NEONATAL BEEF CALF MORBIDITY AND MORTALITY IN MISSOURI

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

NEONATAL BEEF CALF MORBIDITY AND MORTALITY IN MISSOURI

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a candidate for the degree of Master of Science,

and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

This thesis is dedicated to my parents whose unwavering support throughout my entire career has made it possible for me to achieve my dreams. Mom and Dad, thank you for providing support and always encouraging me to go after my goals. Throughout this entire experience you both have been amazing listener whether that be about an excellent day doing research or a particularly frustrating experience. You have been sounding boards that have allowed me to realize what truly matters and I would not be here without you. I love you.

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ABSTRACT

Neonatal morbidity and mortality are major causes of economic loss for US beef cattle producers. The Missouri cattle industry represents 19% of the total agriculture sales in the state and is valued at 1.6 billion dollars. It is estimated that calf sales can account for up to 55% of a farm's gross cash income which highlights the economic importance of calf health to producers. The overarching aim of this body of work was to characterize morbidity and mortality of neonatal beef calves with a focus on bacteremia. An additional goal was to determine if any prognostic factors could be identified that may indicate a calf is likely to survive a critical illness. This body of work is comprised of two studies, one retrospective study on necropsy findings in neonatal beef calves presented to the University of Missouri's Veterinary Medical Diagnostic Laboratory and one prospective study conducted on critically ill neonatal beef calves presented to the University of Missouri's Food Animal Clinic.

The objective of the retrospective study was to describe lesions and abnormal test results among neonatal beef calves presented to a veterinary diagnostic laboratory. Overall, 1,060 reports were reviewed and inclusion criteria were bovine, 2 to 21 days of age, and of a non-dairy breed. Statistical analysis was performed to compare age, system affected, and pathologic agent types in addition to interactions between each system affected. A total of 95 cases met the inclusion criteria. Median age of enrolled calves was 9 days (range: 2 to 21). A total of 252 lesions were

identified with a median of 3 lesions/calf (range: 0 to 7) and 2 different body systems involved/calf (range, 0 to 5). The most common disorders were classified as digestive (42.1% [106/252]), respiratory (12.7% [32/252]), and multisystemic (11.1% [28/252]). With respect to age and system affected, calves with neurologic lesions were significantly younger (mean age: 5.1 days) than calves with digestive lesions (mean age: 9.6 days).

For the prospective study, the study objectives were to determine the prevalence of bacteremia in neonatal beef calves, identify factors predictive of bacteremia, and identify factors associated with survival. A total of 27 calves were enrolled in the study. Median age of calves was 7 days (range, 1 to 21). Diarrhea was the most common presenting complaint and was present in 26% (13/50) of calves. Most calf deaths occurred either during hospitalization or by 2 weeks after discharge. A lower prevalence of bacteremia (17%) was found in this study compared to previous reports. In this study, no bacteremic calves survived. Hypothermia, hypercapnia, and hyperfibrinogenemia were associated with decrease likelihood of survival.

The results of this thesis suggest that calves submitted to a veterinary diagnostic laboratory have a high prevalence of infectious diseases, mainly digestive, respiratory and multisystemic in origin. In addition, critically ill calves are more likely to die during hospitalization or in the first 2 weeks after discharge and that hypothermia, hypercapnia, and hyperfibrinogenemia were associated with decreased likelihood of survival. These findings could help guide producers and veterinarians when assessing factors contributing to neonatal beef calf loss and establishing prognosis for critically ill calves.

CHAPTER 1: LITERATURE REVIEW

1.1 Mortality in Neonatal Beef Calves

Neonatal morbidity and mortality are major causes of economic loss for US beef cattle producers. [1] In 2017, the majority of US beef operations (51.2%) had at least 1 calf that was born alive but died or was lost prior to weaning [2]. Overall, 2.2% to 3.5% of beef calves born alive die before weaning. [2-4] Any calf lost is detrimental not only to the health of the herd but also to the economics of the individual producer and the state of Missouri. In 2021 there were an estimated 1.9 million calves born in the state of Missouri [5]. It is estimated that up to 55% of gross cash farm income comes from cattle sales in cow-calf production systems [6]. The Missouri cattle industry as a whole was valued at 1.7 billion dollars in 2020 which is approximately 19% of the total agriculture sales in the state [7]. This significant economic contribution to the state's economy warrants investigation into what diseases contribute to neonatal calf morbidity and mortality.

Based on producer surveys, the most common causes of death in calves < 3 weeks of age are calving-related problems (24.7%), unknown (24.0%), predators (13.8%), weather related (12.5%), digestive diseases (11.9%), and respiratory diseases (8.1%). [2, 3] Overall, a high proportion of calves die from unknown reasons, leaving an outcome related knowledge gap for producers. Additionally, while producer driven data are valuable, since many neonatal calf diseases are tentatively diagnosed and treated by producers, determining causes of death based on history and clinical signs alone can be misleading. [8]

A postmortem assessment of tissues can provide clarity regarding underlying disease processes. Necropsy data can be useful to confirm disease status of animals especially when morbidities and mortalities are higher than expected, help producers understand outcomes when there is a perceived treatment failure, provide confirmation of a presumptive diagnosis, and potentially identify outcomes that could be controlled through adjusted management strategies. [9, 10] A comparison of expected postmortem findings based on clinical signs and administered treatments and actual postmortem findings obtained at necropsy found limited agreement, indicating that necropsies and additional laboratory-based testing should be part of a systematic postmortem examination of causes of death. [8] That said, in the US, only half of beef cow-calf operations consult with a veterinarian for any reason. [11] In a Canadian study, [12] only 9.8% of producers had a veterinarian perform a postmortem examination on ≥ 1 calf in 2010 and 5.2% had veterinarians submit tissues or biological samples to a veterinary diagnostic laboratory. Producers need to work with veterinarians to aid in the diagnosis of disease through appropriate test selection and then implement prevention measures to reduce future animal losses and antimicrobial usage.

There are limited numbers of studies that have evaluated beef calf deaths unrelated to birthing or reproductive diseases by use of gross necropsy, histopathology, and laboratory tests. In 1 prospective study that evaluated 203 herds in western Canada, among 558 calves that were > 3 days of age, the most common final diagnoses based on history, field necropsy, and histologic examination were starvation (16%); abomasal ulcer, torsion, and perforation (12%); and enteritis (11%). In this study multisystemic infections (sepsis) were the third most common infectious diagnosis and was found in 5% of the sampled calves. Although starvation was the most

common summary diagnosis, most of the cases had additional lesions, suggesting the calves were compromised by underlying disease. [13]

1.2 Sepsis and Bacteremia in Neonatal Calves

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [14]. Infection in this sense is defined as a pathological process caused by invasion of tissue, fluid, or body cavity with pathogenic micro-organisms [15]. While sepsis and bacteremia are often used interchangeably sepsis can arise from a variety of infectious etiologies that are not bacterial or bloodstream in origin and not all cases of bacteremia progress to a dysregulated host response. Sepsis is diagnosed in human medicine by the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score [14]. A score increase of greater than 2 is associated with an in-hospital mortality greater than 10% and those patients are defined as septic. The SOFA score evaluates the respiratory system through paO2/FiO2 ratio, the nervous system through the Glasgow Coma Scale, the cardiovascular system through mean arterial pressure and the need for vasopressors, the urinary system through creatinine and urine output, the hepatobiliary system through bilirubin measurements, and coagulation through platelet counts [14]. Since the SOFA score requires time and testing to be performed, physicians developed the quick SOFA (qSOFA) which can be performed bedside for quick assessment of patients. The qSOFA evaluates respiratory rate, mentation, and systolic blood pressure. If the qSOFA leads to suspicion of sepsis (score greater than 2) then the SOFA can then be performed. In bovine medicine the proposed sepsis definition evaluates 3 factors: the rapidity of blood culture growth, the bacteria cultured, and the complete blood count [16]. Two of the three factors must be abnormal to consider an animal as septic. The current proposed definition in bovine medicine does not evaluate organ

dysfunction and it requires blood cultures which pose significant challenges and potentially delays aggressive treatment of septic patients.

The gold standard for antemortem diagnosis of bacteremia in all species is a positive bacterial blood culture. Although blood cultures are vital for a confirmation of bloodstream infections, contaminated samples are common, complicating interpretation and potentially leading to misdiagnosis. A number of studies have been conducted to determine best practices regarding blood cultures in humans, and these have been applied to veterinary medicine [15]. Critical factors in the optimal recovery of pathogenic organisms from a blood culture include appropriate disinfectant and site preparation, appropriate volume of blood cultured, number and timing of blood cultures, dilution of blood, subcultures, and duration of incubation [15]. Overall, volume of blood is the most important of the critical factors in increasing rates of recovery of pathogens from blood. There is a direct relationship between the volume of blood cultured and the rate of microbial recovery due to the small number of pathogens present in blood [17]. Another key component is a sufficient number of blood cultures. Results of a single blood culture can be difficult to interpret, especially with organisms that may be either pathogens or contaminants. Therefore, it is recommended that a minimum of two, and preferably three or four blood cultures are collected. Although common practice, there is no evidence for the need to draw blood over an arbitrary interval of time or during febrile spikes, as it has not been shown to increase microbial recovery rate [18, 19].

Although current recommendations for recovery of pathogenic organisms from the blood have been outlined, to date no study has been published that follows the criteria regarding bloodstream infections in beef calves. The majority of studies previously conducted in calves only collected a single blood culture [20-22]. These studies have found positive bacterial cultures in 40% to 70% of calves, however, they potentially had a high rate of contamination that was not accounted for appropriately [20-22]. One study, in which two samples were collected from two different blood vessels found a lower prevalence of positive blood cultures with 24% to 31% of sampled calves being culture positive [23]. However, this study that performed blood cultures twice did not say whether different vessels were utilized for sample collection and it also did not have a clear definition of what constituted bacteremia when analyzing discordant results of multiple blood cultures. With that information, it is challenging to determine if the higher prevalence rates among studies in which only one sample was collected are due to true positives or contamination [15]. A more recent study of bacterial cultures in ill neonatal dairy calves found a lower prevalence of bacteremia in ill calves but a higher prevalence of bacteremia in their control group, which highlights the concern of contaminated blood samples [24]. Therefore, data regarding the incidence of bacteremia in calves collected using appropriate methods is needed. Blood cultures from critically ill calves have shown that Gram-negative bacteria account for approximately 80% of the bacterial isolates, with *Escherichia coli* being the most commonly isolated organism [16]. Other common bacterial isolates include *Salmonella*, *Campylobacter*, *Klebsiella*, and several *Staphylococcus* species. [16]

Most of the studies on bacteremia in neonatal calves have been performed primarily on dairy calves. Half of the published studies only included dairy calves. [21, 23] The other half of the

studies previously conducted included beef calves, however beef calves made up less than 30% of the total study populations in these studies [20-23]. Because of the different management styles, environments, and stresses, it is highly possible that beef and dairy calves have different blood culture pathogen profiles and different incidences of bacteremia.

