DIAGNOSIS AND MANAGEMENT OF BOVINE RESPIRATORY DISEASE

by

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B.S., Wichita State University, 1997
D.V.M., Kansas State University, 2009

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

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Abstract

Bovine respiratory disease (BRD) is the most costly disease of cattle in US feedyards and diagnosis based on clinical signs of illness is challenging. Over the course of five independent studies we evaluated the precision of multiple observers assigning clinical illness scores (CIS) to calves with induced *Mycoplasma bovis* pneumonia. We also evaluated the accuracy of CIS in relation to lung lesions at necropsy. Agreement among observers over all five studies was slight ($\kappa = 0.16$; 95% confidence interval, 0.10 to 0.24) and ranged from 0.10 to 0.21 for individual trials. The accuracy of CIS varied based on the pulmonary consolidation score chosen to represent a truly ill animal.

Inflammation associated with BRD can lead to significant pulmonary damage and reduced lung function. Treatment for BRD frequently involves antimicrobial administration and occasionally non-steroidal anti-inflammatory drugs. We evaluated how calves experimentally challenged with *Mannheimia haemolytica* respond to treatment with flunixin meglumine, alone or in combination with the antimicrobial florfenicol. Individual calf response to bacterial pneumonia was highly variable in this study. None of the changes in serum biomarkers, CBC or chemistry parameters provided reliable indicators of the pulmonary inflammation associated with the mild severity of bronchopneumonia in our study.

Metaphylaxis is frequently administered to manage the risk of BRD within cohorts of cattle. We evaluated the impact of metaphylactic antimicrobial administration 10 days prior to experimental *Mannheimia haemolytica* inoculation to mitigate pulmonary lesions. We found that calves receiving tildipirosin had less lung damage and fewer clinical signs of illness compared to calves treated with tulathromycin or saline.

Finally, the ability to predict those animals that would not finish the production cycle normally would provide benefits in effectively managing cattle. We evaluated the ability of classification algorithms to accurately predict an individual calf's outcome based on data available at first identification of and treatment for BRD. We found accuracy of classifiers was dependent on the data recorded by the feedyard and there are sub-groups of calves within feedyard populations where classifiers were highly accurate. These data suggest the importance of pairing the proper classifier with the data available.

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Chapter 1 - Review of the Diagnosis and Management of Bovine Respiratory Disease

Introduction

Bovine respiratory disease complex (BRDC) is the most costly disease in U.S. cattle accounting for 70% to 80% of the morbidity and 40% to 50% of mortalities in feedlot placed cattle. (Griffin, 1997; Smith, 1998) As reported in the National Animal Health Monitoring System (NAHMS) Feedlot 2011 study, 16.2% of cattle placed in feedlots developed BRD and treatment cost averaged $23.60. (USDA, 2013) These estimates are higher than the previous 1999 study that estimated BRD incidence at 14.4% and treatment costs associated with a single case of BRD at $12.59. (USDA, 2000a)

The epidemiology of BRD is multifactorial with complex interactions among the host immune system, viral and bacterial agents, and the multiple phases of beef production resulting in environmental challenges for these animals. A calf's immune system contains innate and acquired responses that work to prevent infection by decreasing adherence and migration of pathogens as well as mounting antibody responses against invading pathogens. (Edwards, 2010) Common commensal bacterial organisms associated with BRD are *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis*. (Griffin et al., 2010) Infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), parainfluenza-3 (PI3), and bovine respiratory syncytial virus (BRSV) are among the most important viral pathogens associated with BRD. The importance of bovine coronavirus in relation to BRD has received attention in recent years. (Fulton et al., 2011) Depending on the viral agent, primary infections can damage tracheal, bronchial, and bronchiolar epithelial cells leading to decreased mucociliary clearance and opportunities for bacterial colonization and infection. (Griffin et al., 2010)

Most cohorts of cattle arriving at the feedlot contain animals from multiple sources with diverse pathogen exposures. The immune status of these animals varies greatly and environmental factors and activities such as weaning, comingling, and transport can negatively impact an animal's ability to fight infection. (Edwards, 2010) In a retrospective study evaluating

32,646 calves arriving at a feedlot between 1985 and 1988, an average truckload of 60 steers comprised calves from as many as 20 to 30 farms. (Ribble et al., 1995) That same study also found a significant relationship between the mean number of calves arriving on a truck and subsequent risk of fatal fibrinous pneumonia. (Ribble et al., 1995) Cernicchiaro *et al*. (2012) evaluated body weight loss during transportation in 16,590 cohorts of cattle and found it was significantly associated with BRD morbidity; however, this association varied by calf gender, season of transportation and the average arrival body weight of the group. (Cernicchiaro et al., 2012b) Ribble *et al*. (1995) concluded that transportation distance from market to feedlot was not correleated with risk of fatal fibrinous pneumonia; however, others have found associations ($P < 0.05$) between distance traveled and BRD morbidity. (Cernicchiaro et al., 2012a; Ribble et al., 1995b) Understanding the associations between these common stressors and BRD could allow for better management of BRD within feedlots.

Diagnosis of Bovine Respiratory Disease

Calves suffering from BRD are frequently identified based on clinical signs such as depression, lack of rumen fill, ocular and or nasal discharge, and labored breathing. (Buhman et al., 2000; Perino and Apley, 1998; Smith et al., 2001) These criteria are highly subjective, and agreement among multiple observers is poor at best. Diagnosis of morbid animals using these subjective criteria is also frequently inaccurate. In a longitudinal study evaluating 469 steers from birth to harvest, 35% received treatment for BRD with 72% having pulmonary lesions at slaughter. (Wittum et al., 1996) Others evaluated pulmonary lesions at slaughter as an indicator of previous respiratory disease and found in a population (n=1,665) with an 8% incidence of clinical BRD, greater than 61% of the calves had pulmonary lesions. (Schneider et al., 2009) In a study of 2,036 calves in two South African feedlots, 22.6% of the calves were treated for BRD; however lung lesions were present in 43% of the calves at slaughter. These studies indicate that observational methods for identifying BRD may not be sensitive enough to identify all cases within the population.

Calves visually identified as suffering from BRD will frequently have rectal temperatures measured. (Perino and Apley, 1998) The goal of applying these two tests in series (visual appraisal and rectal temperature) is to further discriminate among true positive and false positive

animals. There is no consensus on the appropriate temperature cutoff that should be used. Temperatures ranging from > 39.44 °C (103 °F) to > 40.28 °C (104.5 °F) are used based on the management practices in place at individual facilities. The sensitivity and specificity of using clinical signs of illness plus rectal temperature has been estimated to be 61.8% and 62.8%. (White and Renter, 2009) These studies all highlight the imperfection of current methods used to identify calves suffering from BRD and the need for more accurate diagnostic tests.

Feedlot management of disease

To manage the perceived risk of BRD within a cohort of calves, metaphylactic antimicrobials are sometimes administered upon feedlot arrival. In the NAHMS 2011 Feedlot study, 71% of feedlots with ≥ 8,000 head capacity administered metaphylaxis compared to 39.1% of those feedlots with 1,000 to 7,999 head capacity. (USDA, 2013) These numbers have decreased from those published in the NAHMS 99 Feedlot study where 82.1% of large feedlots and 46.2% of smaller feedlots administered metaphylactic antimicrobial therapy (USDA, 2000b) A survey in 2000 of US feedlots that represented 2,495,439 cattle on feed indicated 17% received population antimicrobial therapy upon arrival. (Woolums and Hawkins, 2005) The overall goal of metaphylaxis is to decrease the pathogen burden in clinical and subclinical cases within the population and has been proposed as a method to compensate for the inability to correctly identify the health status of calves. (Nickell and White, 2010)

Cattle identified as suffering from BRD have limited proven treatment options available, and the primary course of therapy is often focused on administration of antimicrobials. According to the NAHMS 2011 Feedlot study, almost all feedlots that treated cattle for respiratory disease used an injectable antibiotic and 55.9% of the feedlots surveyed used some type of nonsteroidal anti-inflammatory drug. (USDA, 2013) After an extensive review of available literature, Francoz *et al.* determined that available studies on the use and efficacy of nonsteroidal anti-inflammatories in relation to BRD lack reliability and validity. (Francoz et al., 2012) Additional supportive therapies such as vitamins, probiotics, and electrolytes are reportedly used in relatively small percentages of cattle suffering from BRD. The 2011 NAHMS feedlot study reported that 39.3% of those feedlots surveyed administered some type of respiratory vaccination when treating calves for BRD (USDA, 2013)

Several authors have evaluated various risk factors for developing BRD (Cernicchiaro et al., 2012a, b; Cusack et al., 2007; Sanderson et al., 2008; Taylor et al., 2010); however, little literature exists evaluating the ability to predict if an individual animal will develop respiratory disease. Others have used models to predict the cumulative risk of BRD and found the accuracy of the models was dependent on the individual characteristics of each cohort evaluated. (Babcock et al., 2013) The lack of literature evaluating individual animal predictions may be due to several factors including but not limited to, access to relevant data, ability to manage the large quantities of data needed to make these predictions, tools necessary to develop predictions, and accuracy of such predictions. Predicting individual events appears to be more common in the human literature and warrants further research within production animal medicine. (Andrews et al., 2002; Chapman et al., 2001; Maroco et al., 2011; Mofidi et al., 2006)

Conclusions

Bovine respiratory disease is a common and important syndrome of calves in confined feeding environments. Multiple risk factors contribute to the development of BRD, and both population and individual animal management are important control methods. Visual observation of clinical signs is the most common method employed for BRD diagnosis, but this system appears to have relatively poor accuracy. The ability to predict outcomes at the time of disease identification may be a helpful tool to improve current diagnostic modalities. Further research is needed evaluating current diagnostic methods and prognostic indicators.

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Chapter 2 - Selected excerpts from "Remote Noninvasive Assessment of Pain and Health Status in Cattle"

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Introduction

The ability to remotely identify cattle that require an intervention due to pain or disease is important for animal health providers and researchers. Behavior is frequently monitored to measure potential changes in animal well being.[1] Stress, pain, or disease may alter animal behavior relative to optimal wellness status, but monitoring these changes is challenging without a clear definition of the expected behavioral response to an adverse event.[2] Some behavioral definitions are vague and are not specifically tied to one pain or disease response. Improvement in behavioral monitoring techniques is needed for remote monitoring of activity to be useful as diagnostic or research tools.

