.* A 60% mortality rate was observed. Calves that ended up surviving possessed higher serum lactate on day 0 than calves that subsequently died, but the authors offered no biological explanation. Calves with fatal BRD had greater serum cortisol concentration than surviving calves, which is indicative of a greater stress response which then led to poorer immune function. Iron and glucose were both greater in surviving calves on day 4 than in dead calves, which is likely due to those animals having better appetite through the disease challenge. A limitation of this study was the small sample size, but the results regarding elevated lactate and cortisol are worth noting.

Haptoglobin

Haptoglobin is a prominent acute phase protein in cattle that has been studied extensively, but the serum concentration of haptoglobin can be misleading because it can bind with free hemoglobin and the resulting haptoglobin-hemoglobin complexes may not be detected in assays. Serum haptoglobin is known to be an indicator of trauma, but this author is unaware of any evidence of its utility as a predictive tool. Wittum et al. (1996) observed serum haptoglobin concentration in calves at feedlot arrival and slaughter between receiving standard antimicrobial therapy for any respiratory disease or observation of the disease process only. Incidence of lung lesions was recorded at slaughter. The authors reported the calves that had received antibiotics had lower

haptoglobin at slaughter, but haptoglobin concentrations were similar between groups with or without lesions, independent of antimicrobial administration. Therefore, they conclude haptoglobin is an "all or nothing" transient response, not indicative of disease severity (Wittum et al., 1996). Petersen et al. (2004) stated the following circumstances where elevated serum haptoglobin has been reported: infection with BVDV and *P. haemolytica,* infection with *P. multocida* and *P. haemolytica,* infection with BRSV, foot-and-mouth virus infection, mastitis, castration, fatty liver, fasting, 2-day transport, and numerous others. This indicates the non-specific nature of haptoglobin and the unsuitability of serum haptoglobin as anything other than an indicator of tissue damage. Young et al. (1996) attempted to use haptoglobin as a predictor of BRD and reported poor efficacy. They obtained blood for serum analysis from 366 mixed-breed steer and heifer calves upon yard arrival, day 40, and day 65, then observed calves daily for the following ten days for clinical signs of BRD. Ten mg/dl was used as the haptoglobin threshold, and concentrations greater than this were considered elevated. None of the calves with greater than 10mg/dl haptoglobin at arrival were diagnosed with respiratory disease in the first ten days. Thirty-seven calves were reported to have increased haptoglobin on sample day 40, seven of which developed clinical BRD in the next 10 days. The last sample day at 65 DOF showed 27 calves with elevated haptoglobin, of which seven became clinically ill with BRD. The authors reported that the percentage of calves diagnosed with clinical BRD tended to increase with increasing haptoglobin concentration. Haptoglobin is a transient acute phase protein, so it is unlikely that all the animals that possessed elevated haptoglobin were captured in this study. Regarding

clinical BRD, the authors conclude that serum haptoglobin is an inadequate predictor, given that some of the subjects had "extreme" values yet did not succumb to BRD. They also observed lung lesions at slaughter for a subset of the animals and reported 58% of the calves with elevated haptoglobin had lung lesions while 39% of calves that never possessed elevated haptoglobin had pulmonary lesions. The authors concluded that though more animals with greater haptoglobin developed lesions, it was not a large enough proportion to indicate an association with subclinical illness (Young et al., 1996). Burciaga-Robles et al. (2009) reported that calves had greatest haptoglobin concentrations upon first treatment, but concluded it is best used as a diagnostic measure.

Hemocytometers

Complete blood count variables are frequently obtained by hemocytometer machines. These machines analyze blood in different ways: impedance analysis, quantitative buffy coat analysis, and flow cytometry (Welles, 2012). As the quantitative buffy coat method is the least automated and takes seven minutes per run, it is not commonly used. Cells are differentiated based on density, or location in the test tube, post-centrifugation by how each layer fluoresces. Another short-coming is that it cannot complete a full differential; for example, all granulocytes are grouped together (Welles, 2012). The most common analysis method is impedance technology which differentiates cell types based on the voltage passing through each single-file cell as they pass between two electrodes and quantity is determined by number of electronic pulses the machine detects (Welles, 2012). This type of machine is more reliable, and has a faster analysis time of approximately two minutes. Another commonly used method is flow cytometry.

Cells pass single-file through a laser beam and refracted light scatter is measured and analyzed to determine cell type and quantity with a cycle time of two minutes (Welles, 2015). The ProCyte Dx (IDEXX Laboratories, Inc. Westbrook, ME) uses laser flow cytometry, laminar flow impedance, and optical fluorescence (Idexx Laboratories, Inc.). Laser flow cytometry directs white blood cells single file through a flow cell where a semiconductor laser at 633 nm wavelength makes contact with each cell and photodiodes interpret the way the light scatters, identifying the cell type. Laminar flow impedance provides a RBC count. Optical fluorescence stains sample cells then measure refracted light scatter to count immature RBC, or reticulocytes (Idexx Laboratories, Inc.). Together those technologies produce an in-depth cell differential for diagnostics.