Few studies have evaluated the antimicrobial susceptibility profiles of organisms isolated from calf blood cultures. Of the two that have been conducted, the study population was comprised of only dairy calves. One study reported susceptibility profiles for fifteen *E. coli* isolates [21]. In that study, 67% of the isolates were resistant to tetracycline, 40% to ampicillin, and 20% to amoxicillin-clavulanic acid (or clavulanate), and 20% trimethoprim/sulfa (TMS). Another study reported susceptibility profiles *for Staphylococcus, E. coli, Salmonella, and Klebsiella* isolated from blood [23]. Out of all of the isolates within that study, 56% were resistant to ampicillin, 20% to amoxicillin-clavulanic acid, 24% to ceftiofur, 52% to gentamicin, 88% to tetracycline, and 44% to TMS. One study evaluated changes in susceptibility profiles for *E. coli* isolates over time by performing blood cultures on critically ill calves on the same farm over two years [23]. A difference in the susceptibility of the *E. coli* isolates was noted, with a greater percentage of isolates showing susceptibility to gentamicin and a smaller percentage of isolates being susceptible to ceftiofur. Due to farm management decision, during the second year of the study, the farm used more ceftiofur and less gentamicin. This demonstrates the effects of antimicrobial selection pressure on susceptibility profiles of *E. coli* isolates. Although these previous reports have proven useful in the treatment of bacteremia in calves, no studies have been published since 1997 on the antimicrobial susceptibility patterns of bacterial isolates from bacteremic calves. Over the last 20 years, the food animal antimicrobial landscape has changed dramatically with

more widespread use of third generation cephalosporins as well as the introduction of new macrolides. Therefore, there is a need for current susceptibility profiles on bacterial isolates from bacteremic calves to help veterinarians and producers make better decisions when it comes to empirical antimicrobial therapy of critically ill calves.

1.3 Acute Phase Proteins and Prognostic Factors in Calves

Acute phase proteins (APPs) are part of the acute phase response or the body's initial physiologic, metabolic, and neuroendocrine response to factors that endanger normal homeostasis such as infection or injury [25]. Acute phase proteins can be utilized to verify and assess the immune response to infection [26]. The bovine APPs include haptoglobin, serum amyloid A, fibrinogen, albumin, transferrin, lipopolysaccharide binding protein, α-1 acid glycoprotein and, ceruloplasmin [27, 28]. Acute phase proteins are classified as major positive, minor positive, or negative depending on to the direction and magnitude of the changes in their concentrations during the acute phase response. Major positive APPs have a 10- to 100- fold increase in their concentration, minor positive APPs have a 2- to 10-fold increase in their concentration and negative APPs have a decrease of at least 25% in the concentration [25, 29]. For cattle, haptoglobin and serum amyloid A are considered as major positive APPs and fibrinogen, lipopolysaccharide binding protein, α -1 acid glycoprotein, and ceruloplasmin as minor positive APPs [27, 28]. The negative APPs of cattle include albumin and transferrin [28, 30]. Haptoglobin (Hp), serum amyloid A (SAA) and fibrinogen (Fib) are considered to be the APPs of clinical interest in cattle [27, 28].

During inflammation, Hp binds hemoglobin before bacteria can degrade the hemoglobin, thereby limiting access of bacteria to the free iron molecules that are essential for bacterial growth [31, 32]. In addition, work has shown that Hp possess anti-inflammatory capabilities as well as antioxidant properties [33, 34]. The concentration of Hp begins to increase within hours of an experimental inflammatory stimulus, accelerating after one day, and peaking at three to four days after the challenge [35, 36]. Serum amyloid A is an apolipoprotein that shows considerable similarity in structure between domestic species [37, 38]. Bovine SAA has multiple isoforms [39-41], including an extrahepatically produced SAA isoform that can be detected in mastitic milk [40]. The main function of SAA remains unknown but various functions have been identified including binding and transporting cholesterol, acting as a chemoattractant of leukocytes, and opsonization of Gram-negative bacteria [42-44]. Serum amyloid A concentration starts to increase almost immediately after an experimental bacterial infection, accelerating within hours, and peaks within two days [35, 45]. Fibrinogen is a very large glycoprotein that mainly functions by promoting blood coagulation or fibrin formation [46]. Unlike most of the other APPs, Fib is present in the plasma of healthy animals at fairly high concentrations [47]. This may help explain the moderate 2- to 3- fold increase in its concentration that is seen during inflammation [36, 48, 49]. The concentration of Fib increases three to four days after a momentary challenge, similar to Hp [36]. As long as inflammation continues, Fib concentrations will remain elevated [48]. Although Fib peaks simultaneously with Hp, it appears to be less sensitive in identifying inflammation in cattle when compared to Hp or SAA, likely due to its moderate increase in concentration [50, 51].

While APPs have been utilized as biomarkers of inflammation for decades, only one study has attempted to use them as prognostic indicators in neonatal calves. This study evaluated 102 calves of which 95% were a dairy breed and found that haptoglobin did not have any prognostic value [52]. Other ruminant studies have been conducted, like one in sheep that found that Hp was a useful prognostic indicator in cases of dystocia [53]. This study found that Hp concentrations above 1.0 g/L represented poor surgical candidates as those with high Hp concentrations died shortly after caesarean section. A study on horses with colic found that horses with an inflammatory cause of colic that failed to survive the colic episode had significantly higher SAA concentrations compared to horses that survived [54]. With regards to cattle, two studies on feedlot cattle with bronchopneumonia found that higher concentrations of Hp predicted more antimicrobial treatments [55, 56]. Another study in feedlot cattle with chronic respiratory disease found that cattle that died or were euthanized had significantly higher SAA and Hp concentrations, suggesting these APPs could be used as a prognostic indicators in these cases [57]. The primary focus of previous studies has been on feedlot cattle; however, there is a paucity of data on the use of APPs on neonatal beef calves.

One complicating factor to consider when evaluating APPs in neonates is that these proteins have been shown to fluctuate in the first three weeks of life [58, 59]. Haptoglobin increases after birth, with some animals having values above the reference range before nine days of life, followed by the mean concentration returning to within reference range after nine days of life [58]. Serum amyloid A also increases in early life, but then returns to normal levels by three weeks of age [60, 61]. Acute phase proteins have been evaluated in neonatal beef calves from a prognostic perspective, but most of the study subjects were healthy and the focus of the study

was on growth, not survival. The study found that calves with elevated serum amyloid-A concentrations at the age of 16 days had decreased weaning weights at 200 days of life [62]. Based on the lack of information on the use of acute phase proteins in sick neonatal beef calves, more research is needed to determine if they can be used as prognostic indicators.

The research on the acute phase response has been extensive but, with the exception of fibrinogen, practical utility of APPs is lacking in bovine medicine [67]. This may be due to the fact that diarrhea and pneumonia, common inflammatory conditions of neonatal calves, are usually easily diagnosed and therefore verifying inflammation in these cases, which are often already financially risky for the producer, may be fiscally irresponsible. However, due to the quantitative nature, APPs have been shown to have the capability of assessing the severity of the inflammation and estimating prognosis in other species [68]. Since prognosis is a critical factor for a producer to consider when contemplating treatment or euthanasia in a neonatal calf, being able to provide that information through a simple blood test could be extremely beneficial to the decision-making process. In addition, they may help veterinarians decide when antimicrobial intervention is necessary. To our knowledge, no studies on sick neonatal beef calves have been performed that identify if APPs can be utilized as prognostic indicators.

Research has also evaluated other factors that may be utilized as prognostic indicators in calves. One study in premature calves with acute respiratory distress syndrome found that venous blood lactate and pCO2 have prognostic value [63]. This study found that calves with lactate levels and pCO_2 values above 7.5 mmol/L and 63.5 mmHg, respectively, were associated with a negative prognosis. Another study also found prognostic value in lactate measurements in calves

undergoing abdominal surgery, but the predictive ability of this measurement was improved by considering the age of the calf with calves less than 1 week of age having a higher likelihood of a negative outcome [64]. One study in 24 dairy calves with naturally occurring diarrhea found that calves with blood urea nitrogen levels above 13.07 mmol/l and potassium concentrations above 5.63 mEq/l were 5.6 and 4 times more likely to die, respectively [65]. Interestingly, a study which looked at 1,400 calves with diarrhea did not reach the same conclusions [66]. This study found that a venous blood pH < 6.85 was the only abnormality with significant prognostic value. Instead, they concluded that certain disease processes like neurologic disease, abdominal emergencies, cachexia, and orthopedic problems such as septic arthritis offer more prognostic value than laboratory abnormalities.

1.4 CONCLUSION

In conclusion, several knowledge gaps have been identified regarding lesions associated with calf mortality, the incidence and isolated of bacteremic beef calves, and the use prognostic indicators like APPs. First, there is a gap in knowledge related to common lesions associated with mortality in neonatal beef calves in the United States. Producer surveys and limited data from Canadian studies tell us that the majority of calf deaths are not infectious in nature. When considering infectious causes, enteritis, pneumonia, and multisystemic infections like sepsis are the most commonly identified causes. Since sepsis is a life-threatening condition with high mortality, identifying factors that increase the risk of a calf being septic are important. Currently the definition of sepsis in cattle relies on blood cultures and altered white blood cell counts so assessing bacteremia using appropriate culture methods is important. Current data on the prevalence of bacteremia in sick neonatal beef calves is lacking. Additionally, identifying factors

related to positive and negative outcomes in sick neonatal calves may help producers and veterinarians when evaluating these patients.

CHAPTER 2: Gross necropsy, histopathology, and ancillary test results from neonatal beef calves submitted to a veterinary diagnostic laboratory

Rivero LA, Zhang S, Schultz LG, & Adkins PRF. (2022). Gross necropsy, histopathology, and ancillary test results from neonatal beef calves submitted to a veterinary diagnostic laboratory, *Journal of the American Veterinary Medical Association*, *260*(13), 1690-1696.

2.1 Aims

Describe lesions and abnormal test results among neonatal beef calves presented to a veterinary diagnostic laboratory.

2.2 Materials and Methods

2.2.1 Case selection: Electronic medical records from the University of Missouri's Veterinary Medical Diagnostic Laboratory (VMDL) were retrospectively reviewed. Dates included were from May 15, 2015, to April 8, 2020. Animals were eligible for inclusion if they were a bovine, between 2 and 21 days of age, and of a nondairy breed.

2.2.2 Medical records review: The search terms used included the following: bovine, necropsy (gross, histopathology, and labs), necropsy (gross and histopathology), necropsy (gross), and necropsy (mail-in). Data collected from the medical record included the following: breed, age (days), sex, weight, type of necropsy performed, lesions identified, and number of lesions per animal. All gross examination and histopathologic based lesions were categorized based on

system, and the associated pathogen type was recorded. All ancillary test results were recorded, including culture and susceptibility of bacterial agents when available. Bacteria identification was completed by the VMDL Bacteriology Laboratory. Bacteria genus and species were determined using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. Undifferentiated coliforms were defined based on phenotypic appearance alone and were evaluated on Tryptic Soy Agar with 5% sheep blood and MacConkey agar.