Multiple methods are available to monitor cattle behavior, including subjective visual observation, objective measures of cattle activity, or determination of cattle location within the housing area. Subjective measurements of pain and cattle well being include behavioral, depression, or illness scores based on observer impression of the animal's current wellness state. The challenges with using subjective measures to determine cattle wellness state are related to potential differences between observers and among observers over time.

An opportunity exists to more discretely identify potential behavioral changes via collection of data using remote sensing technologies. Objective, continuous behavioral monitoring using accelerometers and pedometers has been used to assess cattle behavior in a variety of scenarios.[3–9] Monitoring cattle location within a defined environment has also been used in an effort to identify and monitor potential behavioral changes.[8,10]

The objective of this article is to describe potential benefits and challenges of remotely monitoring cattle behavior with available methodologies, including clinical illness scores (CISs), visual monitoring, accelerometers, pedometers, feed intake and behavioral monitoring, global position systems (GPSs), and real-time location systems (RTLSs). Although all of these remote monitoring systems are not directly applicable in a clinical setting, the results from research based on these technologies provides valuable insights to practitioners on the associations between behavioral changes and pain and wellness states.

Observer monitoring clinical illness

One of the most common methods to determine wellness or painful state of an animal is having a trained observer monitor cattle for clinical signs of pain or disease. Multiple clinical signs and subjective assessments can be used to determine the animal's overall wellness status. Often a combination of findings can be categorized into a single value, or CIS, which represents the current state of the animal. The potential benefit of determining a CIS is presumably that it correlates with the need for an intervention or the probability of a specific outcome.[11] Scoring systems that assign a value based on degrees of illness are relatively common[12] and are frequently used in disease research.[3,10,13] Even when quantitative measurements, such as rectal temperature, are combined with subjective assessment, the final disease classification remains subjective.[14,15] This subjectivity may affect how the results are interpreted if the CIS is used as one of the criteria in a treatment or preventative health program.

Research has shown limited agreement among observers using the same CIS to identify calves with respiratory disease.[16] Potential sources of variation include differences among the experience and training of observers, cattle type, and environmental conditions. When a subjective scoring system is applied and interpreted by more than one individual, it should be repeatable among those individuals. Other research has evaluated agreement among veterinarians assigning body condition scores to cows and determined even small amounts of training among

the observers can increase the overall agreement.[17] A clear case definition and educational programs can decrease the variation between observers and make the results more clinically applicable.

Although CIS are frequently utilized, true accuracy relative to disease state is difficult to determine. There is no gold standard to diagnose respiratory disease in cattle, but the presence or absence of pulmonary lesions at harvest has been compared with ante mortem diagnoses of clinical respiratory disease.[18-20] Results from these studies illustrate low correlations between lung scores and diagnosis of clinical illness. White and Renter[21] estimated the sensitivity and specificity of using clinical signs of illness combined with rectal temperature to diagnose respiratory disease to be 61.8 % and 62.8 %, respectively. A test with imperfect sensitivity and specificity can underestimate or overestimate morbidity, thus leading to errors in the interpretation of preventative or therapeutic treatment efficacy.[16]

One way to improve CIS agreement among observers is the implementation of a refined scoring system with limited categories. The objective of assigning CIS to cattle is to accurately identify those animals that need an intervention (sensitivity) and those that do not (specificity); therefore, the system could be condensed to those two categories. If calves are deemed to require an intervention, the selection of the intervention would be based on clinician's judgment of the case. For example, a calf that was deemed to have clinical respiratory disease may require an intervention with an antimicrobial; whereas euthanasia may be a more appropriate intervention for an animal severely ill enough to become moribund and nonresponsive to human approach. Dichotomizing the results would increase agreement among observers and could potentially increase accuracy of comparison of CIS among individual observers. As previous research has illustrated, distinguishing illness severity based on CIS is challenging.[10,16] Much of the analysis of CIS data is based on the dichotomization of an animal into healthy or sick; therefore, systems that have more than two main levels serve a limited purpose.

Monitoring clinical illness by visual appraisal is a common procedure and the specific implementation of the scoring system influences final data interpretation. Although CIS are qualitative, they may not be repeatable between or among observers and do not provide an objective measure of the degree of clinical illness. Care should be taken to limit potential sources of variability among observers through training and selection of the appropriate scoring system for the situation.

Monitoring activity with accelerometers

Accelerometers are devices that continuously measure gravitational force in multiple axes; these values can be processed to determine activity and postural behaviors. Fig. 2.1A, B show a three-dimensional accelerometer attached with the horizontal, vertical, and diagonal axes the accelerometer monitors gravitational force. Before remote continuous monitoring technology can be used to assess the physiologic and behavioral patterns that cattle display, the technology requires validation.[44,45] Accelerometers have been shown to accurately monitor calf behaviors of standing, lying, or walking with 97.7% agreement to video analysis.[6] This high accuracy allows the user to effectively rely on the accelerometers to determine posture behavior compared with using a labor intensive process of analyzing video.

Assessing postural changes may be important in evaluating calf wellness or pain status and several studies have illustrated differences in postural behavior following painful stimuli. Calves have been shown to increase the percentage of time standing in the hours immediately following castration based on accelerometer analysis.[46] However, Pauly and colleagues[5] determined that calves spent more time lying down and less time walking in the 5-day period following castration. The difference between these two studies may be due to the length of the monitoring period and a potential time-dependent change in behaviors. Theurer and colleagues[8] determined that calves administered the nonsteroidal anti-inflammatory drug, meloxicam, before cautery dehorning spent more time lying down for 5 days after dehorning compared with control calves that did not receive analgesia as commonly performed in production practice.[47] Lying behavior decreased in calves after being induced with experimental lameness using an amphotericin B synovitis-arthritis induction model.[48] Accelerometers are an effective tool for continuous monitoring of behavior changes in response to pain.

Accelerometers (GP1 SENSR, Reference LLC, Elkader, IA, USA) have also been used to monitor disease and wellness state of cattle. Calves challenged with *Mannheimia haemolytica* spent more time lying down compared with unchallenged control calves.[7] This agrees with a common assumption that a primary clinical sign of respiratory disease is depression. In another respiratory disease trial, there was no difference in the amount of time morbid calves spent lying down or walking compared with baseline data collected before challenge.[3] These findings suggest that the postural activity of cattle may be influenced by disease or pain state, but changes in standing and lying behavior may not be a specific response to changes in wellness status.

Daily environmental conditions, differences among individual calves, and circadian rhythms also affect the amount of time calves spend lying[37]; therefore, it is important to make comparisons of behavioral activities of calves housed in the same environmental conditions. Monitoring control animals allows the observer to distinguish between the behavioral changes associated with administering a procedure from daily variation due to environmental conditions.[37,49,50] The placement of the accelerometer on the animal and accelerometer size and weight may transiently alter normal gait and behavior. A brief acclimation period may be needed for the cattle to adjust to having the accelerometer attached to their legs.

Limitations of using accelerometers to monitor behavior include cost, data processing, and technological constraints. Accelerometers are relatively expensive compared with other behavior monitoring techniques, such as video analysis. Transforming the accelerometer into useable behavioral measurements can be achieved with validated algorithms; however, generating the data processing technique is time consuming. The accelerometers must have sufficient battery life, on-board memory storage (or the ability to wirelessly transmit data), and be small enough to be easily affixed to the animal in some method. The objective quantification of cattle postural behavior as determined by accelerometers provides valid data to compare potential changes in behavioral patterns associated with pain or wellness status.

Monitoring step-count frequency with pedometers

Pedometers have been used to objectively quantify the number of steps traveled and total distance traveled. An on-board algorithm calculating the number of steps from the raw data is contained within the pedometer. Pedometers are relatively easy to attach and use, but the number of steps each calf travels varies considerably among days and environmental conditions.

The distance calves travel may be associated with painful and stressful procedures. The amount calves travel following a painful procedure such as castration may vary because some research demonstrated calves traveled fewer steps for 4 days after castration[51] whereas other work was unable to detect a difference in the number of steps traveled in calves after castration.[52] Stress may also influence the distance traveled because calves have been shown to take more steps for 3 days after weaning.[53] Bulls travel more steps than steers per day indicating the need for accounting for gender in the analysis.[51] In properly designed experiments, pedometers may be useful in determining changes in behavior following a painful procedure.

Pedometers have been used to detect early lameness in dairy cattle, but a 15% decrease in activity was needed before the pedometer could accurately identify 92% of lame cattle.[54] The biologic significance of a 15% decrease in activity has not been established, but there may be clinical implications in detecting cattle before a change this large is detected. O'Callaghan and colleagues[55] demonstrated that lame dairy cows traveled 22.5 fewer steps per hour compared with cows that were not lame based on visual locomotion score throughout most of the lactating period. Because pedometers are directly measuring locomotion, they are a valuable tool in identifying and monitoring musculoskeletal pain. However, changes in step counts as measured by pedometers are not specific for identifying pain; due to increased activity levels, pedometer technology has also been able to accurately detect the onset of estrus in cows.[56,57]

Pedometers can be effective monitoring devices for evaluating pain response and health status of cattle. The relative lower cost of investment and labor intensity compared with other technologies makes pedometers an attractive tool to objectively monitor potential behavioral changes.

Feed intake and behavioral monitoring

Systems are available to measure individual cattle feeding behavior and intake in group-housed situations. These systems have been used to identifying morbid cattle from healthy cattle based on differences in feeding behaviors.[58] Feed and water intake, duration, and frequency are specific behaviors that can be monitored with these systems. Systems that monitor feeding and watering behaviors that are commercially available include GrowSafe (GrowSafe Systems Ltd, Airdrie, AB, Canada) and Insentec (Repelweg, Marknesse, Netherlands). GrowSafe uses radio frequency identification (RFID) ear tags to identify individual animals. Insentec, on the other hand, uses transponder collars to identify when animals are at feeding or watering stations. Both systems have integrated software that allows for real-time monitoring and analysis of animal feeding or watering behavior.