Conclusions from the Literature

Substantial literature supports the consensus that sustained stress with the associated sustained increase in cortisol synthesis via HPA-axis stimulation is detrimental to calf immunity and disease resistance. The processes of weaning, transporting with associated dehydration, castration, vaccination, implantation, dehorning, and branding all may occur within days of each other and cause significant stress in an additive manner. When the calf is at his most immunologically vulnerable, he then is sent to a new pen with new herd mates and fed novel feed in a novel method. These conditions predispose animals to developing BRD. However, not every calf that undergoes these circumstances becomes ill. Industry standard dictates when a high-risk cohort is processed; every calf should receive antimicrobial metaphylaxis. This management technique is clearly demonstrated to improve health and performance in high-risk cattle; however, only the

portion of the cohort with bacterial infection during the duration of therapy of the selected antimicrobial benefits from the procedure. Simultaneously, the public perception is that animal agriculture injudiciously uses antimicrobials in their production practices, leading to antimicrobial resistant bacterial infections conferred in humans. The same elevated cortisol that creates vulnerable calves, also leads to other stress markers in the blood. Haptoglobin, serum amyloid A, eosinophils, and other biomarkers have been shown to have utility in identification of stressed calves. This information may ultimately lead to a chute-side test that can be performed to dictate which calves in a high-risk lot are actually biologically high-risk and require metaphylaxis. Millions of dollars are invested into research each year for natural treatments and preventions of BRD. Nutritional supplements of various vitamins and minerals have shown efficacy; hopefully, when coupled with the ability to identify vulnerable individuals through physiological biomarkers, the problem of BRD may be mitigated. However, unless changes are made to the way beef cattle are marketed, i.e. bring the feed to the calf instead of vice versa, the animals will still experience compounding stressors with novel pathogen exposure that can result in BRD.

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Chapter III:

Abstract

This study sought to elucidate if one or more hematologic variables determined upon arrival could predict individual calves at increased risk for BRD compared to cohorts more likely to remain healthy. Utilizing hematologic information derived from 502 steers and bulls with unknown previous health history, we categorized individuals for each complete blood count (CBC) variable in two ways: 2-level categorization determined by receiver operating characteristic (ROC), and 3-level categorization determined by simple quartile division. A reduced eosinophil status upon arrival, expressed as both a percentage of total leukocytes or as concentration, was correlated with increased odds (OR>1.6; P<0.05) of BRD morbidity outcome for 2- and 3-level categorizations. Animals with reduced hemoglobin (CI 0.40-0.95) and/or reduced red blood cells (RBC; CI 0.43-0.95) were determined to be at lesser risk (OR<0.7; P<0.03) to be treated once for BRD based on 2-level categorization. Those in the lowest quartile for relative neutrophils (CI 1.18-3.81) and neutrophil:lymphocyte ratio (NLR; CI 1.07-3.78) had increased risk (OR>2.0; P<0.02) of being treated once for BRD in the 3-level categorization. Likewise, decreased percentage of lymphocytes in peripheral blood was associated with decreased mortality risk (OR<0.2; P<0.01) in 2-level (CI 0.09-0.63) and 3-level (CI 0.02-0.42) categorization methods. In concert with low percentage lymphocytes, a low NLR was also associated with increased mortality risk (OR>5.0;

P<0.04; CI 1.33-20.12) in the 3-level model. Because eosinopenia has been previously reported in multiple mammalian species to be predictive of poor health outcomes, this particular variable may hold promise for use in feedlot health management decisions upon arrival.

Introduction

Bovine respiratory disease (BRD) is the most common and costly health disorder in the US cattle feeding industry. The term BRD describes a complex illness potentially caused by multiple pathogens including: bovine viral diarrhea virus (BVDV), bovine herpesvirus-1 (BHV-1), bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI-3V), *M. Haemolytica, P. multicida, H. somnus*, and varieties of *Mycoplasma.* Chronic stress can cause immunosuppression, and the stressful processes of weaning, transport, and social realignment as a calf leaves the ranch of origin frequently present the opportunity for affliction. If transport time is longer than a few hours, associated fasting, dehydration, and fatigue are additional stressors. Although antimicrobial metaphylaxis has been proven to be effective in reducing BRD morbidity rate and improving performance, modern beef consumers are demanding a reduction or cessation of antimicrobial use in food animals. A reliable predictive test for BRD risk added to the arsenal would allow infected animals to be treated before they become clinically ill and would aid feedlot personnel in better managing the disease, thus improving animal welfare and ensuring judicious use of antimicrobials used to control or treat BRD.

Materials and Methods

Animals and General Processing Procedures

This study used data collected from cattle for a companion study described in Pillen et al. (2016; IACUC Protocol No. 02-10-13). Crossbred beef steers (n=135) and bulls (n=367) weighing 179.4 ± 26.2 kg arrived at a commercial feedlot near Hereford, TX in five groups that were procured from south Texas auction markets (est. 970 km distance of transport). The groups used in this study arrived to the feedlot on 24NOV14 (block 1), 15APR14 (block 2), 28OCT14 (block 3), 17FEB15 (block 4), and 09JUN15 (block 5), were processed the following day, and evaluated for 56-d post-processing. During initial processing, bulls were castrated with an elastic band, all calves were branded on the left hip with a hot iron, an ear notch was collected for identification of calves persistently infected (PI) with BVDV, horns were tipped, pedometers were placed proximal to the metatarsus of the right leg to collect behavior data used in the companion study, a jugular blood sample was collected, and calves were administered 1.1 mL/45.5 kg BW ivermectin-clorsulon (Ivomec Plus; Merial Limited, Duluth, GA) and 2 mL/45.5 kg BW tilmicosin phosphate (Micotil, Elanco Animal Health, Greenfield, IN). Vaccination procedures included: parenteral trivalent respiratory vaccination containing bovine herpes virus-1 (BHV-1) and BVDV types 1 and 2 (Titanium 3, Elanco Animal Health), an intranasal trivalent (BHV-1, BVDV-1, BVDV-2) respiratory vaccine (Inforce 3; Zoetis, Kalamazoo, MI), autogenous *M. haemolytica* bactrin (American Animal Health; Grand Prairie, TX) and multivalent *Clostridial*-tetanus toxoid (Covexin 8; Merck Animal Health, Madison, NJ). Additionally, calves were implanted in the

caudal aspect of the right ear with 20 mg estradiol benzoate, 200 mg progesterone, and 29 mg tylosin tartrate (Component ES; Elanco Animal Health) and a subset (n=269) from blocks 2, 3, 4 and 5 were nasal swabbed. Additional detail on the arrival procedures associated with the companion study is reported in Pillen et al. (2016).