2.2.3 Definitions: A lesion was defined as an abnormal change in a tissue. Both gross and histopathologic lesions were categorized. Terminology used to describe each lesion was based on the terminology stated by the board-certified pathologist that originally reviewed the case. System categories included the following: urogenital, CNS, digestive, hemolymphatic, cardiovascular, muscloskeletal, multisystemic, respiratory, and other known system. Urogenital system was utilized to categorize calves that had lesions associated with the urogenital tracts, including umbilical disease. The hemolymphatic system was used to categorized those cases with lesions in the spleen, thymus, or other lymphoid organs. Multisystemic disease was used to categorize cases with either confirmed or suspected sepsis on the basis of the pathologist's report. Sepsis was determined by either isolating the same bacterial pathogen in 2 or more sites or by identifying multiple foci of inflammation. Calves were categorized only as septic if classified as such by the pathologist and the above conditions were met. Other known systems categorized calves with disorders in other body systems that were known but did not fall into one of the primary categories listed above, which included integument, endocrine, special senses, and peripheral nervous system. Gross necropsy was used to describe the evaluation that was based on visual inspection of the organ systems. Necropsy with histopathology included visual inspection

as well as histopathologic examination of the tissues. Ancillary tests were requested based on the pathologist's findings and could include the following: bacterial culture, bacterial antimicrobial susceptibility testing, fecal examination for parasites, PCR, or ELISA testing. *Cryptosporidium* spp were identified using a direct smear of fresh feces followed by a modified acid-fast strain and microscopic examination, while rotavirus and coronavirus were identified using PCR. Pathogens identified were classified by pathogen type (bacterial, viral, parasitic, fungal, unknown, or none identified).

2.2.4 Statistical analysis: Results were descriptively analyzed and reported as medians and range. Results were normally distributed with equal variances and therefore data was analyzed using 1-way ANOVA with statistical software (Stata IC version 13.1; StataCorp) to compare age, system affected, and pathological agent types. If an interaction was identified, then a Bonferroni pairwise comparison was used to identify differences. The $\chi 2$ or Fisher exact test was used to compare system categories. Analysis was completed using commercial software (SigmaPlot 14.0; Systat Software), and significance was determined at $P < 0.05$.

2.3 Results

A total of 1,060 bovine necropsies were performed during the study period. Of these, 95 (8.9%) animals met the inclusion criteria, with a median of 15 cases/y (range, 13 to 19 calves/y). Median calf age was 9 days (range, 2 to 21 days). Median weight was 33.5 kg (range, 11.5 to 58.3 kg). Overall, 51.6% (49/95) of included calves were male, 42.1% (40/95) were female, and 6.3% (6/95) had no sex reported. More calves were submitted for necropsy in the spring (34/95

[35.7%]), followed by the fall (30/95 [31.5%]), winter (21/95 [22.1%]), and summer (10/95 [10.5%]). Animals represented included mixed breed (42/95, 45.1%), Angus (36/95, 38.7%), Charolais (5/95 [5.3%]), Simmental (3/95 [3.2%]), Limousin (2/95 [2.1%]), South Poll (2/95 [2.1%]), Longhorn (1/95 [1.0%]), Salers (1/95 [1.0%]), and Dexter (1/95 [1.0%]). There was a total of 252 lesions identified, with a median of 3 lesions/calf (range, 0 to 7). When more than 1 lesion was identified within 1 system, duplicate representation of each system were removed to represent the number of system-based lesions. A total of 190 system-based lesions were identified with a median of 2 systems affected/calf (range, 0 to 5). A total of 3 (3.2%) calves had no identified lesions.

Overall, gross necropsy only was performed on 6.3% (6/95) of cases. Gross necropsy and histopathology were performed on 10.5% (10/95) of cases. Gross necropsy, histopathology and ancillary testing were performed on 82% (78/95) of cases. Most cases (99%; 94/95) were from calves submitted to the VMDL while 1% (1/95) of cases were from field necropsies with samples submitted by the referring veterinarian. Among the calves with no identified lesions, 1 was examined by gross necropsy and histopathology only and 2 were examined by gross necropsy, histopathology, and ancillary laboratory tests.

Among the 252 lesions, digestive system (42.1% [106/252]) lesions were the most common (Table 1), followed by respiratory (12.7% [32/252]), multisystemic (11.1% [28/252]), and CNS (8.3% [21/252]) lesions. Among all systems, congenital abnormalities were noted among 8.4% (8/95) of calves. The most common congenital abnormalities identified included patent foramen ovale (3/8) and patent ductus arteriosus (3/8). When considering the system-

based categorization (n = 190), most calves (57.9% [55/95]) had 2 or more system affected while only (38.9% [37/95]) had only 1 affected system. With regard to the calves that had 2 or more systems affected, 26.3% (25/95) had 2 affected systems, 20% (19/95) had 3 affected systems, 9.5% (9/95) had 4 affected systems, and 2.1% (2/95) of calves had 5 affected systems. The majority of calves with only 1 affected system were diagnosed with digestive lesions (73% [27/37]), followed by respiratory lesions (8.1% [3/37]). Among calves with 2 affected systems, the most common combination was digestive and respiratory system lesions (36% [9/25]), followed by a digestive system and other known system lesions (12% [3/25]) and digestive system and multisystemic lesions (12% [3/25]). Among calves with 3 systems involved, the most common combinations included the following: digestive, respiratory, and multisystemic (26.3% [5/19]); digestive, respiratory, and CNS (10.5% [2/19]); and digestive, multisystemic, and other known system (10.5% [2/19]). Among calves with 4 systems involved, the only combination represented more than once was digestive, CNS, musculoskeletal, and multisystemic (22.2% [2/9]). Among calves with 5 systemic involved, 1 of 2 had digestive, respiratory, musculoskeletal, hemolymphatic, and CNS lesions and 1 of 2 had urogenital, hemolymphatic, cardiovascular, CNS, and multisystemic lesions. Among calves with ≥ 3 affected systems diagnosed (n = 30), 43.3% (13/30) were not classified as multisystemic by the pathologist.

Among calves with a digestive lesion (70.5% [67/95]), 43.2% (29/67) had multiple lesions within the digestive tract (Table 2). A total of 106 digestive lesions were identified, with enteritis being the most common (45.3% [48/106]) followed by rumenitis (13.2% [14/106]), peritonitis (5.7% [6/106]), and hepatitis (5.7% [6/106]). Overall, 50.5% (48/95) of calves that were included in this study had enteritis. Among the 48 cases of enteritis, the majority of cases (60.4% [29/48])

had 1 infectious agent type identified, followed by identification of 2 different infectious agent types (35% [17/48]), and identification of 3 different infectious agent types (4.2% [2/48]). The most common infectious agents associated with enteritis were bacteria (54% [26/48]), viruses (44% [21/48]), parasites (27% [13/48]), and unknown agents (15% [7/48]). A total of 77 infectious agents, including bacteria, parasites, and viruses were detected in the small intestines of calves with enteritis. The most common bacteria isolated from the small intestines of calves with enteritis were *E coli* (79.2% [38/48]) and *Clostridium perfringens* (43.8% [21/48]). While these bacterial agents were commonly identified, they were only rarely associated with histologic lesion (Table 3). *Cryptosporidium* spp was the only parasitic agent and was identified in 22.9% of calves with enteritis (11/48). *Salmonella* spp were identified in the small intestines of 4 calves with enteritis. Among the *Salmonella* isolates identified, *S enterica* serotype Newport was detected in 2 out of 4 calves, while the remaining samples were identified as *S enterica* serotype Agona (1/4), and *S enterica* serotype Give (1/4). Viral PCR testing was not performed in all calves with enteritis, including no rotavirus or bovine viral diarrhea virus (BVDV) testing for 8 of 48 and no coronavirus testing for 6 of 48 cases. Among those that were tested for viral enteric pathogens, 26% (11/43) were positive for coronavirus, 34% (14/41) were positive for rotavirus, and 0% (0/41) were positive for BVDV.

The most common respiratory lesion identified was pneumonia (72% [23/32]) followed by pleuritis (6% [2/32]). Other respiratory abnormalities identified included bronchiolitis (1/32), laryngitis (1/32), neutrophil sequestration in the lungs (1/32), pulmonary congestion (1/32), pulmonary edema (1/32), pulmonary hemorrhage (1/32), and tracheitis (1/32). Pneumonia cases were further classified as bronchopneumonia (26% [6/23]), multifocal (26% [6/23]), interstitial

(18% [4/23]), embolic (4% [1/23]), and other (26% [6/23]). Of the 23 animals with pneumonia 13 had a bacterial culture performed on the lungs and common isolated organisms included *E coli* (31% [4/13]), undifferentiated coliforms (31% [4/13]), and *Mannheimia haemolytica* (15% [2/13]). The presence of these bacterial species was not frequently associated with histopathologic lesions (Table 3). The most common reason for the system-based categorization of multisystemic was because of signs of sepsis (79% [22/28]). Other lesions that were characterized as multisystemic included bacteremia (3/28 10.7%), neutrophil sequestration in multiple organs (3.6% [1/28]), petechia (3.6% [1/28]), and subcutaneous hemorrhages (3.6% [1/28]). Among calves with a multisystemic diagnosis (n = 23), bacteria were cultured from several organ systems outside the gastrointestinal tract, including the lungs, liver, umbilicus, joints, brain, spleen, and kidneys. The most common bacterial species identified was *E coli* (78% [18/23]), followed by undifferentiated coliforms (22% [5/23]), and *C perfringens* (13% [3/23]). *S enterica* was identified among 17% (4/23) of calves with multisystemic categorization; however, among these cases, *Salmonella* spp was isolated only from the gastrointestinal tract. Among calves with a system categorization of multisystemic disease, common concurrent lesions included enteritis (61% [14/23]), pneumonia (39% [9/23]), meningitis (17% [4/23]), and septic arthritis (17% [4/23]). Bacterial cultures were performed on samples from 81% (77/95) of the necropsied calves (Table 3). Among the 287 bacterial isolates recovered, the most common body sites of origin included the small intestines (33% [96/287]), large intestines (15% [43/287]), lung (14% [39/287]), liver (10% [28/287]), brain/CSF (8% [22/287]), and umbilicus (7% [20/287]). The most common bacteria species identified were *E coli* (37% [106/287]) and *C perfringens* (16% [46/287]). A total of 12 *Salmonella* isolates were recovered from 7 (7% [7/95]) calves, with most of the isolates originating from the intestines or feces (75%

[9/12]). *Salmonella* was also isolated from the liver (17% [2/12]) and abomasum (8% [1/12]). Many calves that were culture positive for *Salmonella* spp also had enteritis (71% [5/7]). Antimicrobial susceptibility testing was performed on 7 *Salmonella* isolates, but not all isolates were tested for the same antimicrobials. The number of resistant isolates for the following antimicrobials are as follows: penicillin (7/7), sulfadimethoxine (5/5), oxytetracycline (4/7), ampicillin (3/5), ceftiofur (0/5). Antimicrobial susceptibility testing was performed on 9 *E coli* isolates from septic calves but not all isolates were tested for the same antimicrobials. The number of resistant isolates for the following antimicrobials is as follows: penicillin (9/9), sulfadimethoxine (4/8), oxytetracycline (8/9), ampicillin (6/8), and ceftiofur (0/9). While bacteria was often isolated, depending on organ site, only 25% to 64% of isolates were determined to be associated with a histologic lesion (Table 3).