RFID technology has been used to document a reduction in the frequency of visits to feeders.[24] Researchers evaluating residual feed intake found distinct differences in feeding behaviors among high and low residual feed intake calves using both the GrowSafe and Insentec monitoring systems.[59,60] Because feed inputs represent one of the largest costs in producing beef, monitoring behaviors that may identify calves with less than ideal feed efficiencies may be

beneficial.[61] Monitoring the feeding behavior of an animal over a period of time allows establishment of a baseline against which deviations in subsequent behavioral patterns can be evaluated. Investigators have used algorithms with 7-day rolling average feeding times as baselines to identify behavioral changes correlated with painful locomotive conditions in dairy cows days before farm staff were able to diagnose lameness.[62]

Monitoring animal feeding behavior and intake can provide insight into potential changes in wellness or pain status. Setup, maintenance, training, and expense are all potential disadvantages that must be considered when evaluating remote feed intake and behavior systems. However, the feed intake and frequency data collection capabilities make these systems an attractive monitoring tool to use because feed costs are important to the producer.

Location determination: GPS

GPSs have been used to remotely monitor movement of wildlife and domestic animals.[63,64] Advances in GPS technology have created lighter and more accurate receivers, but monitoring multiple animals in varied geographic regions is often cost-prohibitive.[64] Three of the largest challenges when monitoring cattle with GPS technology are the ability to have real-time updates, decreased battery life, and spatial accuracy.

Current technology allows for the location of a GPS receiver to be updated every second, but this update rate exceeds the power sources available in most animal monitoring units.[65] Custom units with real-time updates once every minute have been developed. However, battery life was only 3.7 days.[66] Others using non–real-time receivers have successfully monitored cattle for longer durations (11 days) by only waking the system up from a deep sleep mode every 600 seconds; however, depending on the environment, these infrequent readings may not provide the level of data necessary to define specific behaviors.[67]

Positional accuracy of the systems are also an issue and some research shows a discrepancy between visual and tag positions of an average plus or minus standard deviation of 9 m plus or minus 7 m.[66] Other work illustrates that 99.9% of positional fixes fell within 20 m and 97.3% within 10 m of a known point.[67] Based on these accuracies, the GPS can give approximate location of individuals, but readings are not discrete enough to delineate specific activities such as eating or drinking.

The tradeoffs of battery life and positional update frequency limit the potential uses of GPS systems in situations in which the behavior needs to be continually monitored for longer periods of time. Accuracy of 10 m may be sufficient for questions of pasture usage and grazing activities, but is not sufficient for monitoring feeding and watering behaviors. These limitations make GPS difficult to use to monitor changes in pain or wellness status in cattle.

Monitoring movement in a defined system with RTLS

RTLS are designed to locate the position of an item anywhere within a defined area. The architecture of an RTLS consists of receivers spaced around the desired monitoring space, active or passive tags that are placed on the objects to be monitored, computer hardware, and software to receive and translate positional data. Tags used with most RTLSs are smaller and have considerably longer battery life than current GPS technology. Like GPS, most RTLSs require line-of-sight from tags to sensors for accurate readings. Fig. 2.2 demonstrates a calf within the sensor area and shows how three receivers locate the animal and triangulate its position. Amount of time is calculated by subtracting the time of arrival at that location from the previous time of arrival documented. Although similar to RFID behavior and intake systems, RTLS has the distinct advantage of being able to monitor an animal's location anywhere within the pen, thus not restricting evaluation to only feeding and drinking behaviors.

The system monitors location within the pen at preset intervals and not the specific behavior the calf is engaged in while at that position. Therefore, for data to be useful, the positions must be matched with a known diagram of the facility structure with specific areas of interest (proximity to feed, water, shelter) identified on the same scale of axes as measured by the RTLS. Depending on the frequency of measurements, the RTLS can be used to document the percent of time animals spend in specific locations within the housing environment.

An advantage of RTLS is the ability to measure levels of activity such as distance traveled and time spent within a given proximity to other calves. By measuring location over discrete time intervals, the data can be compared to determine the distance an animal traveled over a given period with results similar to measurements taken using pedometers. Social interactions with other calves (or the lack thereof) can be monitored by comparisons of the proximity of individual calves to other animals within the pen. Real-time location systems have

been used to monitor potential changes in cattle behavior that may be associated with pain or alterations in wellness status.

Investigators have used RTLS technology (Ubisense, Denver, CO, USA) to determine that certain behaviors, such as time spent at the feed bunk and distance traveled, were associated with CISs.[10] The distance traveled by calves as monitored with RTLS was also associated with the level of lung consolidation, indicating that monitoring movement may be a reasonable tool for wellness status evaluation.[10] Theurer and colleagues[8] identified calves that were dehorned and given pain medication had different feeding behaviors when measured by RTLS technology compared with calves dehorned without pain medications. These associations with behavior changes indicate that RTLS technology is a valid tool to generate quantitative measurements of cattle activities that can be used to monitor potential changes in wellness or pain status in response to an intervention.

Limitations of RTLS technology include expense and technological constraints. The RTLSs are able to monitor animal behavior within a specific area, but those areas need to be equipped with multiple sensors to accurately monitor behavioral activity; this may be cost prohibitive in many situations. Installation and calibration of an RTLS requires significant investment in time and resources and, although the use of this technology for monitoring animals is relatively new, these systems have been used successfully for many years for monitoring assets in large complex manufacturing environments.

Summary

Determining animal wellness status is frequently based on visual appraisal or performance parameters. The use of multimodal, remote, quantitative monitoring techniques will become more critical in determining the physiologic, behavioral, and performance responses cattle experience in different scenarios. Interpretation of multiple behavioral responses as an aggregate indicator of animal wellness status instead of as individual outcomes may be a more accurate measure of true state of wellbeing. Individual animals differ greatly in behavior and accurate interpretation of behavioral changes depends on the ability to establish normal baseline activity in calves in a specific housing environment.

Behavioral data should be interpreted carefully because none of the commonly monitored behaviors are truly specific for one type of illness or pain response. Statistical analyses should

account for the hierarchy of repeated measures on individual calves, the effect of having multiple observers, housing effects, time of day, and seasonality. If these potential sources of variability are not included in statistical analysis, differences between treatment groups may be falsely detected or there may be differences that are undetected.

There are numerous remote monitoring methods available to assess the pain or wellbeing status of an animal; however, determination of the specific behavior needed to monitor, labor, and expense all need to be taken into consideration before deciding which behavioral monitoring device to use. The selection of the appropriate system for the situation depends on the expected benefits compared with costs of operating the system. Use of a remote monitoring system provides basic information on cattle behavioral changes that can be translated to other aspects of clinical practice and animal wellness evaluation.

Figure 2.1 Position of the three-dimensional accelerometer (measured X, Y, and Z axes) on the lateral aspect of the right rear limb in a standing (A) and lying (B) calf. (From Robert B, White BJ, Renter DG, et al. Evaluation of three-dimensional accelerometers to monitor and classify behavior patterns in cattle. Comput Electron Agr 2009;67(1–2):80–4; with permission.)

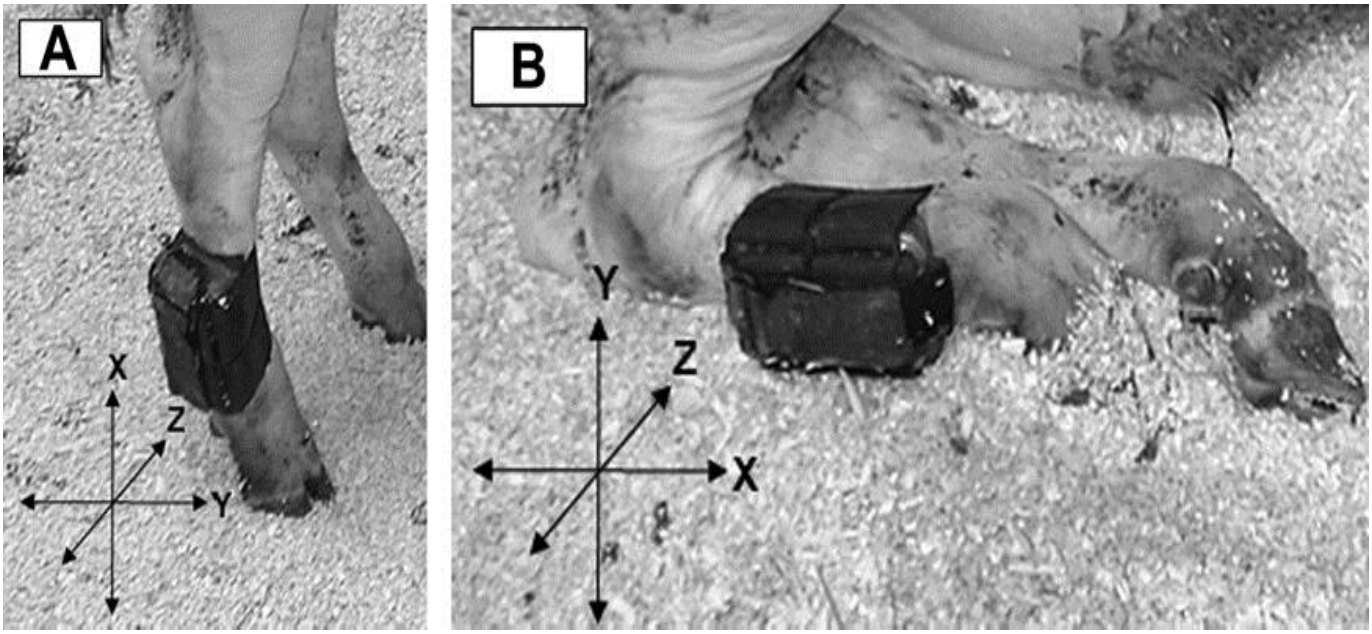
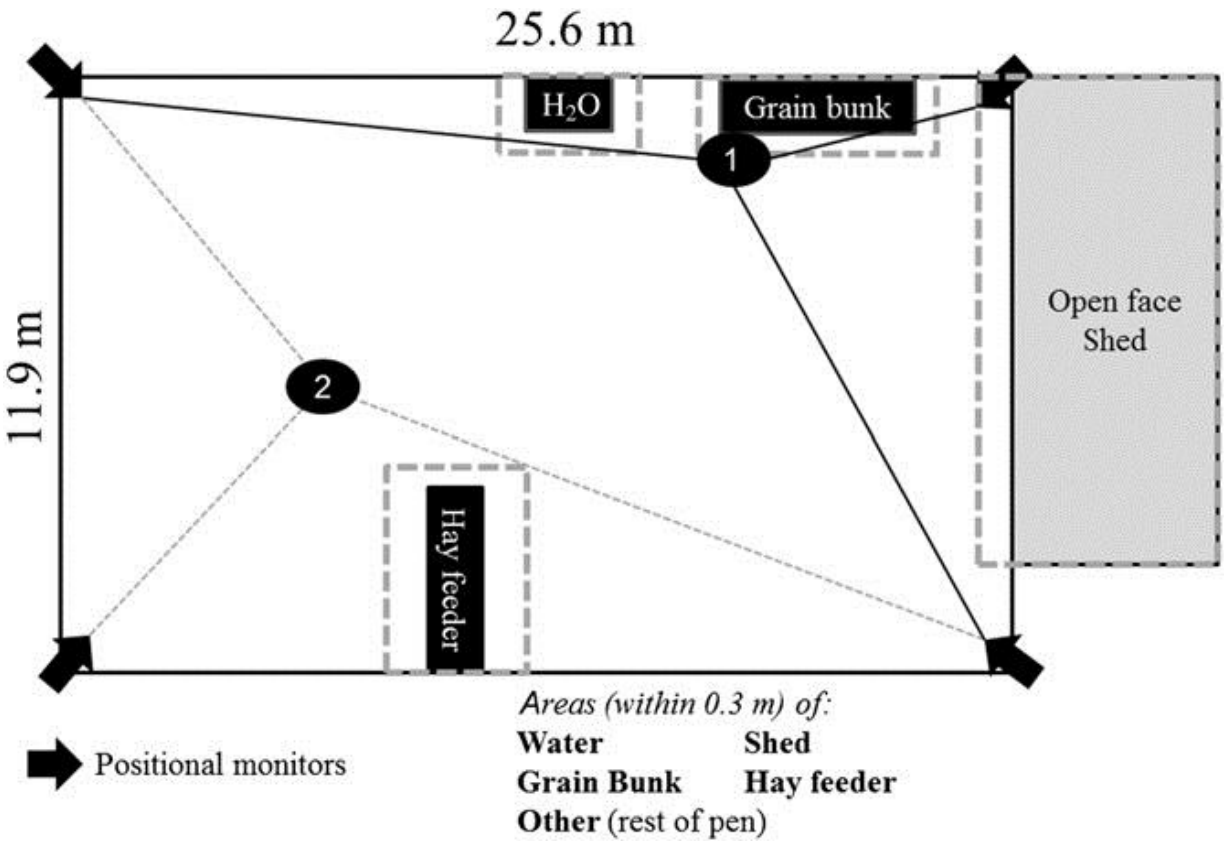