Clinical Illness

Morbid calves were classified as such using a combined system of clinical illness score (CIS) and a depression score (DS). The CIS scale was from 0 to 4 with a CIS of 0 indicating a healthy, normal calf, a 1 indicative of a slightly ill calf with nasal discharge and a gaunt appearance, a 2 indicating moderate illness with nasal discharge, self-isolation, and respiratory difficulties, a 3 indicating a severely ill calf with purulent discharge, labored breathing, and a minimal flight zone, and a 4 indicating a moribund animal near death. The DS scale was also 0 to 4 with a DS of 0 being a normal calf, a 1 being slightly depressed calf that lags behind its cohorts but is still alert, a 2 indicating a moderately depressed calf with a lowered head that may be uncoordinated, a 3 being an obviously weak animal with a nearly non-existent flight zone, and a 4 being unresponsive to humans. If an animal was identified as being a 1 for both methods or ≥ 2 for either method, it was classified as a BRD case and was treated according to the antimicrobial treatment regimen suggested by the consulting feedlot veterinarian. A BRD case was administered 1.5 mL/45.5 kg BW ceftiofur crystalline-free acid (Excede; Zoetis) upon first treatment, 6 mL/45.5 kg BW florfenicol (Nuflor; Merck Animal Health) if a second treatment was required, and oxytetracycline at 5 mL/45.5 kg BW (Oxytet 100; Norbrook Inc., Lenexa, KS) for the third and final antimicrobial treatment. All medication was

given in accordance with label guidelines. Treatment and other health records were obtained from the feedlot data management system (Animal Health International, Inc., Greeley, CO) and used for statistical analyses.

Complete Blood Count and Virus Detection

Two blood samples were collected via jugular venipuncture, one in a serum separator tube used for analyses in the companion study and a second tube containing 7.2 mg EDTA (BD Vacutainer, Franklin Lakes, NJ). The anti-coagulated blood samples from blocks 1 and 2 were submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) in Amarillo, TX for complete blood count (CBC) with an automated hemocytometer (Cell Dyn, Abbott Laboratories, Inc., Abbott Park, IL) and histological blood smear analysis. Blood samples obtained from blocks 3 to 5 were analyzed at the West Texas A&M University Animal Health Laboratory using an automated hemocytometer (ProCyte Dx, IDEXX Laboratories, Inc., Westbrook, ME). Hematological data were tabulated in spreadsheets and included the following variables: total white blood cells (WBC), percent neutrophils (PERNEU), neutrophil concentration (NEU), percent lymphocytes (PERLYM), lymphocyte concentration (LYM), percent monocytes (PERMONO), monocyte concentration (MONO), percent eosinophils (PEREOS), eosinophil concentration (EOS), red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT). Variables reported as percentages are relative to the total leukocyte count, and all variable

concentrations are n × 10^3 cells/μL, with the exception of RBC which is reported as n × 10^6 cells/μL.

The procedures employed by personnel from the TVMDL to detect virus in nasal swab samples is described elsewhere (Richeson et al., 2016). In brief, nasal swabs were collected on a subset (n = 269) and analyzed via RT-PCR to detect the presence of PI-3V, BRSV, BHV-1, and BVDV. Due to low frequency of positive samples, the nasal swab data is reported in terms of demographics and these data were not statistically analyzed.

Statistical Analyses

Calf identification data, morbidity and mortality, CBC variables, and performance data was organized in a spreadsheet (Microsoft Corp, Seattle, WA) and analyzed using statistical software (SAS version 9.4, SAS Inst. Inc., Cary, NC). Associations between CBC variables and subsequent BRD diagnosis were determined using multivariate logistic regression. Animals were classified for each hematological variable by 2- and 3-level categorization. Three-level categorization was achieved by categorizing individuals into low, middle, and high quartiles for each CBC variable. The variables were then converted to ordinal data, with a "1", "2", or "3" denoting individuals in the low, medium, or upper quartile. Two-level categorization was achieved by using ROC curves generated with the PROC LOGISTIC function of SAS. The thresholds were selected by observing the most ideal specificity at given sensitivities, i.e. the greatest combined values. The variables were converted to ordinal data with a "1" denoting individuals below the calculated threshold, and a "2" denoting animals above that threshold.

With thresholds determined for each categorization method, the GLIMMIX procedure of SAS was used to calculate odds ratio for each CBC variable in relation to the indicated health outcome. Originally, the model contained the CBC variable of interest, block, sex, pen, and initial weight, but data failed to converge for several CBC variables. A reduced model was subsequently used, including CBC variable, sex, and block. Only the CBC variables with a *P*-value <0.05 and confidence intervals (CI) not including 1.0 were considered significantly associated with morbidity or mortality outcomes. Variables with a *P*-value <0.10 are discussed as tendencies.