There was a significant association between median age and system affected ($P = 0.012$; Figure 1). Pairwise comparisons indicated that calves with CNS lesions were younger than calves with digestive lesions ($P = 0.029$). The χ^2 or Fisher exact test (when needed) also identified that calves with digestive lesions were less likely to have urogenital lesions ($P = 0.001$) and cardiovascular lesions ($P = 0.003$). Calves with CNS lesions were less likely to have musculoskeletal lesions ($P = 0.002$), while calves with urogenital lesions were more likely to have musculoskeletal lesions ($P = 0.014$). No seasonal effects were identified.

2.4 Discussion

Overall, the majority of lesions in this retrospective study were digestive, respiratory, and multisystemic in origin. This is in contrast to producer-driven data that identified weather-related causes, unknown causes, and digestive problems as the most common causes of calf loss in the first 3 weeks of life unrelated to birthing problems. [11] This discrepancy could be based on case selection as calves that have died during a difficult weather event are unlikely to be presented for necropsy. However, the addition of gross and histopathologic findings were able to help classify the death of necropsied calves that would otherwise likely be reported as unknown by producers. It should be noted that most calves had at least 1 lesion identified, while only 3 (3.2%) calves had no lesions identified. This finding supports the utility of necropsy data to help classify likely causes of death in neonatal calves.

Enteritis was the most common reported digestive disorder in this study. This is similar to a Canadian study that reported enteritis as the most common digestive disorder; however, that study had a much lower prevalence than our study [13]. Overall, 45.3% of cases with digestive lesions included in this retrospective evaluation had enteritis, while only 16% of older calves (those that lived > 3 days) had enteritis in the Canadian study [13, 69]. A high proportion of enteritis cases in this study were associated with *E. coli, C. perfringens,* and *Cryptosporidium* sp. The significance of the identification of these bacterial organisms on culture of the intestines is questionable since *E coli* and *C perfringens* are normal inhabitants of the gastrointestinal tract and the latter can also rapidly overgrow shortly after death. Additional characterization of these bacterial species would be necessary to determine their virulence potential. Additional

characterization of *C. perfringens* was pursued in only 2 cases of suspected clostridial enteritis by enterotoxin ELISA, both of which were negative. Viral pathogens, coronavirus and rotavirus, were commonly identified among enteritis cases, 26% and 34%, respectively. Reports of prevalence of both coronavirus and rotavirus in beef calves are sparse, but a previous study found a higher prevalence of coronavirus (62%) in Canadian beef calves than what is reported in this study [13].

Respiratory disease is one of the most common causes of disease in preweaned calves and is one of the major reasons for antimicrobial usage in calves prior to weaning [2, 12]. Most previous studies base their classification of respiratory disease on producer records. Few studies have characterized the lesions within the lungs, and in the 1 study that did, cases were classified broadly as pneumonia [2, 12, 13]. While bronchopneumonia is thought to be a common cause of disease in preweaned beef calves and is often associated with summer heat, little data can be found to determine the prevalence of bronchopneumonia among young calves. In this study, the majority of lesions classified as pneumonia were not characterized as bronchopneumonia, with 74% of cases being classified as either interstitial, multifocal, other, or embolic pneumonias. Multifocal and embolic lesions are likely related to a septic spread of disease rather than originating from the respiratory tract. Factors that have been proposed to have an impact on respiratory disease in preweaned calves include maternal transfer of immunity, dystocia, environmental stressors, and maternal nutrient restriction [70].

Multisystemic disease was the third most common system categorization in the population of necropsied calves in this study. This retrospective study has a higher prevalence of septic calves

than what has been previously reported for neonatal beef calves (7.4%) [13]. Enteritis, pneumonia, meningitis, and septic arthritis were often identified among cases with septic lesions in this study, which is similar to previous data [71]. The data supports the notion that a focal site of infection is an important risk factor for sepsis in neonatal calves [72, 73].

Major risk factors for illness in neonatal calves include dystocia, calving in confinement, failure of transfer of passive immunity, poor maternal milk production, or weather-related conditions that make it challenging for the calf to nurse [74-76]. While no studies in the US have investigated the prevalence of failure of transfer of passive immunity in beef calves, studies from Ireland have reported a failure of transfer of passive immunity prevalence of 22% to 29% which is higher than the 19.2% reported for US dairy herds [77-79]. Identified factors that contribute to failure of transfer of passive immunity in beef calves include dystocia, poor mothering ability, parity of the dam, and nutrition of the dam [10, 76, 77]. One study found that even mild complications in the birthing process that do not require assistance increase the risk of failure of transfer of passive immunity, highlighting how small difficulties in calving can impact the life and productivity of a beef calf [77]. It is estimated that up to 20% of 2-year-old heifers may abandon their calf or not allow them to nurse and 12% of neonatal beef calf losses could be attributed to mothering problems [4, 80]. Furthermore, primiparous dams and those dams that have been nutrient restricted in the prepartum period have been shown to have decreased colostrum quality, which increases the risk of failure of transfer of passive immunity [76]. Unfortunately, in this study, transfer of passive immunity status was not available for review.

Overall, lesions that likely contributed to illness were identified in a high proportion of cases. Previous research has identified noninfectious disease processes to be more common than infectious among necropsies done across all age groups of calves (abortions, still-births, perinatal calves and neonatal calves) [13]. The results of this study differ in that infectious diseases were common. This is not surprising because the previously mentioned study found that infectious causes increased as calves aged. Among perinatal calf deaths this previous report found lesions in the thyroid, cardiac muscle, and skeletal muscle, and therefore the role of trace minerals and vitamins in overall disease was speculated [13]. Lesions in these organ systems were not common among the sample population in our study, but underlying trace mineral imbalances may be a factor to consider. No trace mineral profiles were performed among cases included in this study.

Limitations of this study include its retrospective nature and lack of uniformity of necropsy data. Standardizing nomenclature based on postmortem findings in simple and consistent terms can provide more useful information about significant lesions that may be associated with death [81]. Additionally, the results of the gross, histopathologic, and ancillary tests are all listed in this study, however these results do not always agree, making interpretation challenging. For example, bacterial species are frequently isolated from body sites; however, they are less frequently associated with a lesion at that specific location. While, having access to common organisms identified from different organ systems may be useful to veterinarians when addressing sick calves, it is important for veterinarians to evaluate whether the bacterial species is associated with a lesion and therefore significant. Furthermore, there is an inherent bias towards calves that presented for necropsy evaluation, as these may have been calves coming

from outbreak situations or situations in which a presumptive diagnosis of the cause of death was made on farm and may not accurately represent the overall calf population. Finally, the inclusion criteria for this study required an age to have been recorded for each calf. This criterion may have led to a bias, as this selects for producers that maintain accurate records and delivered that information to the laboratory.

2.5 Conclusion

This study reports gross necropsy, histopathologic, and ancillary test results from beef calves submitted to a veterinary diagnostic laboratory. Common systems affected are consistent with previous reports of digestive and respiratory diseases being common systems affected. Multisystemic diseases (ie sepsis) may be more common in calf populations than previously thought and could account for the high proportion of calf losses when producers cannot identify a cause of death. Future studies should focus on clinical prospective investigation to identify risk factors for these disease processes in neonatal beef calves.

2.6 Tables

2.6.1 Table 1

Number and percentage of lesions (n = 252) identified that were categorized into each system category and number of beef calves (95) with at least 1 lesion within each category. Most calves had multiple lesions impacting multiple body systems.

Body system category	Total No. of lesions identified (%)	No. of calves with lesion(s) identified within each system
Digestive	106 (42.1%)	67 (70.5%)
Respiratory	32 (12.7%)	31 (32.6%)
Multisystemic	28 (11.1%)	23 (24.2%)
CNS	21 (8.3%)	17 (17.9%)
Urogenital	19 (7.5%)	16 (16.8%)
Cardiovascular	14 (5.6%)	6 (6.3%)
Musculoskeletal	13 (5.2%)	12 (12.6%)
Other known systems	11 (4.4%)	11 (11.6%)
Hemolymphatic	8 (3.2%)	7 (7.4%)

2.6.2 Table 2

Total number and percentage of each digestive lesion identified among 95 necropsied beef calves. The percentage represents the number of each specific lesion per total number of digestive lesions (n = 106) and the number of each specific lesion per total number of calves (95).

Diagnosis	No. of lesions	Percentage of lesions (n = 106)	Percentage of calves with lesion (n = 95)
Enteritis	48	45.3%	50.5%
Rumenitis	14	13.2%	14.7%
Peritonitis	6	5.7%	6.3%
Hepatitis	6	5.7%	6.3%
Abomasitis	5	4.7%	5.3%
Diarrhea	5	4.7%	5.3%
Colitis	4	3.8%	4.2%
Omasitis	4	3.8%	4.2%
Rumen acidosis	2	1.9%	2.1%
Hepatic lipidosis	2	1.9%	2.1%

Diagnosis	No. of lesions	Percentage of lesions (n = 106)	Percentage of calves with lesion (n = 95)
Abomasal ulcer	1	0.9%	1.1%
Atresia ani	1	0.9%	1.1%
Duodenal ulcers	1	0.9%	1.1%
Enterocolitis	1	0.9%	1.1%
Esophagitis	1	0.9%	1.1%
Peritoneal effusion	1	0.9%	1.1%
Reticulitis	1	0.9%	1.1%
Ruminal drinking	1	0.9%	1.1%
Typhlocolitis	1	0.9%	1.1%
Salmonellosis	1	0.9%	1.1%

2.6.3 Table 3

Number of each microorganism identified at various body sites among the 95 beef calves that were presented to a veterinary diagnostic laboratory. Within each body site, the number of each organism associated with a lesion at the specific organ site is listed over the total number of each microorganism identified at each organ site location. Body site locations were included if bacteria were identified from a specific location > 10 times. Bacterial genus was included if identified ≥ 3 times. Bacteria were listed at the species level if identified > 10 times. If multiple species were identified but each species was identified < 10 times, they were grouped at the genus level. Isolates identified as coliforms were not identified to the genus or species level by the laboratory.