Figure 2.2 A remote triangulation system with positional monitors (arrows) able to triangulate animal position and compare with marked areas of interest including grain bunk, hay feeder, shed, and water. Calf position is determined by the relative distance between the calf tag and at least three readers (lines from the readers to the points within the pen). Amount of time at a location is determined by calculating the difference between time of arrival at specific coordinates and previous triangulation time point. (Circle 1) Calf at the grain bunk. (Circle 2) Calf in the pen, but not next to a location of interest. (Adapted from Theurer ME, White BJ, Coetzee JF, et al. Assessment of behavioral changes associated with oral meloxicam administration at time of dehorning in calves using a remote triangulation device and accelerometers. BMC Vet Res 2012;8(1):48; with permission.)



Chapter 3 - Precision and accuracy of clinical illness scores, compared with pulmonary consolidation scores, in Holstein calves with experimentally induced Mycoplasma bovis pneumonia

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Abstract

Objective—To determine the precision of a clinical illness score (CIS) system for identification of clinical signs in calves with experimentally induced *Mycoplasma bovis* pneumonia and to evaluate the accuracy of CISs in relation to pulmonary consolidation scores assigned at necropsy.

Animals—178 Holstein bull calves that were 52 to 91 days of age at the time of pneumonia induction.

Procedures—5 trials involved calves challenged with *M bovis* and scheduled for euthanasia and necropsy 12 to 24 days afterward. Nine veterinarian observers with various degrees of experience simultaneously assigned CISs to calves within 48 hours before necropsy. The precision of the CIS system among observers was evaluated via the Cohen κ statistic. The accuracy of each observer's CISs relative to 6 cutoffs (≥ 5%, ≥ 10%, ≥ 15%, ≥ 20%, ≥ 25%, and ≥ 30%) of percentage pulmonary consolidation was determined by comparing pre-necropsy CISs with the gross pulmonary consolidation scores assigned at necropsy. Estimates for sensitivity and specificity were calculated relative to the 6 pulmonary consolidation cutoffs.

Results—A slight level of agreement was evident among observers (κ range, 0.10 to 0.21 for the individual trials) and overall (κ = 0.16; 95% confidence interval, 0.10 to 0.24). Median sensitivity and specificity changed with pulmonary consolidation score cutoff. Median sensitivity for all observers ranged from 81.7% to 98.9%, and median specificity ranged from 80.8% to 94.9% over all cutoff values.

Conclusions and Clinical Relevance—Agreement among observers assigning CISs to calves was low; the accuracy of the CIS system in relation to that of pulmonary consolidation scoring varied with the severity of consolidation considered to represent bovine respiratory disease. (*Am J Vet Res* 2013;74:310–315)

Introduction

Bovine respiratory disease is commonly initially diagnosed in cattle on the basis of clinical signs, with the decision to treat typically made on the basis of a high rectal temperature.[1] *Mycoplasma bovis* is an important pathogen in BRD and one of the causes of calf pneumonia.[2] Although a 2-staged approach is often used to establish whether a calf has BRD, studies[3,4] have revealed that > 50% of pulmonary lesions detected at slaughter are in calves that were never treated for respiratory disease. The sensitivity and specificity of clinical signs of illness followed by confirmatory rectal temperature for diagnosing BRD are reportedly only 62% and 63% respectively.[5] These findings suggest that considerable opportunities exist to improve diagnostic accuracy for respiratory disease in cattle.

A CIS system is commonly used in the clinical assessment of animals and quantification or assignment of values that correspond to the probability of a specific outcome.[6] By having multiple observers evaluate and assign a CIS to the same calf, the agreement among observers can be used to determine the repeatability of that CIS system. Determining the repeatability (a measure of precision) of a diagnostic test allows for a better understanding of the variability among observers, which can be used when evaluating methods to improve agreement and, thereby, allows consistent evaluation of disease status. The accuracy of a test refers to how well the test identifies the true status of an individual and is based on sensitivity and specificity.[7] In BRD, the true disease status can be assessed on the basis of the amount of pulmonary damage at a given time point.

Given that CISs are used to make decisions regarding treatment effectiveness, an estimate of the system's performance as a diagnostic test is necessary to interpret results from any BRD study in which CISs are used to define inclusion criteria, to allocate experimental units, or to classify outcome (eg, case vs noncase). The objectives of the study reported here were to determine the precision of a CIS system as a diagnostic tool to identify clinical signs of illness in

calves with experimentally induced *M bovis* pneumonia and to evaluate the accuracy of that system in relation to pulmonary consolidation scoring at necropsy.

Materials and methods

Animals

Five trials were conducted at the Kansas State University College of Veterinary Medicine between August 2010 and September 2011. Each trial was independent, but clinical observations were conducted in conjunction with procedures within each trial to elucidate the precision and accuracy of CIS assigned by multiple investigators relative to pulmonary consolidation scores. In total, 178 Holstein bull calves were enrolled in 5 trials (Appendix).

Calves were procured from a commercial calf grower, where they were individually housed in commercial hutches and fed a commercial milk replacer and calf starter ration until weaning. All calves were weaned and comingled on the farm of origin approximately 2 weeks prior to relocation to the Kansas State University Large Animal Research Center, with the exception of the calves in trial 3, which were weaned approximately 3 days prior to arrival. During the comingling phase, calves were fed only the starter ration and given free access to water. Calves in trials 4 and 5 received a series of *M bovis* vaccines[a] prior to arrival at the research center.

Upon arrival, all calves received ceftiofur crystalline free acid[b] (6.6 mg/kg, SC, in the base of the ear). Calves in trials 1 and 2 were housed in open-air, dirt-floor pens with an attached, open-face shed on the north end (total area of each pen, 544 m^2). In trial 3, calves were housed in an open-air, concrete-floor pen with an open-face shed attached to the north end. Calves in the remaining 2 trials (trials 4 and 5) were housed in open-air, dirt-floor pens (total area of each pen, approximately 297 m^2). A starter ration[c] was fed to calves in trials 1 and 2, and an alternative ration[d] was fed to calves in the remaining 3 trials. Free access to water and grass hay was made available to all calves in all trials. The study protocol was approved by the Kansas State University Institutional Animal Care and Use Committee.

M bovis challenge trials

Details of the sample collection procedures, laboratory protocols, and trial procedures used are described elsewhere.[8] In all trials, calves were randomly assigned to an inoculation

method or inoculation dose with the aid of a commercial software program.[e] The *M bovis* organism[f] used for pneumonia induction was the same for every trial. After the *M bovis* challenge, the same trained veterinarian (DEA) observed each calf twice daily and assigned a CIS until the calves were euthanized on the scheduled study completion date (Table A.1). By trial design, calves with a CIS of 4 at any point during the trial were immediately euthanized and a necropsy was performed on the same day.

CIS system

The CIS system used was a modified version of one previously reported.[9] Scores ranged from 1 to 4 as follows: 1 = usual behavior; 2 = slight illness (mild signs of depression or cough); 3 = moderate illness (severe signs of depression, labored breathing, or cough); and 4 = severe illness (moribund or failure to respond to human approach). Descriptions of each score were provided at the bottom of the scoring sheet so observers could reference these criteria when observing calves.

Multiple observer clinical illness scoring

Nine veterinarians with various degrees of experience were involved as observers in the assignment of CISs to calves over the course of the 5 trials. Four observers each had ≥ 14 years of large animal (bovine) clinical and research experience and held a faculty position at the Kansas State University College of Veterinary Medicine. The remaining observers were involved in post-graduate training in clinical or production large animal medicine.

To determine the precision of the CIS system, multiple observers evaluated each calf on the same day, 24 to 48 hours prior to the necropsy date (Table A.1). In each trial, observers were provided with identical scoring sheets and asked to observe each calf and assign a CIS for each calf at each assessment point. All observers entered and exited the pen at approximately the same time (4:30 PM) and were unaware of the other observers' scores. Observers were free to move about the pen to visually inspect all calves.

Necropsy and pulmonary consolidation scoring

Calves in all trials were euthanized at a predetermined point after challenge (Table A.1), in accordance with AVMA euthanasia guidelines regarding use of penetrating captive bolt.[g] Necropsy was performed on all calves, including gross examination of all major organ systems.