Results

Of the 502 calves included in this trial, 230 were treated at least once for BRD (45.8%), 86 were treated two or more times (17.1%), 22 were treated three times (4.4%) and 25 calves died (5.0%) during the 56-d evaluation period. Summarized CBC data is displayed in Table 1. Demographic data including morbidity and mortality rate is shown in Table 2. Out of the 269 nasal swabs collected, 75 (27.9%) were positive for at least 1 of the 4 respiratory viruses evaluated. Fourteen (5.20%) calves were positive for BHV-1, two (0.74%) were positive for BVDV, 37 (13.8%) for BRSV, and 23 (8.55%) for PI-3. Block 4 had the most calves with viruses detected in nasal swabs with 28 total positives, 19 (29.69%) of which were positive for PI-3. Only three calves (4.84%) in block 2 were positive for more than one virus, two calves (3.13%) from block 4 were positive for more than virus, and three calves (3.70%) from block 5 were positive for more than one virus. Of the 75 calves with virus-positive nasal swabs, 26 (34.67%) were treated at least once for BRD. These observational results are summarized in Table 3.

There were no differences between means for bulls and steers, other than bulls having (P<0.05) reduced eosinophil concentration and eosinophil percentage, and tending (P<0.10) to have reduced WBC compared to steer cohorts. Compared with the published reference ranges (Merck, 2015), these cattle had an abnormally high PERMONO with an overall mean of 11.2%, and low PERLYM with an overall mean of 57.2%. Thresholds calculated with ROC analysis for CBC variables predictive of a BRD event are reported, along with sensitivities and specificities for each (Table 4). The P-values reported are for univariate analysis of predictive value and indicate the difference between the CBC variable AUC and the chance AUC. Mean corpuscular hemoglobin concentration (MCHC) and PLT were the only CBC variables associated (P≤0.04) with any health outcome when the variables were analyzed as continuous data, but AUC for all variables was relatively low, ranging from 0.50 for hematocrit to 0.57 for platelet count. The moderate to low AUC values are indicative of limited predictive value. We then progressed to multivariate regression of the data in categorical form for further analysis.

To generate odds ratios via multivariate logistic regression, the model included CBC variable, sex, and block. Significant BRD-predictive CBC variables and their odds ratios for 2-level categorization are summarized in Table 5. The odds of a calf with reduced PEREOS (CI 1.01-2.57) and EOS (CI 1.06-2.50) at arrival being treated at least once for BRD were 1.6 (P<0.05). Further, odds of calves with reduced PEREOS (CI 1.12-3.32) and EOS (CI 1.15-3.29) at arrival being treated twice or more for BRD were 1.9 (P<0.02). Calves with reduced RBC (CI 0.43-0.95) and HGB (CI 0.40-0.95) at arrival were less likely (OR<0.64; P<0.03) to be treated once for BRD than their peers with

above-threshold levels. Calves with decreased PERLYM were at decreased odds (OR<0.6; *P*<0.04; CI 0.34-0.96) of being diagnosed with BRD at least once, and were also at decreased odds (OR<0.3; *P*<0.01; CI 0.09-0.63) of mortality than their cohorts with increased PERLYM. Those animals with decreased HGB were at 0.6 the odds (*P*≤0.04; CI 0.34-0.98) of being treated at least twice for BRD than their above-threshold cohorts. Cattle below the threshold for PLT were more likely to be diagnosed at least once for BRD (OR>1.65; *P*<0.05; CI 1.01-2.71) and tended to be at increased odds for BRD 2 (OR>1.9; *P*<0.04; CI 1.04-3.54). For 3-level categorization, animals in the lower quartile for PEREOS count (CI 1.12-5.26) and EOS (CI 1.34-5.43) had more than 2.4 greater odds (*P*<0.04) to be treated once than animals in the upper quartile (Table 6). Animals in the lower quartile for NLR (CI 1.07-3.78) and/or PERNEU (CI 1.18-3.81) were twice as likely (OR>2.0; *P*<0.02) to be treated once than animals in the upper quartile for those variables. Interestingly, odds of mortality of animals in the lower quartile for NLR were 5.17 (*P*<0.04; CI 1.33-20.12) times their upper quartile counterparts. Lower quartile PERLYM calves were at less odds (OR<0.10; *P*<0.003; CI 0.02-0.42) than calves in the upper quarters for mortality.

Odds ratios were generated to determine if castration status at arrival had an impact on subsequent BRD diagnosis. There were no significant interactions, but calves that were bulls at arrival tended to be at increased risk for being treated at least once for BRD (OR=1.49; *P*≤0.07; CI 0.97-2.30). It is plausible to assume that calves that have not been castrated prior to feedlot arrival are probably not vaccinated or weaned prior to sale

either, which is a significant predisposing factor for increased disease risk, in addition to adding another inflammatory and stressful procedure to initial processing.

With the hematological data in continuous form, differences in LS Means were observed between animals that remained healthy and those treated only once, only twice, three times, and those that died, for each CBC variable. Platelets were the only variable with differences between the animals that remained healthy and animals that were diagnosed once or twice (Table 7). Thrombocytopenia, or decreased platelet number, has been associated with BVDV, especially in the animals that develop bloody diarrhea as a symptom (Rehun et al., 1989). Downey-Slinker et al. (2016) also found animals subclinically infected with BVDV to be thrombocytopenic, even though none developed diarrhea.