Bacteria identified	**No. of isolates***	**Small Intestine (%)**	**Large Intestine (%)**	**Lung (%)**	**Liver (%)**	**Umbilicus (%)**	**Brain /CSF (%)**	**Joint (%)**
Escherichia coli	106	15/50 (30)	10/17 (59)	5/7 (71)	8/11 (73)	3/5 (60)	4/5 (80)	2/2 (100)
Clostridium perfringens	46	1/24 (4.2)	1/14 (7.1)	0/0 (0)	1/3 (33)	0/0 (0)	0/0 (0)	0/0 (0)
Coliforms	27	1/2 (50)	0/5 (0)	2/7 (29)	0/5 (0)	0/1 (0)	1/2 (50)	1/1 (100)

Bacteria identified	No. of isolates*	Small Intestine (%)	Large Intestine (%)	Lung (%)	Liver (%)	Umbilicus (%)	Brain /CSF (%)	Joint (%)
Streptococcus spp	17	0/3 (0)	0/1 (0)	0/5 (0)	0/1 (0)	1/4 (25)	0/2 (0)	0/0 (0)
Pseudomonas spp	12	1/4 (25)	0/0 (0)	1/3 (33)	0/0 (0)	0/1 (0)	0/4 (0)	0/0 (0)
Salmonella spp	12	4/4 (100)	4/4 (100)	0/0 (0)	2/2 (100)	0/0 (0)	0/0 (0)	0/0 (0)
Proteus spp	8	0/0 (0)	0/1 (0)	0/1 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/0 (0)
Staphylococcus spp	8	0/0 (0)	0/0 (0)	1/2 (50)	0/0 (0)	0/1 (0)	1/2 (50)	2/3 (67)
Campylobacter spp	7	2/4 (50)	0/1 (0)	0/0 (0)	0/0 (0)	0/1 (0)	0/0 (0)	0/0 (0)
Klebsiella spp	5	0/1 (0)	0/0 (0)	0/3 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/0 (0)

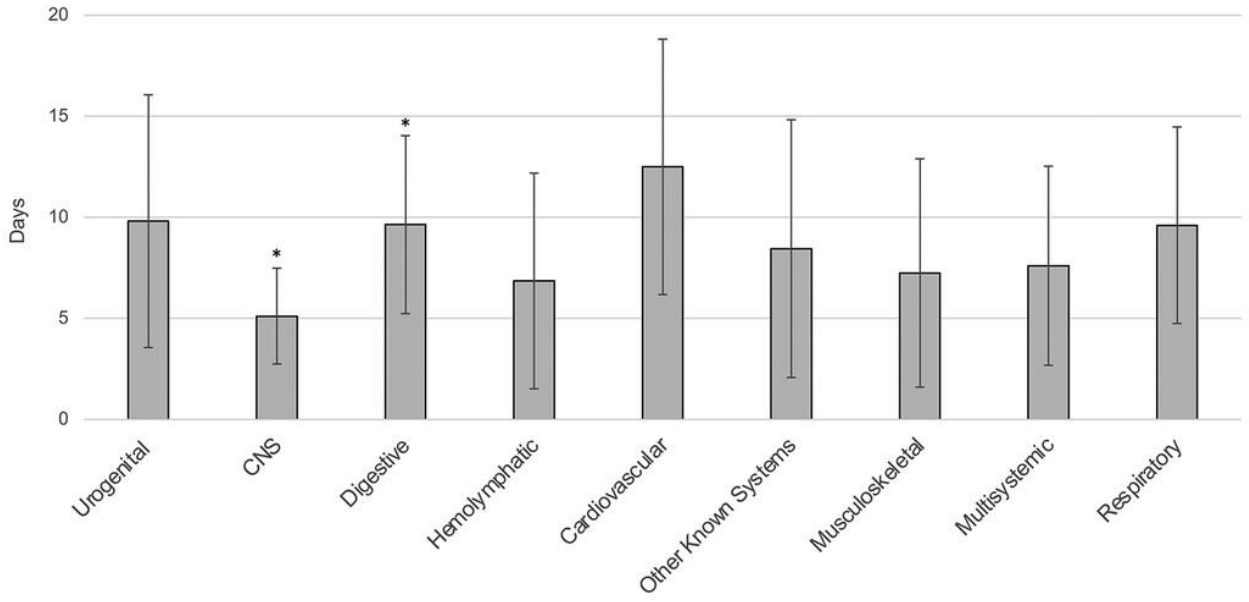
Bacteria identified	No. of isolates*	Small Intestine (%)	Large Intestine (%)	Lung (%)	Liver (%)	Umbilicus (%)	Brain /CSF (%)	Joint (%)
Mannheimia spp	4	0/0 (0)	0/0 (0)	1/2 (50)	0/0 (0)	0/0 (0)	0/1 (0)	0/0 (0)
*Acinetobacte*r spp	3	0/1 (0)	0/0 (0)	0/0 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/0 (0)
Bacillus spp	3	0/0 (0)	0/0 (0)	0/1 (0)	0/0 (0)	0/0 (0)	0/0 (0)	1/2 (50)
Enterococcus spp	3	0/1 (0)	0/0 (0)	0/0 (0)	0/0 (0)	0/2 (0)	0/0 (0)	0/0 (0)
Moraxella spp	3	0/0 (0)	0/0 (0)	1/1 (100)	0/0 (0)	0/0 (0)	0/1 (0)	0/1 (0)
Truperella pyogenes	3	0/0 (0)	0/0 (0)	0/0 (0)	0/0 (0)	1/1 (100)	0/0 (0)	1/1 (100)

Bacteria identified	No. of isolates*	Small Intestine (%)	Large Intestine (%)	Lung (%)	Liver (%)	Umbilicus (%)	Brain /CSF (%)	Joint (%)
Total*	**287**	**24/96 (25)**	**15/43 (35)**	**11/39 (28)**	**11/28 (39)**	**5/20 (25)**	**6/22 (27)**	**7/11 (64)**

*The number of isolates and the total number represents all isolates identified, including bacterial genus and species not shown in this table.

2.7 Figure 1

Mean age (± 1 SD) of calves (n = 95) included in the study by system-based lesion categorization. *Significant ($P < 0.05$) differences among mean ages.



CHAPTER 3: Blood Cultures and Outcomes in Sick Neonatal Beef Calves

3.1 Hypothesis and Aims

The objectives of this study were to (1) determine the prevalence of bacteremia among neonatal beef calves with focal sites of infection, (2) identify factors that could predict bacteremia in neonatal beef calves, and (3) identify factors associated with survival in sick neonatal beef calves. The experimental hypothesis was that neonatal beef calves sampled at the time of hospital admission that were blood culture positive and had elevated acute phase proteins were less likely to survive compared to blood culture negative calves or those with lower acute phase proteins.

3.2 Materials & Methods

3.2.1 Case Selection: Any beef calf ≤ 3 weeks of age that was presented to the University of Missouri Veterinary Health Center Food Animal Clinic for illness was eligible for enrollment. Following owner consent, a complete treatment history was collected, including duration of illness, clinical signs, therapies administered, and response to treatments. Enrolled calves received a complete physical examination. Based on the physical exam findings, calves were divided into two groups, those with and those without a focal site of infection. A focal site of infection was defined as gross signs of omphalitis, septic arthritis, diarrhea, abnormal thoracic auscultation indicating respiratory disease, or hypopyon.

3.2.2 Sample Collection: Blood samples were collected from all enrolled calves for complete blood count (CBC) and acute phase protein assays. Additional blood samples were collected for culture from calves with a focal site of infection.

For calves with a focal site of infection, 3 blood culture samples from three different vessels were aseptically collected upon admission. The vessels utilized for sample collection were both left and right jugular veins and one of the cephalic veins. Alternative to cephalic vein sampling, the lateral saphenous vein was utilized on some calves. Prior to sample collection, the skin overlying the vessels was clipped using a #40 clipper blade. The skin was scrubbed with chlorhexidine for a minimum of 4 minutes and then wiped with alcohol. Next, utilizing sterile gloves, a surgical scrub with chlorhexidine was performed for an additional 3 minutes for a total scrub time of 7 minutes. The chlorhexidine was wiped off with dry sterile gauze. Prior to sample collection, the plastic seal from the blood culture media bottle (Oxoid Signal System Blood Culture Media;Oxiod Limited) was removed. If any defects were seen in the septum or if the media was cloudy, it was not used. The septum was cleaned with an alcohol-soaked gauze pad and allowed to dry. Following aseptic preparation of the skin, 10 mL of blood was collected into a sterile syringe attached to a sterile 20-gauge needle. Following sample collection, the needle was changed using aseptic technique and the blood was inoculated into the blood culture media. This procedure was repeated for each collection site, for a total of three aseptically collected blood samples per enrolled calf. During both jugular venipunctures an additional 10 mL of blood (a total of 20 mL) was collected. These additional 10 mL would be utilized for CBC and acute phase protein assays. The samples were transferred to the appropriate tube (see below) only after 10 mL of the sample was inoculated into the blood culture media.

For those calves without a focal site of infection, only two blood samples were collected. One sample was collected for a CBC and one for acute phase protein assays. No blood cultures were performed since a focal site of infection has been well recognized as a risk factor for bacteremia. Blood samples were collected from either the left or right jugular vein utilizing a syringe and a 20-gauge needle. For the CBC, 10 mL of whole blood was collected and immediately transferred to a BD EDTA Vacutainer Tube (Thermo Fisher Scientific) and submitted to the Clinical Pathology Service at the University of Missouri Veterinary Medical Diagnostic Laboratory (VMDL) at room temperature for analysis. For the acute phase protein assays, 10 mL of whole blood was collected and transferred into a vacutainer additive free red top tube (Thermo Fisher Scientific). The blood was allowed to clot at room temperature. Once the clot was formed, the tubes were centrifuged at 2,500 rpm for 10 min and the serum was separated. Serum was aliquoted and frozen at -80°C in polyprolene tubes (Sarstedt) until further processing.

3.2.3 Blood Culture Processing: The inoculated media bottles were swirled gently for 30-60 seconds and then a growth indicator device (Oxoid Signal System Blood Culture; Oxoid Limited) was attached to each bottle. The bottles were placed in the aerobic incubator at 37°C. Each bottle was checked every 18-24 hours for one week for fluid in the growth indicator device or cloudiness in the Oxoid bottle as indicators of bacterial growth. Culture on solid media was performed for all samples after 18-24 hours of incubation regardless of visible indicators of growth. If there was no growth on the solid media after approximately 72 hours, a second set of solid media was inoculated.

Culture on solid media was performed on tryptic soy agar with 5% sheep blood (Thermo Fisher Scientific) and MacConkey agar (Thermo Fisher Scientific) under aerobic and anaerobic conditions. To obtain a sample from the Oxoid bottles, first, the top of the signal chamber was opened. A sterile syringe was used to remove 1 mL of fluid from the Oxoid signal chamber. Each agar plate was inoculated with 1-2 drops of liquid media. Following inoculation, each plate was struck for isolation with a sterile cotton tip applicator. Inoculated aerobic plates were incubated at 37°C in ambient air and anaerobic plates were incubated at 37°C/ < 1% oxygen in a Mitsubishi box (Thermo Fisher Scientific) with an anaerobe sachet (Thermo Fisher Scientific). Aerobic plates were incubated for a total of 72 hours and examined for bacterial growth every 18-24 hours. Anaerobic plates were incubated for a total of 72 hours and examined for growth at 48 and 72 hours. After incubation, if more than one colony type was identified on the plate each unique colony type was subcultured prior to preservation and further characterization. If one distinct colony type was identified on the plate, representative colonies were selected for preservation and characterization. All isolates selected for preservation were transferred to phosphate buffered glycerol and stored at -80ºC.