The lungs and trachea were removed from each calf for pulmonary scoring, which was based on the percentage of each lung lobe with pneumonic change.[10] Percentage pulmonary consolidation was calculated by the proportion of the lung that a given lung lobe represented, and the percentage consolidation for each lung lobe was multiplied by the fraction each lobe represented of the whole lung. Lobe values were totaled and multiplied by 100 to yield the reported consolidation score. The accuracy of the CIS system relative to pulmonary consolidation scoring was determined by comparing CISs assigned within 48 hours prior to necropsy with the gross pulmonary consolidation scores assigned at necropsy.

Statistical analysis

The precision (or agreement) of CISs among observers was calculated on an individual trial basis and overall (all trials) as the Cohen κ statistic and associated 95% CIs with the aid of statistical software.[h] Observers' scores for each calf were transformed into a dichotomous variable, with 0 representing an apparently healthy state (CIS, 1) and 1 representing a diseased state (CIS > 1) for all κ calculations. The following scale[11] was used to interpret values of $\kappa \leq 0$, poor agreement; 0.01 to 0.20, slight agreement; 0.21 to 0.40, fair agreement; 0.41 to 0.60, moderate agreement; 0.61 to 0.80, substantial agreement; and 0.81 to 1.00, almost perfect agreement.

To evaluate the accuracy of the CIS system versus the pulmonary consolidation system, CISs assigned within 48 hours prior to necropsy were compared with pulmonary consolidation scores obtained at necropsy, with consolidation scores considered to represent a calf's true disease status (ie, the reference standard). Because no standard exists for defining a percentage of pulmonary involvement that would require medical intervention, 6 cutoff values were used, ranging in intervals of 5% from ≥ 5% to ≥ 30%.

Pulmonary consolidation scores for each calf were considered positive (greater than or equal to the cutoff) or negative (less than the cutoff) at each of the 6 cutoff values. A dichotomous variable was created and populated with a value of 1 when a calf's pulmonary consolidation score was greater than or equal to the cutoff, otherwise the variable contained a value of zero. Clinical illness score was treated as a dichotomous variable (healthy vs diseased) as well, as described for precision calculations. For each calf, comparisons were then made of the CIS dichotomous value with each of the pulmonary consolidation cutoffs to determine the

accuracy of the CIS system at each cutoff. A calf with a CIS status of healthy but a pulmonary consolidation status of diseased was considered to have a false-negative result, and one with a CIS status of diseased but a pulmonary consolidation status of non-diseased was considered to have a false-positive result.

Statistical software[i] was used to perform generalized linear mixed modeling (binomial distribution and logit link function) to estimate the probability of misclassification of disease state. The probability of an observer assigning a calf a false-positive or false-negative result was considered the outcome of interest at each cutoff value. Repeated measures on calf and trial were included in each model to account for a lack of independence between samples. The sensitivity of the CIS system was subsequently calculated by subtracting from 1 the probability of an observer falsely scoring a calf as healthy at a given cutoff. Specificity was calculated by subtracting from 1 the probability of an observer falsely scoring a calf as diseased at a given pulmonary consolidation cutoff. Sensitivity and specificity were estimated with data from only trials 2 through 5 because scoring of these calves was done in the afternoon before necropsy, contrary to trial 1, in which scoring was done several days prior to necropsy. Because data calculated for sensitivity and specificity at each cutoff were not normally distributed, median (range) values are reported.

Results

Animals

Clinical illness scores were assigned to 178 calves over 5 trials by 9 observers. The same 4 observers scored calves in all 5 trials, and the remaining 5 observers scored calves in 1 to 3 trials on the basis of their availability. No calf in any trial received a CIS > 3. In trials 2 through 5, 87 of 154 (56%) calves had pulmonary consolidation scored as ≥ 5% and 24 of 154 (16%) received a score ≥ 30% (Figure 3.1).

CIS precision

Precision, or agreement beyond chance, for all 9 observers over all trials was 0.16 (95% CI, 0.10 to 0.24), indicating slight to fair agreement **(**Table 3.1**)**. Accuracy of the CIS system relative to the pulmonary consolidation scoring system was assessed via the calculated sensitivity and specificity at each pulmonary consolidation cutoff. The median (range) calculated sensitivity

for all observers ranged from 81.7% (55.4% to 96.4%) at the ≥ 5% consolidation cutoff to 98.9% (93.9% to 99.8%) at the ≥ 30% consolidation cutoff **(**Figure 3.2**)**. Median (range) specificity also varied by pulmonary consolidation score cutoff and ranged from 94.9% (81.3% to 97.3%) at the ≥ 5% cutoff to 80.8% (48.5% to 93.8%) at the ≥ 30% cutoff **(**Figures 3.3 and 3.4**)**.

Discussion

Nine veterinarians with various backgrounds and experience participated in assessing the precision and accuracy of CISs relative to pulmonary consolidation scores assigned at necropsy in calves inoculated with *M bovis*. Overall, the precision or agreement among observers was poor; accuracy varied by the degree of pulmonary consolidation chosen to identify a truly diseased calf.

In the absence of a gold standard, agreement between observers is used to estimate the precision of a test.[12,13] The interobserver agreement among all observers for all trials in our study was considered slight as defined elsewhere,[11] indicating the repeatability of the CIS evaluated as a diagnostic test was limited among observers. All observers were veterinarians; therefore, they all had prior training that should have biased them toward correctly identifying a calf as clinically ill or not. Our results did not support the hypothesis that these observers scored the calves similarly. No effort was made to train observers on the scoring system, and it is possible that observers with more experience than the others at identifying ill cattle scored the calves on the basis of previous experiences more than on the CIS system.

Multiple observers are often called upon to diagnose illness on farms, and our results suggested that the CIS system lacks precision as a diagnostic test. Improvement of the case definition of a truly ill calf along with training of observers might improve agreement, as was found in a study[14] in which multiple observers evaluated lameness in cattle. Because there were multiple raters per calf and the number of raters was not consistent across trials, no attempt was made to determine whether an individual observer's bias affected κ values through use of bias-adjusted κ values.[7] The κ statistic has been used in veterinary medicine to estimate the agreement between clinicians in relation to lameness[15,16]; however, to our knowledge, interobserver agreement with regard to clinical illness scoring for the diagnosis of induced respiratory disease in calves has not been reported.

Assessment of clinical signs of illness to detect respiratory disease in cattle is generally practiced, but few reports exist to quantify the accuracy of this method versus pulmonary consolidation scoring. Two studies[3,17] revealed that nearly 70% of calves with lesions at slaughter had never received treatment for respiratory disease, suggesting that not all calves with respiratory disease can be identified by clinical signs alone. However, in those studies, the temporal relationship between clinical signs of illness and pulmonary lesions was not known. In another study,[5] the diagnostic sensitivity and specificity of use of signs of clinical illness followed by rectal temperature to diagnose disease in sick cattle were 61.8% and 62.8%, respectively. In our study, at the ≥ 5% cutoff, median sensitivity (81.7%) and specificity (94.9%) were higher than previous estimates, although the observers were all veterinarians and the study population was significantly different from that in the other study[5] in regard to age and breed.

Median sensitivity (81.7%) was lowest at the ≥ 5% cutoff, where the range of values (55.5% to 96.4%) among observers was the largest. A threshold of pulmonary damage at which BRD is subclinical likely exists. Given the large increase in median sensitivity between the 5% (81.7%) and ≥10% (95.1%) cutpoints, several calves made the transition from subclinical to clinical disease and observers were more likely to identify the calves as clinically ill. As the cutoff for pulmonary consolidation became ≥10%, calves were generally more clinically ill. As calves' clinical signs of illness worsened, all observers more accurately identified these calves as diseased, which led to higher sensitivity values with narrower ranges of values among observers. The objective of the study was not to determine a specific cutpoint beyond which treatment is no longer beneficial because none of the calves were treated after pneumonia induction. Instead, an objective was to highlight some challenges inherent to a subjective method of identifying and quantifying disease in calves.

Median specificity (94.9%; range, 81.3% to 97.3%) was highest at the ≥5% cutoff, which indicated that observers less commonly falsely identified calves as having disease, compared with at other cutoff values. One explanation for this is that the lowest cutoff resulted in classification of > 50% of all calves as sick; therefore, fewer healthy calves were available to be falsely identified as disease positive in relation to higher pulmonary consolidation cutoff values. All calves were challenged with *M bovis*, and previous work[8] with this challenge method yielded highly variable degrees of pulmonary consolidation and clinical illness. Therefore,

although observers were aware that calves had been challenged, they had no reason to believe all calves would be ill immediately prior to necropsy.

A tradeoff in sensitivity and specificity was identified as the cutoff for determining a truly diseased calf increased. When deciding on an optimal cutoff for a diagnostic test, consideration should be given to the importance of false-negative and false-positive test results.[7] When the extent of BRD in a herd is measured through clinical observation (as is commonly done in clinical trials), a test with imperfect sensitivity would not identify all truly ill animals. No previous studies have been conducted to specifically evaluate the impact of false negative test results, but several investigators[3,18,19] found a negative association between the presence of pulmonary lesions at slaughter and average daily gain. Conversely, if calves are falsely identified as disease positive by a test with < 100% specificity, then the extent of disease will be overestimated, the effectiveness of preventative treatment will be falsely underestimated, and the effectiveness of disease treatment will be falsely overestimated.

Limitations to the present study existed that weaken its external validity. All calves used were young Holstein bulls inoculated with *M bovis*. Although observers knew that all calves had been exposed to *M bovis* and their scores may have been influenced by this knowledge, not all calves responded similarly and observers did not believe all calves would become sick because of previous work with this challenge model. Observers were aware that some calves in trials 4 and 5 had received prechallenge treatment (vaccination) but were not aware of treatment status for individual calves.

Table 3.1 Median (range) pulmonary consolidation scores and agreement (95% CI) with CISs in calves with experimentally induced *Mycoplasma bovis* pneumonia in 5 trials that differed in dates of score assignment.

Trial	Pulmonary consolidation score (%)	Agreement (κ)
1 (n = 24)	2.4 (0–19.5)	0.16 (0.03–0.43)
2 (n = 42)	6.3 (0–13.4)	0.13 (0.05–0.28)
3 (n = 16)	1.8 (0.2–45.2)	0.21 (0.05–0.47)
4 (n = 43)	6.3 (0.2–47.4)	0.17 (0.05–0.37)
5 (n = 53)	6.1 (0.2–50.3)	0.10 (0.01–0.25)
Overall agreement for all trials	—	0.16 (0.10–0.24)

— = Not applicable.
See Appendix for information on how the 5 trials differed.