Discussion

Nasal swabs and molecular analysis to determine virus presence may provide insight for managers in the disease risk of an arriving group of cattle, but the current processing time is not timely to aid in decision-making at initial processing. Additionally, only 34.7% (26/75) of the cattle identified as having at least 1 of the 4 common viral agents (BHV-1, BRSV, PI-3V, BVDV) in their naris were treated for BRD, suggesting that presence of one of those viral pathogens in the nasal passages does not exclusively indicate that animal will succumb to clinical presentation of disease.

Eosinopenia, both in terms of relative eosinophils and percentage of eosinophils, was predictive of morbidity for both types of categorization. For 2-level categorization, animals below the calculated threshold for PEREOS (0.2%) and EOS (0.02 $x10^3$ /μL)

were 1.6 times more likely to be treated for BRD at least once, and 1.9 times more likely to be treated at least twice, respectively. Likewise, Richeson et al. (2013) reported an association between low eosinophil quartile and BRD treatment. Stress decreases eosinophil concentration by increasing apoptosis of those cells in peripheral blood and by decreasing eosinopoiesis in bone marrow (Fonteque et al., 2013). Therefore, it is possible that those calves were at increased odds of BRD not directly because of their reduced eosinophils, but because eosinopenia is a biomarker of more intense stress in those animals. Potentially supporting this idea is a study in humans where eosinopenia was correlated to pediatric mortality independent of infection, possibly indicating the children that died were under a greater degree of physiologic stress than the surviving children (Kim et al., 2013). However, Isobe et al. (2012) reported that eosinophils do have an active role in inflammation mediation; eosinophils are recently known to produce the lipid mediators PD1 and RvE3, which directly inhibit neutrophil translocation and promote phagocytosis of degrading neutrophils by macrophages. By these mechanisms eosinophils resolve inflammation, suggesting a lack of or reduction in those cells would result in greater tissue damage. Elevated cortisol concentration was also associated with eosinopenia in parturient goats (Fonteque et al., 2013), humans (Ray and Kanagasabapathy, 1993), bulls (Gupta et al., 2007), and cats (Middleton et al., 1987). This does not exclusively occur in mammals; Wojtaszek et al. (2002) observed this same phenomenon in carp, with the fish developing eosinopenia and neutrophilia within 24 hours of intraperitoneal cortisol injection.

Calves with elevated RBC were found to be at increased odds for BRD in this study for 2-level categorization, and this was also observed in Richeson et al. (2013). Elevated RBC, or polycythemia, can indicate dehydration, which has been shown to decrease salivary and mucosal antimicrobial proteins (Fortes et al., 2012). Polycythemia can also be an indication of lung damage; as the lungs' ability to absorb oxygen is decreased, more gas-trafficking red blood cells are produced in an attempt to maintain oxygen delivery to tissue. Calves in this study may have had elevated RBC for both reasons, but particularly due to dehydration since they were transported long-distances from their purchase origin and likely withheld from water for extended time. Hemoglobin was also elevated in high-risk calves at arrival, presumably for the same reasons as RBC. Transport is known to increase serum cortisol in cattle (Chulayo et al., 2016; Crookshank et al., 1979; Hulbert et al., 2011; Ishizaki and Kariya, 2010) and Parker et al. (2003) observed a diuretic effect of increased cortisol in cattle, exacerbating the dehydration status of stressed, water-restricted calves.

Increased relative lymphocyte concentration was observed to be associated with increased odds of mortality for both categorization types. This association may indicate the animals in the upper quartile for lymphocyte percentage had a pre-existing viral infection and were producing abundant natural killer cells and T-lymphocytes in response to the viral infection. A CBC does not differentiate between innate NK cells and adaptive lymphocytes (i.e., T- and B-cells), so it is not plausible to distinguish whether these calves were in the early or late stages of disease. Abundant NK cells would be indicative of a viral infection in early stages, while elevated T- and B- cells would indicate the

animal is launching humoral and cell-mediated immunity for protection against future similar infections (Abbas et al., 2007).

The 3-level categorization of NLR revealed a correlation between low NLR and BRD diagnosis and death. The majority (72.5%; 87/120) of the calves in the low quartile for NLR were low due to being in the lowest quartile for neutrophils. This contradicts results from a meta-analysis in human cancer patients, where NLR>4 was associated with 1.81 greater odds of death (Templeton et al., 2014), and another human study observing chronic obstructive pulmonary disease (COPD) patients possessed greater NLR than healthy controls (Günay et al., 2014). It is possible in the present study that the correlation between elevated percent lymphocytes and death artificially inflated the effect of a low NLR; further research in the bovine model is necessary to elucidate these implications. In addition to the calves possessing elevated percentage of lymphocytes being at increased odds for BRD, a low PERNEU was also indicative of greater odds of BRD treatment in the 3-level categorization. The associations could be due to those animals' neutrophils already having migrated to inflamed pulmonary tissue, thus resulting in low blood concentrations. Neutrophils are the first leukocytes to respond to an infection (Abbas et al., 2007), so the animals possessing reduced PERNEU may have been infected upon arrival.