3.2.4 Bacterial identification and characterization: Bacterial characterization included identification to the genus and/or species level and antimicrobial susceptibility profiles. MALDI-TOF mass spectrometry was utilized for bacterial identification. Bacterial Test Standard (Bruker Daltonics Inc.) was used as a control and to calibrate the mass spectrometer. In duplicate, the Bacterial Test Standard and one colony of each isolate was placed on the MALDI-TOF target. Target spots were then overlaid with 1 µL of 70% formic acid. Once dried, 1 µL of matrix

solution, consisting of α-cyano-4-hydroxycinnamic acid diluted in a solution of 50% acetonitrile and 2.5% trifluoroacetic acid, was overlaid over each spot. Samples were analyzed on a Microflex MALDI-TOF mass spectrometer (Bruker Daltonics Inc.) using proprietary software (Flex Control, Bruker Daltonics Inc.). The mass spectrometer was calibrated using the automatic calibration and validation procedure prior to the classification run. The Biotyper matching algorithm computed three separate values for three fundamental characteristics of the sample and the reference spectra. First, the number of signals in the reference spectrum that had a closely matching partner in the unknown spectrum were determined. Second, the number of signals in the unknown spectrum that had closely matching partners in the reference spectrum were calculated. Third, the symmetry of the matching signal pairs was computed. If both high- and low-intensity signals of the unknown spectra were matched to the high- and low-intensity signals of the reference signals, it resulted in high symmetry. For each characteristic, a complete match returned a value of 1, and no matches returned a value of 0. The three values were multiplied, and the results normalized to 1000. The score value was the common (decadic) logarithm of this result. The minimum score was 0, and the highest score was 3 (= log 1000). The higher the score, the more probable the classification of the species. Species identification cut-off values were applied according to the manufacturer's instructions which required a score ≥ 2 to indicate a species-level identification. A score < 2 but ≥ 1.7 was utilized to identify isolates to the genus level. A score < 1.7 was classified as inconclusive and yielded no identification.

Susceptibility profiles of the isolates were performed to determine the minimum inhibitory concentration of antimicrobials routinely used in bovine medicine. The antimicrobial susceptibility profile was performed using the Sensititre™ bovine plate (BOPO7F; Thermo

Fisher Scientific) and included the following antimicrobials: ampicillin, ceftiofur, enrofloxacin, florfenicol, gentamicin, penicillin, sulfadimethoxine, tetracycline, and tulathromycin. Minimum inhibitory concentrations for aerobic and anaerobic bacteria were determined following 18-24 hours of incubation using an automated system (Sensititre ARIS system [Thermo Fisher Scientific]). The isolates were first grown on Columbia blood agar for 24 to 48 hours at 37ºC under aerobic or anaerobic conditions. Isolated colonies were transferred to sterile deionized water and additional colonies transferred to reach a 0.5-McFarland standard. Ten microliters of the resulting solution was inoculated into Mueller-Hinton broth (Thermo Fisher Scientific) for aerobic isolates and into Brucella broth (Thermo Fisher Scientific) for anaerobic isolates. Following inoculation, the samples were vortexed to ensure mixing. An automated inoculator (Sensititre auto-inoculator, Thermo Fisher Scientific) was utilized to inoculate 96-well plates. Plates were incubated in aerobic or anaerobic conditions at 37°C for 18 to 48 hours. The MIC was reported according to the manufacturer's guidelines (Thermo Fisher Scientific) and the Clinical and Laboratory Standards Institute. CLSI VET08 was utilized to classify isolates as resistant, intermediate, or susceptible. Antimicrobials that were on the microdilution plate but are used in porcine medicine only or would be illegal were not included for interpretation. These included clindamycin, danofloxacin, neomycin, spectinomycin, tiamulin, trimethoprim-sulfa, and chlortetracycline.

3.2.5 Complete Blood Count Processing: All BD EDTA blood samples were submitted to the Clinical Pathology Service at the University of Missouri VMDL within 1 hour of collection for CBC analysis. All CBCs were performed on an automated analyzer (Advia 2120 Hematology System, Siemens Health Care Diagnostics SL) using standard methods performed by trained

personnel at the VMDL which included a manual white blood cell differential count. Any results that were flagged as abnormal were verified by a licensed clinical pathology technician or a board-certified clinical pathologist. Fibrinogen concentration was determined using the heat precipitation method [82]. Briefly, two microhematocrit tubes were filled with freshly drawn and anticoagulated blood and were centrifuged at 2,500 RPM for 5 minutes. One tube was broken just above the RBC/Plasma interface and the plasma protein was measured with a refractometer. The other tube was incubated at 58°C for 3 minutes. After incubation the tube was centrifuged again at 2,500 RPM for 5 minutes. After centrifugation, the tube was broken just above the RBC/fibrinogen interface and the protein concentration was measured via refractometry. The fibrinogen concentration (in g/dL) was calculated by determining the difference in total protein between the first (non-incubated) and the second (incubated) microhematocrit tube. The fibrinogen heat precipitation method and concentration calculations were conducted by VMDL laboratory personnel.

3.2.6 Serum Sample Processing and Acute phase protein assays: Haptoglobin and serum amyloid A (SAA) concentrations were determined using commercial test kits. For all assays, aliquoted serum samples were thawed at room temperature. Samples were run in duplicate along with a positive and negative control. Optical densities were measured by a microplate absorbance spectrophotometer (Biotek Synergy HT, BioTek Instruments Inc.). If duplicates yielded discrepant results, samples were retested in duplicate until discrepancies were resolved. Standard curves were evaluated for goodness of fit and were repeated if the R^2 value was < 0.99. Duplicate samples with an inter-assay coefficient of variation > 10% were repeated. If multiple

standard curves were utilized an inter-assay coefficient of variation < 10% must have been present between control samples from each standard curve.

Haptoglobin concentrations were determined using the colorimetric PHASE Haptoglobin Assay (Tridelta Development Limited) according to the manufacturer's instructions. A standard curve was created by serially diluting the provided Haptoglobin Calibrator (2.5 mg/mL) with the provided phosphate buffered saline diluent (PBS). The final standard curve concentrations were 2.5, 1.25, 0.625, 0.312, and 0 mg/mL. Following creation of the calibration solution, 7.5 μL of study samples, standards, and kit provided controls were plated in duplicate on a flat-bottom 96-well plate. Next, 100 μL of stabilized hemoglobin and 140 μL of the provided chromogen was added to all wells. The plate was incubated at room temperature for 5 min. Immediately following incubation absorbances were read at 630 nm. The mean absorbance of the standards was plotted against the known calibrator concentrations utilizing computer software (Gen5 Data Analysis Software, BioTek Instruments Inc.). A best smooth curve was drawn through these points to construct the calibration curve. The concentrations of the study samples were determined by the interpolated value in the calibration curve.

Serum Amyloid A was determined using the PHASE Multispecies SAA Assay (Tridelta Development Limited, Maynooth, Ireland) according to manufacturer's instructions. This SAA kit is a solid phase sandwich enzyme linked immunosorbent assay (ELISA). All serum samples were initially diluted 1:500 with the provided manufacturer diluent. Wells were coated by the manufacturer with a monoclonal antibody specific for SAA. The SAA calibrator solution (300

ng/mL) was created by diluting the provided calibrator with 1 mL of the provided diluent buffer. Then a standard curve was created by serially diluting the created SAA calibrator with the provided diluent buffer. The final standard curve concentrations generated were 300, 150, 75, 37.5, 18.8, and 0 ng/mL. Following creation of the standards 50 μL of the samples, company provided controls, and standards, were incubated in the coated wells at 37°C together with 50 μL of the Anti-SAA/HRP for 1 hour. Following incubation, the plates were washed 4 times with the provided wash solution to remove any unbound material. Next, 100 μL of the provided TMB substrate was added and incubated at room temperature in the dark for 15 min. Immediately after incubation, 100 μL of the provided stop reagent was added and absorbances were read at 450 nm. The mean absorbance of the standards was plotted against the known standard concentrations utilizing Gen5 computer software, drawing a best smooth curve through the points to construct the calibration curve. The concentrations of the samples were determined by multiplying the interpolated value by the appropriate dilution factor. Samples that had a signal greater than the top calibrator or fell onto the non-linear portion of the curve were further diluted and repeated.

3.2.7 Bacteremia Classification: A calf was classified as bacteremic when the same bacterial species was isolated from two or more blood specimens.

3.2.8 Failure of Transfer of Passive Immunity Classification: Failure of transfer of passive immunity if it was determined in calves between 2 to 7 days of age and was assessed by

measuring the serum total protein. Failure of transfer of passive immunity was defined as a calf with a serum total protein < 5.5 g/dL [83].

3.2.9 Venous Blood Gas Analysis: While not a criterion for enrollment venous blood gases were commonly performed as part of the diagnostic evaluation. Venous blood gases were performed on the VetScan i-STAT 1 System (Abbot) utilizing the CG8+ cartridges (Abbot). Prior to sample collection that cartridge would be allowed to warm to room temperature for 5 min in its protective pouch. For venous blood gas analysis, blood samples were collected into BD Lithium Heparin tubes (Thermo Fisher Scientific). Following blood collection and cartridge warming, the pouch containing cartridge would be opened and the bar code on the pouch would be scanned by the analyzer. A 1 mL syringe (Thermo Fisher Scientific) was utilized to aspirate approximately 0.2 mL of heparinized blood. This amount of blood was then utilized to fill the sample well of the cartridge to the fill mark making sure to not have any air bubbles. Following filling of sample well the tab on the sample well would be closed and the cartridge would be inserted into the analyzer. Results were generated after 2-3 minutes of analysis. Obtained values from the CG8+ cartridge included: sodium, potassium, ionized calcium, glucose, pH, PCO_2, PO_2, TCO_2, HCO_3^-, BE, SO_2. Since the samples were venous in origin PO_2 and sO_2 values were not evaluated.

3.2.10 Survival Classification: Survival of each calf was determined based on phone interviews with the owners. Survival was classified as: a) died or euthanized in the Food Animal hospital b) survived until discharge but died or was euthanized before the two-week check point, c) was

alive at the two-week check point but died or was euthanized before the three-month check point or d) was alive at the three-month check point.

3.2.11 Statistical Analysis: Data were compiled in a computerized spreadsheet (Excel 2013, Microsoft). Data collected included patient demographics, previous treatment history, physical exam parameters, diagnosis, blood culture results, isolate identifications, antimicrobial resistance profiles, CBC results, Hp concentrations, SAA concentration, and Fib concentrations. Additional laboratory tests that were not part of the study but were run by the clinician overseeing the case were also recorded for each calf. Additionally, survival of each calf (as classified above) was recorded. Absolute numbers and proportions were calculated descriptive data. A single logistic regression model was created to determine which variables were associated with survival. A variable was considered significant if its p-values was < 0.5.