Figure 3.1 Percentage of Holstein calves inoculated with *Mycoplasma bovis* that had pulmonary consolidation scores at various cutpoints as determined at necropsy (n = 154). Pulmonary consolidation for each lung lobe was assigned a percentage by a veterinary pathologist. Total pulmonary consolidation scores were calculated on the basis of a reported formula.[10]

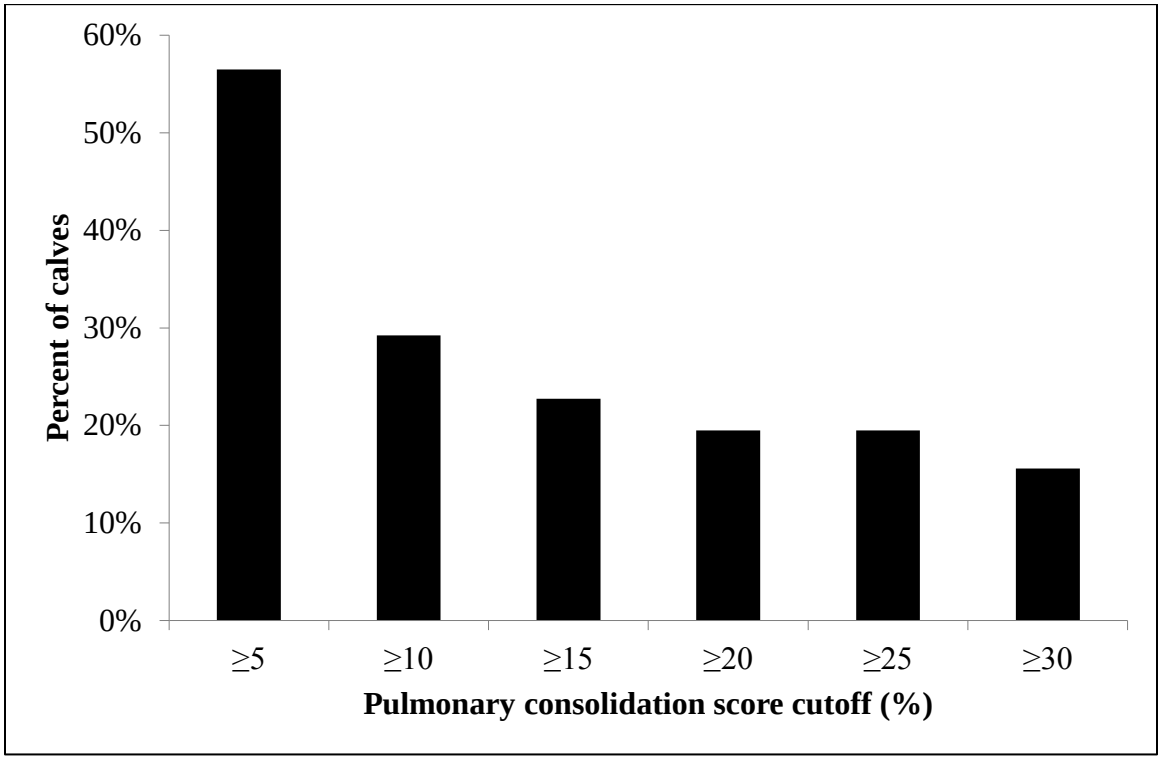


Figure 3.2 Box-and-whisker plots of estimated sensitivity values for CISs assessed in calves (n = 154) by 8 observers at each pulmonary consolidation score cutoff. Boxes represent the 25th and 75th quartiles, and the horizontal line within the boxes represents the median. Whiskers represent the minimum and maximum values. *See* Figure 3.1 for remainder of key.

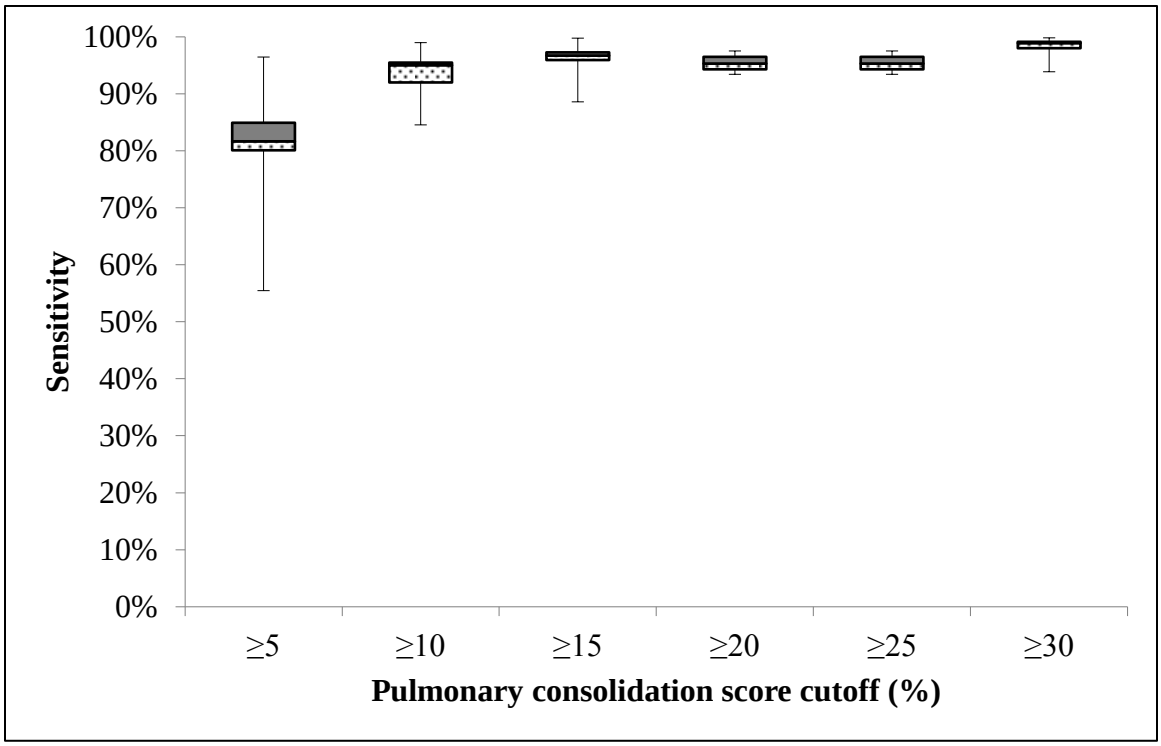


Figure 3.3 Box-and-whisker plots of estimated specificity values for CISs assessed by the observers represented in Figure 3.2. *See* Figures 3.1 and 3.2 for remainder of key

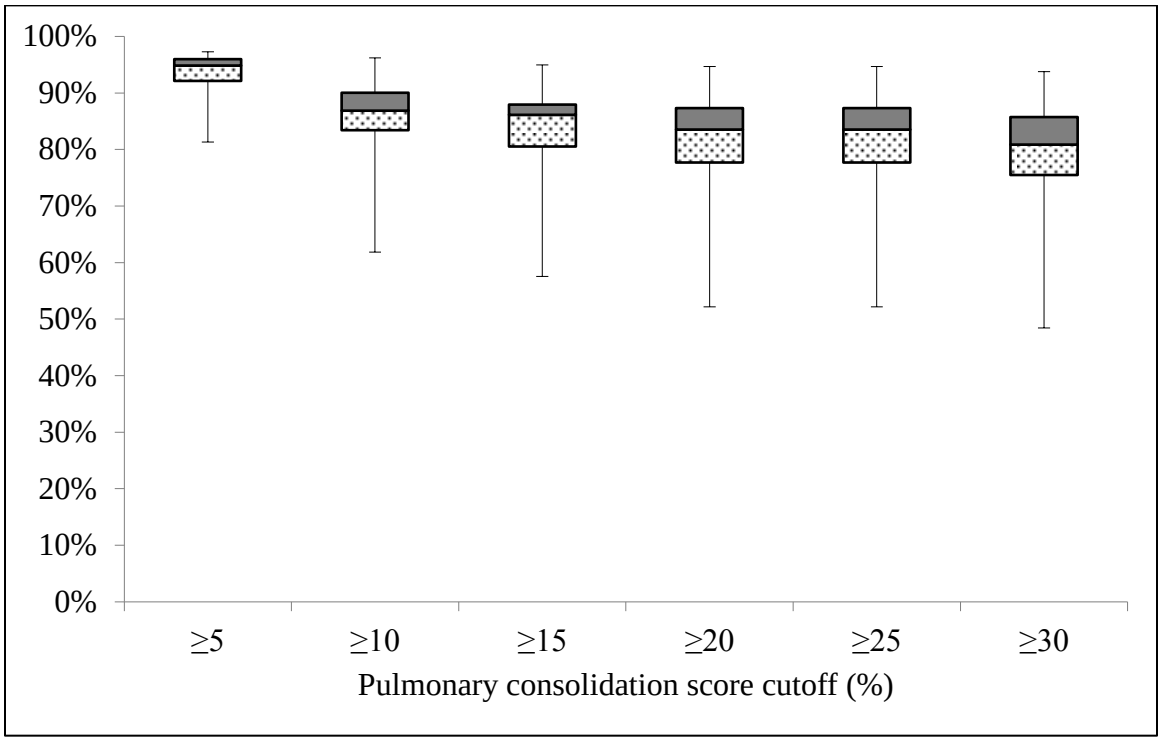
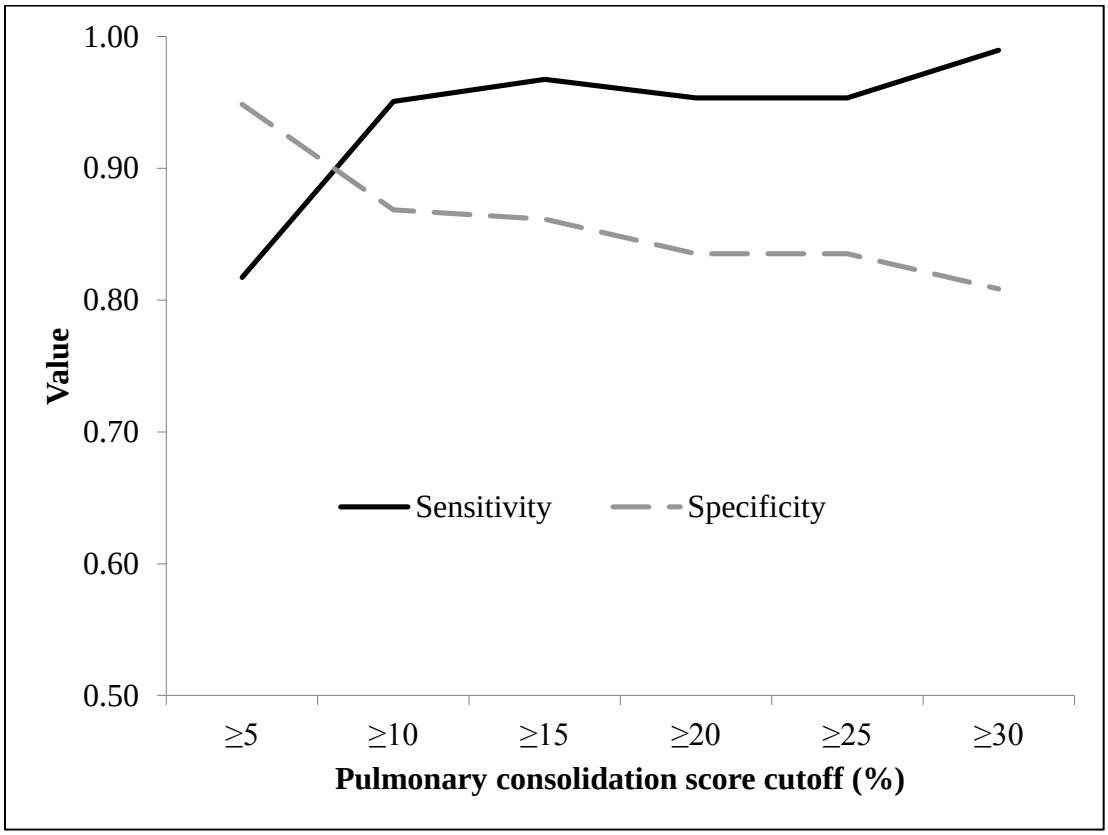