Conclusion

As public demand for decreased antimicrobial use in food animals increases, the need for alternative strategies that can maintain animal health with reduced, or without use of antimicrobials becomes critical. This study determined that eosinopenia, increased

RBC and hemoglobin, and decreased NLR, percent neutrophils, and percent lymphocytes could be promising biomarker candidates to identify newly received feedlot cattle at greater need of antimicrobial metaphylaxis. Additional research is needed to validate if these variables are in fact predictive of BRD events and to determine if a biological causation exists for these variables to alter BRD pathogenesis. Reduced eosinophil count has been associated with BRD morbidity outcome in a previous study and the current one, so this particular leukocyte may warrant special attention as a biomarker to assist with antimicrobial metaphylactic or BRD treatment decisions in the feedlot.

Tables

Table 1. Mean complete blood count variables for 135 beef steers and 367 beef bulls upon feedlot arrival.

				Mean (SD)		Least Square Mean (SE)	
Variable	Mean	S.D	Range	Bulls	Steers	Bulls	Steers
Total WBCs	8.52	2.45	0.01-20.43	8.40 (2.35)	8.85 (2.70)	8.39 (0.28)	8.84 (0.34)*
Neutrophil %[1]	31.0	11.6	4.90-78.6	30.8 (11.5)	31.7 (12.0)	30.7 (2.31)	31.8 (2.43)
Lymphocyte %[1]	57.2	14.4	14.7-94.0	57.5 (14.3)	56.4 (14.6)	57.5 (4.82)	56.5 (4.88)
Monocyte %[1]	11.2	6.73	0-31.5	11.2 (6.75)	11.1 (6.67)	11.2 (2.77)	10.9 (2.78)
Eosinophil%[1]	0.66	0.97	0-8.00	0.58 (0.82)	0.87 (1.28)	0.59 (0.10)	0.86 (0.14)†
Neutrophil[2]	2.67	1.33	0.26-8.73	2.61 (1.28)	2.82 (1.45)	2.61 (0.11)	2.82 (0.15)
Lymphocytes[2]	4.90	1.90	1.31-13.2	4.85 (1.82)	5.02 (2.08)	4.85 (0.54)	5.03 (0.56)
NLR[3]	0.63	0.44	0.05-5.34	0.63 (0.44)	0.66 (0.42)	0.62 (0.09)	0.66 (0.10)
Monocytes[2]	0.93	0.70	0-7.88	0.93 (0.70)	0.93 (0.69)	0.94 (0.21)	0.92 (0.22)
Eosinophils[2]	0.06	0.10	0-1.18	0.05 (0.08)	0.08 (0.14)	0.05 (0.01)	0.08 (0.01)†
RBC[4]	10.22	1.58	0.14-14.1	10.29 (1.47)	10.06 (1.8)	10.3 (0.21)	10.1 (0.25)
HGB[5]	12.9	1.75	0.20-17.7	13.0 (1.57)	12.7 (2.14)	13.0 (0.31)	12.7 (0.34)
HCT %[6]	38.6	5.10	0.40-55.0	38.7 (4.59)	38.5 (6.30)	38.7 (0.50)	38.5 (0.69)
MCV[7]	38.1	4.00	26.0-69.4	38.0 (4.13)	38.4 (3.61)	38.0 (0.67)	38.4 (0.70)
MCH[8]	12.8	1.71	9.60-41.5	12.8 (1.91)	12.7 (0.94)	12.8 (0.12)	12.7 (0.11)
MCHC[9]	33.6	2.30	28.1-50.0	33.7 (2.19)	33.2 (2.56)	33.7 (0.76)	33.2 (0.78)
PLT[10]	756.7	818.4	0- 8435	764.3 (756.1)	736.6 (970.8)	762.7 (230)	746.0 (239)

Cross (†) denotes parameters with $P<0.05$, and asterisk (*) denotes parameters with $P<0.10$ (determined by Wilcoxin test).
Blocks 1 and 2 were analyzed with Cell Dyn (Abbott Laboratories, Inc., Abbott Park, IL), Blocks 3-5 were analyzed using a ProCyte DX (IDEXX Laboratories, Inc., Westbrook, ME)
[1]Percentage of total WBC count [2]$\times 10^3$ cells /µL [3]Neutrophil:lymphocyte ratio [4]$\times 10^6$ cells /µL [5]Hemoglobin, g/dL [6]Hematocrit, % [7]Mean corpuscular volume, fL [8]Mean corpuscular hemoglobin, pg [9]MCH concentration, g/dL [10] $\times 10^3$ platelets /µL

Table 2. Number of individuals by block and sex diagnosed with clinical BRD once, twice, or three times, and BRD-associated mortalities.

	Block 1		Block 2		Block 3		Block 4		Block 5	
BRD Outcome[1]	**Bulls** n=76	**Steers** n=24	**Bulls** n=72	**Steers** n=28	**Bulls** n=70	**Steers** n=31	**Bulls** n=75	**Steers** n=26	**Bulls** n=74	**Steers** n=26
1	22	8	13	3	29	10	33	13	12	1
2	11	1	4	2	14	5	13	5	7	2
3	5	1	1	0	5	0	7	1	0	2
Mortality	3	1	2	0	7	4	5	2	0	1

[1]Bovine respiratory disease (BRD) outcome "1" is the number of animals treated only once, "2" the number of animals treated only twice, "3" the number of animals treated thrice. Mortality is the number of animals that died attributable to BRD. A BRD diagnosis was an animal that had both a Depression Score and Clinical Illness Score of 1, or an animal with ≥2 for either system. Scoring system adapted from Pillen et al. (2016)

Table 3. Virus prevalence in nasal swab samples, by block.