3.3 Results

Twenty-seven calves were enrolled in the study. The median age of calves was 7 days (range 1 – 21 d). There was a total of 12 heifers and 15 bulls. Angus (15/27; 56%) was the most common breed represented followed by crossbred (6/27; 22%), Gelbvieh (3/27; 11%), Charolais (1/27; 4%), Brangus (1/27; 4%), and Hereford (1/27; 4%). All calves were described as being raised for breeding purposes (18/27, 67%) or commercial meat production (9/27, 33%). A total of 56% (15/27) of calves were presented in the months of February (7/27, 26%) and March (8/27, 30%). The remaining calves presented in April (2/27, 7%), June (1/27, 4%), August (3/27, 11%), September (1/27, 4%), October (3/27, 11%), and November (2/27, 7%).

Median duration of clinical signs at the time of presentation was 2 days (range 0.1 – 15 days). Most calves presented with 2 presenting complaints (11/27, 41%), followed by 1 presenting complaint (10/27, 37%), 3 presenting complaints (5/27, 19%), and 4 presenting complaints (1/27, 4%). Presenting complaints included diarrhea (13/50; 26%), lethargy (12/50, 24%), not nursing (8/50, 16%), recumbency (6/50, 12%), squinting (1/50, 2%), coughing, (1/50, 2%), decreased nursing (1/50, 2%), dehydration (1/50, 2%), tearing (1/50, 2%), fever (1/50, 2%), lameness (1/50, 2%), obtundation (1/50, 2%), open mouth breathing (1/50, 2%), pneumonia (1/50, 2%), and umbilical infection (1/50, 2%).

Owners had attempted treatment in 19/27 (70%) of calves. Antimicrobials were the most commonly administered treatment with 15/19 (79%) calves being administered at least one antimicrobial. Among the treated calves, ceftiofur had been administered 7 times, florfenicol 6 times, sulfadimethoxine 6 times, tulathromycin 2 times, and enrofloxacin 2 times. A total of 6/19 (32%) calves had received ≥ 3 antimicrobial protocols, while 4/19 (21%) calves had received florfenicol ≥ 2 times. The second most common owner administered treatment was oral electrolytes (12/19, 63%). Other owner administered treatments included flunixin meglumine (4/19, 21%), colostrum replacer (3/19, 16%), bismuth subsalicylate (1/19, 5%), Bovi-Sera (Colorado Serum Company) (1/19, 5%), BioSponge (Platinum Performance) (1/19, 5%), vitamin E paste (1/19, 5%), vitamin B (1/19, 5%), IV fluids (1/19, 5%), and probiotics (1/19, 5%).

Among calves for which vital parameters were collected (n = 26), median weight, rectal temperature, heart rate, and respiratory rate were 36.6 kg (range 26 – 50), 102.1°F (89 – 106.7), 116 bpm (72 – 200), 30 brpm (6 – 160), respectively. One calf had missing vital signs and 3 calves had temperatures which were too low to read. Calves with a temperature that was determined to be too low to read were recorded as 89°F which was 1 degree lower than the lowest temperature the thermometer could record. Complete blood count data was available for 21/27 (78%) calves (See table 4). Parameter median and ranges were as follows: fibrinogen (g/dL) 0.7 (0.1 – 1.7), hematocrit (%) 37.2 (17.8 – 59.3), white blood cells (x 10^3/µL) 10.61 (3.72 – 26.21), segmented neutrophils (x 10^3/µL) 5.27 (1.07 – 21.62), band neutrophils (x 10^3/µL) 0.2 (0 – 6.57), lymphocytes (x 10^3/µL) 3.45 (1.77 – 8.04), monocytes (x 10^3/µL) 0.49 (0.06 – 4.84), platelets (x 10^3/µL) 662 (218 – 1654). Morphologic abnormalities on blood smear performed during CBC analysis were identified in 20/21 (95%) of calves. Three out of 21 (14%) had 1 morphologic abnormality, 5/21 (24%) had 2 abnormalities, 9/21 (43%) had 3 abnormalities, and 3/21 (14%) had 4 abnormalities. In total 52 morphologic abnormalities were identified which included: few reactive lymphocytes (11/52, 21%), occasional mild neutrophil toxicity (7/52, 13%), mild anisocytosis (7/52, 13%), mild neutrophil toxicity (5/52, 10%), marked poikilocytosis (5/52, 10%), moderate poikilocytosis (4/52, 8%), moderate neutrophil toxicity (2/52 4%), moderate number of reactive lymphocytes (1/52, 2%), moderate echinocytes (1/52, 2%), moderate anisocytosis (1/52, 2%), mild polychromasia (1/52, 2%), mild ovalocytosis (1/52, 2%), mild basophilic stippling (1/52, 2%), mild acanthocytosis (1/52, 2%), metamyelocytes and myelocytes (1/52, 2%), marked echinocytes (1/52, 2%), few small platelet clumps (1/52, 2%), and few giant platelets (1/52, 2%).

While not part of the study protocol, venous blood gas analysis were performed in 18/27 (66%) calves as part of the case work-up (See table 5). Median and ranges for the following variables are as follows: pH 7.3 (6.8 – 7.5), pCO_2 (mmHg) 35.8 (21.6 – 65), HCO_3^- (mmol/L) 18.55 (4.4 – 37.1), BE (mmol/L) -7 (-28 – 14). Failure of transfer of passive immunity was assessed in 12/27 (44%) of calves that were between 2 to 7 days of age. Median serum total protein value was 5.1 g/dL (range 3.8 – 7.2). Based on a cut-off criterion of 5.5 g/dL, 7/12 (58%) calves were classified as having failure of transfer of passive immunity.

Focal sites of infection were identified in 23/27 (85%) calves. Most calves (22/23, 96%) had 1 focal site of infection while 1/23 (4%) had two focal sites of infection (umbilicus and synovial). Identified focal sites of infection included gastrointestinal (16/23, 69%), umbilical (2/23, 9%), ocular 2/23, 9%), synovial (2/23, 9%), and respiratory (1/23, 4%). Of the 23 calves with focal sites of infection in which blood cultures were performed 4 (17%) were considered bacteremic with *E. coli* (3/4, 75%) and *Bacteroides uniformis* (1/4, 25%) being the organisms identified. Based on the results of the susceptibility testing the number of resistant *E. coli* isolates were as follows: ampicillin (3/3, 100%), ceftiofur (0/3, 0%), enrofloxacin (0/3, 0%), florfenicol (0/3, 0%), gentamicin (0/3, 0%), oxytetracycline (1/3, 33%), penicillin (3/3, 100%), sulfadimethoxine (0/3, 0%), and tulathromycin (0/3, 0%). For *B. uniformis*, the results of the susceptibility testing showed the isolate was resistant to ampicillin, oxytetracycline, and penicillin. Haptoglobin concentrations were available for 24/27 (89%) of calves. The results of 5 calves were excluded because the coefficient of variance was > 10%. Median haptoglobin concentration was 0.16 mg/mL (range 0.07 – 0.95). Serum amyloid A concentrations were available for 21/27 (78%) of

calves. The results of 6 calves were excluded because the coefficient of variance was > 10%. Median serum amyloid A concentration was 0.248 mg/mL (range 0.019 – 1.46).

Overall, 13/27 (48%) calves survived to 3 months after discharge while 14/27 (52%) died. When evaluating survival to discharge, 20/27 (74%) survived while 7/27 (26%) died or were euthanized while in hospital. From discharge to 2 weeks an additional 7 calves died bringing the total to 13/27 (48%) calves alive while 14/27 (52%) had died. No deaths were reported from 2 weeks to 3 months after discharge. No calves with positive blood culture (n = 4) survived to discharge.

Results from the univariable logistic regression model showed that admission temperature, admission heart rate, admission pCO_2, admission fibrinogen, and admission haptoglobin were all significant for predicting survival to either discharge, 2 weeks after discharge or 3 months after discharge. Serum amyloid A was not predictive of survival at any time point. Admission temperature was significant for predicting survival to 2 weeks and 3 months after discharge ($p = 0.0421$). As temperature increased calves were more likely to survive to 2 weeks and 3 months after discharge (OR = 1.256). Admission heart rate was significant for predicting survival to 2 weeks after discharge ($p = 0.075$). As heart rate increased calves were more likely to survive to 2 weeks after discharge (OR = 1.04). The measured pCO_2 was significant at predicting survival to discharge ($p = 0.0067$) and to 2 weeks and 3 months after discharge ($p = 0.0228$). As pCO_2 increased calves were less likely to survive to discharge (OR = 0.842) and to 2 weeks and 3 months after discharge (OR = 0.8844). Admission fibrinogen was significant for predicting

survival to 2 weeks after discharge (p = 0.025). As fibrinogen increased calves were less likely to survive to 2 weeks after discharge (OR = 0.0208). Haptoglobin was significant for predicting survival to discharge (p = 0.0233). As haptoglobin increased calves were more likely to survive (OR = 8525.597). Due to the low number of bacteremic calves, no predictive factors were able to be identified.

3.4 Discussion

The findings of this study suggest that the incidence of bacteremia in calves (17%) with a focal site of infection is lower than previously reported (24-70%) [73, 84-86]. One potential reason for the variability in prevalence is the criteria used to define bacteremia. In this study, each animal had 3 different blood vessels samples and at least 2 samples had to be culture positive for the animal to be classified as bacteremic. Among previous reports on bacteremia in calves, only one study collected blood samples from more than one vessel [86]. Another potential explanation for the lower prevalence of bacteremia is improved management practices that enable calves to be healthier. Improved management practices include better colostrum management and nutrition. Many of the previously mentioned studies are at least 20 years old. This is supported by the fact that a more recent study identified a prevalence of bacteremia similar to the one identified in this study [24]. In the study by Garcia et al. (2020), the authors identified a 9% prevalence of bacteremia in diarrheic calves. However, in that study, only one blood sample was used and the blood samples were not obtained in a sterile manner. The collection techniques used could potentially explain why the study reported a higher prevalence of bacteremia in the non-diarrheic group (14%). A final potential reason for the low prevalence of bacteremia was the fact that 55% of calves in this study had received antimicrobials prior to admission. In human medicine,

antibiotic-absorbing resin media is recommended in an attempt to increase organism recovery for patients that received antimicrobial therapy prior to blood sample collection [87].

The most commonly isolated organism in this study was *E. coli*. This is similar to previous work that shows that Gram-negative bacteria, mainly *E. coli*, were the most common organisms identified from calf blood samples [16, 24, 73, 84-86]. The susceptibility profiles of the 3 *E. coli* isolates revealed resistance to ampicillin and penicillin. Gram-negative bacteria like *E. coli* have natural structural characteristics which can make them inherently resistant to beta-lactam antibiotics though this is not always observed in wild-type isolates [88]. One study in bacteremic calves found that use of an antimicrobial was correlated with development of resistance [86]. This finding is not surprising as numerous studies have identified this correlation; however in this study, ampicillin and penicillin were not reported to be administered by owners of enrolled calves [89, 90]. What is most important is that antimicrobial resistance should be monitored over time, so that appropriate antimicrobial usage recommendations can be made. The other isolate identified from a bacteremic calf in this study was *B. uniformis*. While anaerobes are not frequently identified in the blood of calves, *Bacteroides* spp have been isolated from the blood of calves in previous studies [86].