Figure 3.4 Graph of median sensitivity (solid line) and 1 minus median specificity (dashed line) of CISs assigned by the observers in Figure 3.2. *See* Figures 3.1 and 3.2 for remainder of key.



Footnotes

a. CEVA/Biomune, Lenexa, Kan.

b. Excede, Pfizer Animal Health, New York, NY.

c. Herd Maker Supreme B90, Land O' Lakes, Shoreview, Minn.

d. Calf Grower B-68 Medicated, Manhattan, KS Coop, Manhattan, Kan.

e. Excel 2010, Microsoft Corp, Redmond, Wash.

f. Provided by CEVA/Biomune, Lenexa, Kan.

g. Koch Magnum 0.25 Stunner, KOCH Supplies Inc, Kansas City, Mo

h. Stata/MP, version 12, StataCorp LP, College Station, TX.

i. PROC GLIMMIX, SAS, version 9.2, SAS Institute Inc, Cary, NC

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Appendix A - Trial characteristics

Figure A.1 Trial characteristics in a 5-trial study to determine the usefulness of a CIS system for BRD diagnosis in Holstein bull calves inoculated with *Mycoplasma bovis*.

Variable	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
No. of calves	24	42	16	43	53
No. of observers	7	8	7	5	5
Observation day*	6	12	23	11	11
Necropsy day*	14	13 and 14	24	12	13
Time of year	Sept–Dec	Jan–March	June–Aug	June–Aug	June–Sept

*Days are relative to the day on which *M bovis* pneumonia was experimentally induced (day 0).

Chapter 4 - Determination of the potential mitigation of bovine respiratory disease inflammatory response through use of florfenicol and flunixin meglumine.

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Abstract

Bovine respiratory disease (BRD) continues have negative impacts on the production of cattle in the U.S. Once diagnosed as clinically suffering from BRD cattle are frequently treated with an antimicrobial. Treatment of the acute inflammation associate with BRD with non-steroidal anti-inflammatories may decrease some of the pathologic changes associated with BRD. Our goal was to evaluate how calves with experimental bacterial pneumonia respond to early treatment with flunixin meglumine, alone or in combination with the antimicrobial florfenicol. Clinically healthy calves were experimentally inoculated with *Mannheimia haemolytica* and administered their respective treatments eight hours following inoculation. Prior to challenge administration and daily until study termination, calf weights and rectal temperatures were recorded and blood samples were collected. All calves became clinically ill immediately following challenge and those calves necropsied exhibited gross and histopathological signs of pneumonia; however, the variability and extent of lung lesions was

mild in all treatment groups. Changes in serum biomarkers as well as CBC and chemistry parameters were noted but none provided a reliable indicator of the pulmonary inflammation associated with bronchopneumonia. Further studies evaluating potential serum biomarkers and their relationship to pulmonary inflammation are warranted.

Introduction

Bovine respiratory disease (BRD) causes significant economic and production losses in the cattle industry (Smith, 1998; Snowder et al., 2006) and *Mannheimia haemolytica* (MH) is considered to be the predominant bacterial pathogen associated with BRD (Griffin et al., 2010). An infection with bacterial pathogens and the associated inflammation causes long-term deleterious effects; however, early treatment may decrease the adverse impacts. Previous studies have implicated a variety of pro-inflammatory mediators are released in response to bacterial pneumonia and is a major contributor to lung tissue damage associated with BRD (Bannikov et al., 2007; Rice et al., 2007). Some of the pro-inflammatory cytokines released are TNF-alpha, IL-1, IL-6, and, IL-8 (Gifford et al., 2012). Proteinase's are also released from neutrophils and are thought to be associated with breakdown of extracellular matrix and recruitment of neutrophils (Bannikov et al., 2007; Bannikov et al., 2011; Li et al., 1999).

Antimicrobials and anti-inflammatories are available for BRD treatment, but little information exists documenting how early treatment with these agents impacts disease course. To evaluate the efficacy of therapeutic treatments in a field setting, an experimental model replicating infection as it occurs in common production systems is needed to provide a means to reliably evaluate current and forthcoming therapies for BRD. Work at KSU has developed a MH pneumonia induction model that has illustrated changes in concentration of free Haptoglobin, and neutrophil haptoglobin-MMP-9 (Hp)-MMP-9 complexes, TNF-α, cortisol and behavior before induction, after induction and after treatment of induced pneumonia (Theurer et al., 2013). The purpose of this project was to evaluate how calves with bacterial pneumonia, induced using our established model for *Mannheimia haemolytica* pneumonia, respond to early treatment with the anti-inflammatory agent flunixin meglumine, alone or in combination with the antimicrobial florfenicol, in comparison to untreated animals. Specifically, behavior, inflammatory cytokines and biomarkers of pulmonary injury were measured to monitor the acute phase response to infection.

Materials and methods

All study procedures were conducted in accordance with a protocol (#3114) approved by the Kansas State University institutional Animal Care and Use Committee. The study was a blinded, randomized prospective clinical trial to evaluate efficacy of florfenicol and flunixin meglumine as measured by potential differences in markers of inflammation and behavior. Individual calf was the experimental unit. A summary study schedule is presented in Table 4.1. All calves were administered an endoscopic guided bronchoselective MH challenge and then treated 8 hours post challenge based on treatment group. Prior to challenge administration and then once daily, blood, body weight and rectal temperature were collected.

Experimental design

This trial was designed in a 2x2 factorial to evaluate the impacts of flunixin meglumine (Banamine, Schering-Plough, Madision, NJ), florfenicol (Nuflor Gold, Schering-Plough, Madision, NJ), and a combination of the two products (Resflor Gold, Schering-Plough, Madision, NJ) administered to calves induced with MH pneumonia. Calves were randomly assigned to 1 of 4 treatment groups: RES: flunixin meglumine & florfenicol treated (n=10), BAN: flunixin meglumine treated (n=10), NUF: florfenicol treated (n=10), and CNTL: positive controls (n=10). Additionally, 5 calves (Group E) were used as potential replacements for calves that became ill prior to trial initiation (Table 4.2). Calves were monitored for 9 days post pneumonia induction. All calves within the positive control group were euthanized and necropsied upon study termination. Five additional calves from each of the other treatment groups were randomly selected, euthanized and also necropsied upon study termination. Individual calf was the experimental unit and the primary outcomes of interest were physiological and behavior level changes. Secondary outcomes of interest were lung lesions and clinical illness scores (CIS).

Calf housing at KSU

Forty-five Holstein calves arrived at Kansas State University (KSU) 15 days prior to challenge. Forty calves were randomly allocated to 1 of 4 treatment groups, then blocked by treatment group and assigned to 1 of 2 adjacent open-air, dirt-floor pens with a total area of 3200 square feet (297 m^2) per pen. Five calves were held as alternates in the event of illness among study group calves. A starter ration (Calf Grower B-68 Medicated, Manhattan, KS Coop) was

fed for the duration of the trial. The ration contained 14.0% crude protein, 2.0% crude fat, 1.0% calcium, and 0.35% phosphorus. Calves were fed at 2 lbs per head/day for the first three days and then increased to 4 lbs per head/day for the remainder of the trial. Water and grass hay were made available ad libitum. One day after arrival (study day -14) all calves were processed; each calf was weighed, received 2.5mL (200mg/mL) ceftiofur crystalline free acid (Excede, Pfizer Animal Health) SQ in the base of the ear and received a new ear identification tag (Allflex USA, INC., Dallas, TX). Calves were randomly allocated to treatment group and one of two pens so there was an approximately equal number from each treatment group within each pen. No calf had received MH vaccination prior to challenge administration.

Behavioral observations

During processing (study day -14), all calves were equipped with Ubisense tags (Ubisense Series 7000 Compact Tag; Ubisense, Denver, CO) to monitor behavior and activity throughout the remainder of the trial. The animal portion of the system consists of a dual-radio architecture tag that also transmits ultra-wideband radio pulses, which are used to determine the precise location in three dimensions within the containment area. Tags were affixed to a commercial ear tag button placed in the dorsal aspect of the left ear.