Block	BHV-1[1]	BVDV[2]	BRSV[3]	PI-3V[4]
2	6/62 (9.68%)	0/62 (0%)	9/62 (1.45%)	1/62 (1.61%)
3	4/62 (6.45%)	1/62 (1.61%)	4/62 (6.45%)	1/62 (1.61%)
4	1/64 (1.56%)	0/64 (0%)	8/64 (1.25%)	19/64 (29.69%)
5	3/81 (3.70%)	1/81 (1.23%)	15/81 (1.85%)	2/81 (2.47%)
Total	14/269 (5.20%)	2/269 (0.74%)	37/269 (13.8%)	23/269 (8.55%)

A subset of 269 calves were swabbed with nylon-flocked nasal swabs (Puritan Medical; Guilford, ME) of the total 502; 62 from block two and three, 64 from block four, 81 from block five. No samples were collected from block one. Real time- PCR analysis was conducted by Texas A&M Veterinary Medical Diagnostic Laboratory (Amarillo, TX).
[1]Bovine herpesvirus-1 [2]Bovine viral diarrhea virus [3]Bovine respiratory syncytial virus
[4]Parainfluenza-3 virus

Table 4. Thresholds of selected sensitivity and specificity for complete blood count variables determined upon feedlot arrival of calves treated at least once for BRD based on ROC analysis.

Variable	Threshold	AUC[11]	*P*-value	Specificity	Sensitivity
Total WBCs	7.77	0.5240	0.3588	0.4	0.5609
Neutrophil %[1]	33.0	0.5054	0.8362	0.6	0.4739
Lymphocyte %[1]	61.0	0.5312	0.2305	0.6	0.3739
Monocyte %[1]	10.0	0.5551	0.0327	0.5	0.6478
Eosinophil%[1]	0.2	0.5111	0.6634	0.4	0.6478
Neutrophil[2]	2.75	0.5200	0.4430	0.6	0.4087
Lymphocytes[2]	5.21	0.5363	0.1646	0.6	0.3783
NLR[3]	0.596	0.5162	0.5380	0.6	0.4957
Monocytes[2]	0.80	0.5368	0.1551	0.5	0.6174
Eosinophils[2]	0.02	0.5203	0.4257	0.4	0.5870
RBCs[4]	10.24	0.5089	0.7320	0.5	0.5130
HGB[5]	13.5	0.5270	0.2978	0.6	0.3913
HCT[6]	38.6	0.4979	0.9352	0.5	0.5304
MCV[7]	36.9	0.5079	0.7598	0.4	0.6261
MCH[8]	12.9	0.5355	0.1694	0.6	0.3826
MCHC[9]	32.8	0.5535	0.0384	0.4	0.5391
PLT[10]	485	0.5728	0.0045	0.4	0.4565

[1]Percentage of total WBC count [2]$\times 10^3$ cells /µL [3]Neutrophil:lymphocyte ratio [4]$\times 10^6$ cells /µL [5]Hemoglobin, g/dL [6]Hematocrit, % [7]Mean corpuscular volume, fL [8]Mean corpuscular hemoglobin, pg [9]MCH concentration, g/dL [10]$\times 10^3$ platelets /µL [11]Area under the curve

Table 5. Odds of newly received beef steers and bulls being diagnosed with clinical BRD or mortality over a 56-d evaluation period based on arrival complete blood count variables in 2-level categorization

Variable	n[6]	OR[7]	95% CI[8]	*P*-value	BRD Outcome[9]
Lymphocyte%[1]				0.0334	1
<61.0	304	0.567	0.336—0.956		
≥61.0	198	—	—		
Eosinophil%[1]				0.0456	1
<0.2	175	1.597	1.009—2.527		
≥0.2	327	—	—		
Eosinophils[2]				0.0255	1
<0.02	197	1.629	1.062—2.500		
≥0.02	305	—	—		
RBC[3]				0.0280	1
<10.24	248	0.638	0.427—0.953		
≥10.24	254	—	—		
HGB[4]				0.0270	1
<13.5	303	0.612	0.397—0.946		
≥13.5	199	—	—		
PLT[5]				0.0475	1
<485	233	1.652	1.006—2.713		
≥485	269	—	—		
Eosinophil%[1]				0.0187	2
<0.2	175	1.923	1.115—3.315		
≥0.2	327	—	—		
Eosinophils[2]				0.0132	2
<0.02	197	1.943	1.149—3.285		
≥0.02	305	—	—		
HGB[4]				0.0405	2
<13.5	303	0.580	0.344—0.977		
≥13.5	199	—	—		
Lymphocyte %[1]				0.0042	Mortality
<61.0	304	0.231	0.085—0.630		
≥61.0	198	—	—		

[1]Percentage of total WBC count [2]$\times 10^3$ cells/μL [3]$\times 10^6$ cells/μL [4]Hemoglobin, g/dL [5]$\times 10^3$ platelets/μL
[6]Number of individuals in each level [7]Odds ratio generated via multivariate logistic regression [8]95% confidence interval generated via multivariate logistic regression [9]BRD outcome "1" indicates animals diagnosed with BRD at least once, "2" indicates animals diagnosed with BRD at least twice
— = Referent

Table 6. Odds of newly received beef steers and bulls being diagnosed with clinical BRD or mortality over a 56-d evaluation period based on arrival complete blood count variables in 3-level categorization.