With our small number of bacteremic calves we were unable to identify any factors that were predictive of bacteremia. Previous work has been done to create models and identify predictors of bacteremia in calves [24, 72, 73]. Models have focused on both clinical and laboratory factors that predict bacteremia. In all three studies commonly identified clinical variables predictive of

bacteremia included fever, depression, presence of a focal site of infection, poor suckling reflex, and posture/recumbency. In the laboratory model, predictive factors identified included moderate to marked increases in serum creatinine, presence of toxic changes in neutrophils, and failure of transfer of passive immunity. These factors are consistent with reported sepsis scores from other veterinary species although the sepsis scores in other veterinary species have been validated on a larger number of patients and often utilize other laboratory factors including blood lactate and blood pressure [91, 92].

The majority of the calves in this study presented with diarrhea (18/27, 66%) which is consistent with the results of our first study (Chapter 2) that shows that digestive disorders, mainly enteritis, are the most common conditions affecting this age group of beef calves. Initial treatment recommendations for calves with diarrhea primary focus on oral fluid therapy; however, 79% of calves that owners had treated in this study had received antimicrobials prior to admission and 32% of calves that owners had treated had received greater than 3 antimicrobial protocols. Current literature suggests that parenteral antimicrobial administration for calves with diarrhea be reserved for those calves that also demonstrate evidence of systemic illness (depression, poor suckle, fever) [24, 93]. Furthermore, repeated empirical antimicrobial use should be discouraged and producers should be educated and encouraged to seek veterinary care if initial therapies fail. The choice of antimicrobials is an additional topic of education for producers. The common use of ceftiofur is concerning since this 3rd generation cephalosporin is classified as a critically important antimicrobial by the World Health Organization [89]. More concerning is the use of enrofloxacin, another critically important antimicrobial, in the treatment of calves with diarrhea.

Currently enrofloxacin is only labeled for use in ruminating beef cattle for respiratory disease and anaplasmosis; any extra-label drug use of this antimicrobial is considered illegal [94].

While antimicrobials were the most commonly administered treatment, oral electrolytes were administered to 63% of calves that owners had treated in this study. As most calves in this study had diarrhea, this treatment was considered appropriate. It is well documented that calves with diarrhea frequently develop dehydration, strong ion acidosis, and electrolyte abnormalities, and are in a state of negative energy balance [95]. Oral electrolyte therapy is a simple and economical method of addressing all of these potential complications and it is encouraging to see that a majority of calves in this study had received this treatment. However, because diarrhea is so common and producers often treat these calves on farm, further education on the importance of oral electrolyte therapy in calves and the differences in oral electrolyte products should be provided to producers.

This study identified a higher prevalence of failure of transfer of passive immunity compared to what has previously been reported for suckling beef calves [96-99]. Previous reports have identified rates of failure of transfer of passive immunity between 13 – 26%, while 58% of calves in this study were classified as FTPI. The discrepancy in these results could be because other studies have focused on both healthy and sick calves while this study only evaluated sick calves. However, these results highlight the importance of colostrum absorption in the prevention of illness. One study identified that calves with a total proteins less than 5.5 g/dL were 3.07 times more likely to become ill than those with total proteins above or equal to 5.5 g/dL [98]. Irish

studies have used even more stringent criteria and identified that calves with a total protein less than 6.0 g/dL had a 4.3 times greater risk of mortality compared to those above the cut-off point [100]. Since the importance of appropriate colostrum absorption is widely known, educating producers on the importance of making sure calves are up and nursing is important. Based on current literature, calves at high risk of failure of transfer of passive immunity are those born to dystocia, primiparous dams, nutrient restricted dams, or those born during weather related events that make it challenging for a calf to nurse [4, 76, 77]. In this study refractometry was utilized to assess failure of transfer of passive immunity. This was chosen because refractometry can serve as an indirect measurement of immunoglobulin concentration and several studies have identified this as being the best alternative to direct immunoglobulin concentrations [101-105]. One potential confounding factor is the fact that most of the calves in this study had diarrhea and in calves with infectious diarrhea it has been shown that they lose serum total proteins in their diarrhea [106]. Therefore, a more accurate assessment would have been to directly measure immunoglobulins.

Median fibrinogen concentration (0.7 g/dL) and band neutrophil count (0.2 $x10^3/\mu L$) suggested that the majority of calves in this study suffered from a systemic inflammatory response. This finding is not surprising since both these abnormalities are commonly seen in critically ill calves and those suspected of being septic [16, 73]. Median venous blood gas values indicate that this population of calves has a propensity for metabolic acidosis with respiratory compensation. This finding is expected since the majority of the calves in this study had diarrhea which has been shown to cause a strong ion acidosis mainly due to accumulation of d- and l-lactate [107].

Results of the univariable logistic regression model showed that temperature was significant for predicting survival to 2 weeks and 3 months after discharge. As body temperature increased calves were more likely to survive. The majority of the calves with abnormal body temperatures in this study were hypothermic and presented during the colder months of the year (November to April). In human trauma patients, hypothermia is frequently associated with mortality [108]. Proposed mechanisms include diminished function of clotting factors and platelets that predispose patients to increased blood loss. The importance of this mechanism in this calf population is questionable since no cases had clinical evidence of hemorrhage.

Another variable that was significant in the univariable logistic regression model was pCO_2, with an increased value being associated with decreased likelihood of survival. The exact mechanism behind this finding is unclear but one could hypothesize that an increasing pCO_2 was associated with respiratory acidosis which could indicate respiratory disease. This mechanism is not supported by the data since only 2/8 (25%) calves with the highest pCO_2 were diagnosed with respiratory disease. It is possible that this finding is a result of the low sample size since a study conducted on 1,400 critically ill neonatal calves found that the only laboratory value associated with mortality was a venous blood pH < 6.85 [66]. That same study identified that the presence of clinical abnormalities most predictive of mortality were clinical signs of neurologic disease, abdominal emergencies, cachexia, or orthopedic problems such as septic arthritis.

When evaluating the association of APPs with survival, the results of this study are conflicting. As fibrinogen increased, calves were less likely to survive; the opposite effect was seen for

haptoglobin, and no effect was seen for serum amyloid A. Since fibrinogen is an indicator of inflammation in cattle it stands to reason that more inflammation is associated with a negative outcome [48]. This assumption was seen in a study of calves with clinical bronchopneumonia where it was found that calves with fibrinogen concentrations > 8 g/dL died or performed poorly despite treatment [109]. In this study increased haptoglobin concentrations were associated with survival which is in contrast to fibrinogen and in contrast to previously published data that showed that haptoglobin was not associated with outcome in horses with inflammatory conditions or in sick neonatal calves [52, 110]. While one study in neonatal calves did not identify any value in haptoglobin concentrations as a prognostic factor other studies have identified that increased haptoglobin was associated with an increased number of treatments for bovine respiratory disease complex [52, 56]. A possible mechanism for the association with increased haptoglobin and likelihood of survival in this study is that haptoglobin may play a protective role in human sepsis patients who have elevated levels of circulating cell-free hemoglobin due to hemolysis [111]. Since no gross hemolysis was identified in any CBC in this study the importance of this mechanism is not clear. Serum amyloid A was not predictive of outcome which is in line with previously published equine data [110]. The previously cited study identified that while admission SAA was not predictive of outcome, increasing SAA concentrations in the first 72 hours after initiation of therapy was predictive. This tells us that there may be greater value in monitoring acute phase protein concentrations over time than a single measurement. Unfortunately, only one time point was assessed in this study.

All calf deaths in this study occurred during hospitalization or during the two weeks after discharge. This information is of vital importance because it lets producers know that monitoring

of these patients in the 2 weeks after discharge is crucial since calves are at high risk of death during that period. It also tells producers that if a calf makes it past 2 weeks after a critical illness it is likely to survive and not succumb to complications from its previous illness.

The major limitation of this study was the small sample size and the incomplete data-set for all calves enrolled in this study. The sample size was determined by patient presentation to the University of Missouri Food Animal Clinic. Furthermore, patient collection had to be halted during the middle of the study due to the COVID-19 pandemic, which further limited patient enrollment. In addition to limited patient enrollment this study has an inherent bias toward more ill calves that needed intensive care at a referral institution; this data may not reflect other ill calves that were successfully treated on-farm or by primary care veterinarians. Finally, since acute phase proteins are known to change throughout the first days of life, the lack of a control group is another limitation as having one could help distinguish normal acute phase protein variations from inflammatory changes.

3.6 Conclusion:

Diarrhea was the most common presenting complaint and median blood gas values indicated that most calves had metabolic acidosis. Beef calves in this study had a higher prevalence of failure of transfer of passive immunity compared to previous reports. Most calf deaths occurred either during hospitalization or by 2 weeks after discharge. A lower prevalence of bacteremia was found in this study compared to previous reports. In this study, no bacteremic calves survived. Hypothermia, increased pCO_2, and hyperfibrinogenemia were associated with decreased

likelihood of survival. These results may help veterinarians determine prognoses for sick neonatal beef calves.

3.7 Tables

3.7.1 Table 4

Complete Blood Count Results for 21 out of 27 enrolled calves.

Indices (units)	Median (Range)	Normal [112]
Fibrinogen (g/dL)	0.7 (0.2 – 1.7)	0.2 – 0.7
HCT (%)	37.2 (17.8 – 59.3)	22 – 33
WBC (x 10^3/µL)	10.61 (3.72 – 26.21)	4.9 – 12
Segmented neutrophils (x 10^3/µL)	5.27 (1.07 – 21.62)	1.8 – 6.3
Band neutrophils (x 10^3/µL)	0.2 (0 – 6.57)	Rare
Lymphocytes (x 10^3/µL)	3.45 (1.77 – 8.04)	1.6 – 5.6
Monocytes (x 10^3/µL)	0.49 (0.06 – 4.84)	0 – 0.8
Platelets (x 10^3/µL)	662 (218 – 1654)	100 – 800

3.7.2 Table 5

Venous blood gas results for 18 of the 27 enrolled calves.

Indices (Unit)	**Median (Range)**	**Normal [113]**
pH	7.3 (6.8 – 7.5)	7.373 – 7.466
pCO_2 (mmHg)	35.8 (21.6 – 65)	43.3 – 58.6
HCO_3^- (mmol/L)	18.55 (4.4 – 37.1)	26.3 – 34.1
BE (mmol/L)	-7 (-28 – 14)	2.6 – 10.8

CHAPTER 4: Conclusions

The results of this thesis show that gastrointestinal diseases, mainly diarrhea, are the most common infectious problem affecting neonatal beef calves in Missouri. The results of this study also confirm what previous research has shown in that calves with diarrhea develop a metabolic acidosis with respiratory compensation. In addition, there appears to be decreased incidence of bacteremia in this calf population than in previous reports, but the identified pathogens remain similar to previous studies. The majority of calf deaths occurred either during hospitalization or in the 2 weeks after discharge; producers should closely evaluate calves in the immediate two weeks after discharge. Finally, hypothermia, increased pCO_2, and hyperfibrinogenemia were associated with decreased likelihood of survival. The results of this body of work may help veterinarians further understand causes of mortality and provide prognostic factors for treating clinical cases.

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