Variable	n[4]	Ranges	OR[5]	95% CI[6]	*P*-value	BRD Outcome[7]
Eosinophil%[1]					0.0302	1
	148	≤0.00	2.429	1.121—5.264		
	270	0.00—1.0	0.969	0.556—1.687		
	84	>1.0	—	—		
Eosinophils[2]					0.0177	1
	147	≤0.00	2.702	1.344—5.434		
	233	0.00—0.07	0.962	0.545—1.698		
	122	>0.07	—	—		
NLR[3]					0.0144	1
	120	≤0.33	2.015	1.073—3.781		
	258	0.33—0.81	0.963	0.581—1.595		
	124	>0.81	—	—		
Neutrophil%[1]					0.0145	1
	126	≤22.70	2.117	1.175—3.814		
	251	22.70—38.6	1.092	0.666—1.791		
	125	>38.6	—	—		
Lymphocyte %[1]					0.0024	Mortality
	130	≤46.20	0.095	0.021—0.420		
	249	46.20—68.0	0.143	0.043—0.475		
	123	>68.0	—	—		
NLR[3]					0.0329	Mortality
	120	≤0.33	5.173	1.330—20.12		
	258	0.33—0.81	1.779	0.524—6.041		
	124	>0.81	—	—		

[1]Percentage of total WBC count [2]$\times 10^3$ cells /μL [3]Neutrophil:lymphocyte ratio [4]Number of individuals in each level [5]Odds ratio generated via multivariate logistic regression [6]95% confidence interval generated via multivariate logistic regression [7]BRD outcome of "1" indicates animals diagnosed with BRD at least once, "2" indicates animals diagnosed with BRD at least twice

— = Referent

Table 7. Least squares means for arrival hematological variables between beef cattle that remained healthy, or were treated only once, only twice, three times, or subsequently died from bovine respiratory disease.

	BRD 1			BRD 2			BRD 3			Mortality		
Variable	**0**	**1**	***P*-value**	**0**	**1**	***P*-value**	**0**	**1**	***P*-value**	**0**	**1**	***P*-value**
Total WBCs	5.59	8.43	0.4923	8.55	8.33	0.4451	8.54	8.01	0.3190	8.53	8.25	0.5826
Neutrophil %[1]	30.9	31.1	0.8834	31.3	29.6	0.2292	31.1	29.8	0.6059	31.2	26.9	0.0688*
Lymphocyte%[1]	57.8	56.5	0.3001	57.1	57.6	0.7731	57.2	57.2	0.9894	57.0	61.0	0.1732
Monocyte%[1]	10.7	11.8	0.0603*	11.0	12.2	0.1137	11.1	12.6	0.3174	11.2	11.1	0.9389
Eosinophil%[1]	0.69	0.62	0.4265	0.69	0.53	0.1675	0.67	0.50	0.4313	0.64	0.98	0.0913*
Neutrophil[2]	2.68	2.65	0.7775	2.71	2.50	0.1810	2.68	2.47	0.4681	2.69	2.33	0.1902
Lymphocytes[2]	5.00	4.78	0.2043	4.91	4.83	0.7371	4.91	4.61	0.4670	4.89	4.99	0.8088
NLR[3]	0.62	0.65	0.4317	0.64	0.60	0.4652	0.64	0.57	0.5052	0.64	0.51	0.1577
Monocytes[2]	0.92	0.95	0.6843	0.93	0.95	0.7545	0.93	0.89	0.7525	0.94	0.85	0.5227
Eosinophils[2]	0.06	0.05	0.4180	0.06	0.05	0.2683	0.06	0.04	0.4037	0.06	0.09	0.1331
RBCs[4]	10.29	10.2	0.3437	10.3	10.1	0.3182	10.2	10.1	0.8137	10.3	9.71	0.0974*
HGB[5]	13.1	12.8	0.1068	13.0	12.7	0.1896	12.9	12.8	0.7616	13.0	12.1	0.0142
HCT[6]	38.7	38.5	0.6429	38.7	38.3	0.5097	38.6	38.3	0.7290	38.8	36.2	0.0139†
MCV[7]	38.0	38.2	0.7171	38.1	38.2	0.8276	38.1	38.0	0.8796	38.1	37.1	0.2041
MCH[8]	12.9	12.7	0.2142	12.8	12.7	0.6911	12.8	12.8	0.9334	12.8	12.6	0.6112
MCHC[9]	33.7	33.4	0.0922*	33.6	33.5	0.6391	33.6	33.7	0.8443	33.5	34.3	0.1226
PLT[10]	846.7	650.6	0.0073†	794.6	574.2	0.0228†	761.5	655.1	0.5516	763.2	636.0	0.4495

Cross (†) denotes parameters with $P<0.05$, and asterisk (*) denotes parameters with $P<0.10$ (determined by Wilcoxin test).
Blocks 1 and 2 were analyzed with Cell Dyn (Abbott Laboratories, Inc., Abbott Park, IL), Blocks 3-5 were analyzed using a ProCyte DX (IDEXX Laboratories, Inc., Westbrook, ME). BRD 1 is the animals never treated (0) compared to those treated only once (1); BRD 2 is the animals never tr (0) compared to those treated only twice (1); BRD 3 is the animals never treated (0) compared to those treated thrice (1); Mortality is the surviving animals (0) compared to those that died (1)

[1]Percentage of total WBC count [2]$\times 10^3$ cells /µL [3]Neutrophil:lymphocyte ratio [4]$\times 10^6$ cells /µL [5]Hemoglobin, g/dL [6]Hematocrit, % [7]Mean corpuscular volume, fL [8]Mean corpuscular hemoglobin, pg [9]MCH concentration, g/dL [10]$\times 10^3$ platelets /µL

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