Calf observations

Calves were observed by the same blinded veterinarian (DEA) twice daily throughout the study period (morning and evening) for any clinical signs of illness. From arrival to challenge, any calf with an abnormal clinical illness score (CIS > 1) was evaluated by a veterinarian. If treatment was determined necessary, the animal was removed from the other animals, treated and excluded from the trial. Post-challenge, calves were observed twice daily by the same blinded veterinarian and assigned an individual CIS. The scoring system utilized assigned a normal calf as CIS= 1, mild signs of depression +/- cough were assed as CIS= 2, moderate depression +/- cough was assigned CIS= 3 and calves with severe illness were given CIS=4.

Challenge administration

A 110-cm long, 5.9-mm diameter endoscope with a 2-mm diameter biopsy channel was introduced through the nasal passage and subsequently through the glottis into the trachea. The endoscope was passed into the smaller airways until the accessory bronchus was visually

identified. A 195-um diameter polyurethane catheter was passed through the endoscopic biopsy channel and an inoculum containing 5 ml of 1-5 x 10^9 per ml of MH was delivered to calves. The catheter was flushed with 20 ml of Phosphate Buffered Saline (PBS) (HyClone Laboratories, INC., Logan, Utah) before removal from the bronchus of each calf. All personnel who were in daily contact with the animals wore designated coveralls and boots that were only used in the facility.

Treatment administration

Calves were weighed and administered treatment 8 hours post challenge based on previous random group assignment. All calves within the RES group received a subcutaneous injection in the neck at the labeled dose of 40 mg florfenicol/kg body weight and 2.2 mg flunixin/kg body weight. Calves within the NUF group received a subcutaneous injection in the neck at the labeled dose of 40 mg florfenicol/kg body weight. Calves within the BAN group, received an intravenous injection at the labeled dose of 2.2 mg flunixin/kg body weight. Calves within the CNTL group received a subcutaneous injection of 10ml 0.9% sterile saline (Baxter Healthcare Corporation, Deerfield, IL) in the neck. All calves were immediately returned to their assigned pens post challenge administration.

Necropsy

All calves from the CNTL group (n=10) and 5 additional calves from each treatment group (n=15 total) were randomly selected for necropsy and pulmonary evaluation. Euthanasia was conducted following the American Veterinary Medical Association (AVMA) euthanasia guidelines with a penetrating captive bolt (KOCH Supplies Inc., Koch Magnum 0.25TM Stunner, Kansas City, MO). Lungs were removed intact with the trachea and lung lesions were scored using a standardized system (Fajt et al., 2003). A modified scoring system was also developed that focused only on the cranioventral lung lobes, as this is the site of MH selective challenge model and are the predisposed lung lobes, most commonly affected in acute bronchopneumonia. This modified scoring system did not include the right and left diaphragmatic lobes in the calculation of overall lung lesions. Histopathology was performed on lung lesions from each calf. A veterinarian performed a full gross necropsy on each calf to identify any additional abnormalities.

Data collection

Physiological monitoring

Prior to challenge administration and daily until study termination, calf weights and rectal temperatures were recorded (Table 4.3). From d 0 to 9 d post challenge, blood samples were collected once daily between 6 and 8 AM via jugular venipuncture into Vacuette (Greiner Bio-One North America Inc, Monroe, NC) EDTA K2 and serum clot activator tubes. All EDTA tubes were immediately placed on ice. The Veterinary Diagnostic Lab at Kansas State University College of Veterinary Medicine analyzed complete blood counts (CBC), serum biochemistries (CHEM) and total protein and fibrinogen levels. All remaining tubes were centrifuged for 10 min at 1500 x g; serum or plasma was harvested, aliquoted into cryovials, and stored in a minus 62.2 (-80 F) degree Celsius freezer until ready to be analyzed. The ELISAs for bovine Hp and HP-MMP 9 were performed as described previously (Theurer et al., 2013). TNF-alpha concentrations in the serum samples were measured in duplicate using a commercially available bovine TNF-alpha ELISA kit (R & D Systems, Minneapolis, MN, USA). Briefly, 96 well plates (Pierce, Rockford, IL) were coated with the capture antibody and incubated overnight at room temperature. Following the incubation, the wells were washed with wash buffer (0.05% Tween 20 in phosphate buffer saline (PBS). The plates were blocked with 5% Tween 20 in PBS with 0.05% NaN_3 and then incubated for 1hour at room temperature. The wells were washed with washing buffer followed by incubation with samples or standards to each well for 2 hours at room temperature. Subsequently, appropriate detecting antibody was added to each well and incubated at room temperature for 2 hours. After washing with wash buffer, horse radish peroxidase-labeled streptavidin in reagent diluent (pH-7.2-7.4, R & D Systems, Minneapolis, MN, USA) was added and incubated for 20 minutes at room temperature. Substrate solution treatment for 20 minutes (#DY999, R & D Systems, Minneapolis, MN, USA) and, then, addition of stop solution followed this incubation. The absorbance was measured by subtracting reading at 540 nm from the reading at 450 nm.

All serum samples were diluted 1:1 in reagent diluent (pH-7.2-7.4, R & D Systems, Minneapolis, MN, USA) prior to cytokine analysis. A standard curve was generated based on the standards provided in the kit. The concentration of cytokines determined from the standard curve was expressed in pg/ml.

Behavioral data

The real time location (Ubisense) system records an X, Y, and Z position of each tag within a pre-defined area for a given time point. Areas of interest within the pen were the automatic water, feed, hay bunk and shed. Tags were used to map the X and Y coordinates of these areas of interest. Commercial software (SpotFire Miner, Tibco Corporation, Seattle, Washington) was utilized to obtain the location of each calf at a time point in relation to the areas of interest. Given a known XY coordinate for a specific time point, the distance traveled was calculated utilizing Pythagorean theorem. The amount of time each calf spent at the areas of interest was also calculated.

Statistical analysis

Trial data were imported into a commercial statistical software package (JMP and SAS, SAS Inst. Inc., Cary, NC) for descriptive and statistical analyses. The distributions of primary outcome variables (Hp-MMP 9 and Hp) were highly skewed; therefore non-parametric (Kruskal-Wallis) tests were used to evaluate potential differences among all four-treatment groups, and the main effects flunixin meglumine (FLUN) and florfenicol (FLOR) within a study day. Primary outcome variables (Hp-MMP 9 and Hp) were analyzed for interactions among the main effects of FLUN and FLOR. These interactions were not significant; therefore interactions between FLUN and FLOR were not tested among the remaining outcome variables. By trial design, each main effect was evaluated independently and calves that received BAN or RES were evaluated together (FLUN) and the same was true for calves receiving NUF or RES (FLOR). Mean percent lung consolidation among treatment groups was compared using the MULTTEST procedure in SAS with the permutations option. General linear mixed models were used to analyze continuous data for blood parameters, temperatures and weights while accounting for repeated measures on individual calves over time. Fixed effects evaluated in all models included study day and treatment. The interaction of each main effect with study day was included when found to be significant ($P < 0.05$).

Results

During the pre-challenge phase (d -14 to 0), five calves (Calf IDs 6, 13, 18, 33, 41) were removed from study inclusion due to acute onset of natural BRD and replaced with calves out of the control-replacement group (Calf IDs 39, 23, 32, 26, 25).

Clinical illness scores

A total of 40 calves were successfully enrolled in the study (CIS = 1 (normal) at each observation prior to challenge administration). Scores were assigned twice daily (once in the AM and PM) at approximately the same time, from challenge administration through study termination by the same veterinarian (Amrine). A CIS > 1 was considered abnormal and 100% (10/10) of BAN, CNTL and RES calves and 90% (9/10) of NUF calves received an abnormal CIS at least once post challenge. Most (32/40) calves received an abnormal CIS on study d 0, approximately 6 hours post challenge administration (Figure 4.1). Fifteen percent (6/40) of calves received a CIS = 3 and 65% (26/40) received a CIS = 2 on d 0, after MH challenge administration. No calf received a CIS > 2 on the remaining study days.

Necropsy – Lung lesions

Pulmonary lesion scores for each lung lobe were assigned a percentage by a pathologist (DM) (Table B.1). Total percent lung consolidation was calculated based on a formula representing the relative area of each section of lung ranged from 0.4% to 25.3% (Table 4.4). Only 12% (3/25) of calves necropsied had pulmonary consolidation > 20%. Pulmonary consolidation for calves in the CNTL group (n=10) ranged from 1.7% to 25.3%. In the other three treatment groups, consolidation scores ranged from (0.6% to 24.7%), (0.4% to 20.9%) and (2.2% to 9.6%) for BAN, NUF and RES calves, respectively. There were no differences ($P > 0.10$) in percent lung consolidation among treatment groups. Total cranioventral lung consolidation ranged from 1.2% to 65.3% (Table 4.4). Median cranioventral lung consolidation ranged from 11.5% for the CNTL group to 15.2% for the RES group. Using only the cranioventral lobes provided greater sensitivity for detecting differences in percent consolidation, yet the percent lung consolidation was not statistically different ($P > 0.10$) among treatment groups. Percent pulmonary consolidation was not a primary outcome; therefore, each statistical analysis is based on very low power, and limited conclusions can be drawn.

Histopathology was completed on representative samples from each set of lungs. General gross and histopathologic morphologic features were described for each calf (Tables C.1, C.2). There were no clear trends observed in histopathologic morphologic criteria based on treatment status (Figure 4.2).

Hp / Hp-MMP 9

Analysis within study day among main effects revealed no significant ($P < 0.05$) differences for Hp-MMP 9 complexes or Hp. Median levels of Hp-MMP 9 complexes were higher on d 2 compared to all other days. Haptoglobin levels were highly variable, and remained elevated longer than Hp-MMP 9 levels (Figure 4.3). Both Hp and Hp-MMP 9 complexes were elevated on d 1 as compared to d 0.

The primary objective of the current study was to evaluate effects of FLOR, FLUN, and the combination of the two (Resflor) on calves with acute pulmonary inflammation as gauged by levels of Hp and Hp-MMP 9. By trial design the interaction of FLOR and FLUN was initially evaluated for the primary variables (Hp and Hp-MMP 9). This interaction was not significant for either primary variable; therefore, the main effects of treatment with FLOR or FLUN could be evaluated independently and the interaction of FLOR and FLUN was not tested in any subsequent analyses.

Weights

Calves were weighed during processing (d -14), immediately prior to challenge administration, prior to treatment administration and once daily post challenge. The mean (± SD) weight upon arrival was 59.7 kg ± 7.14 for all calves. Prior to challenge administration (d 0) there were no differences ($P = 0.6$) among treatment group mean weights. Average weights (± SD) prior to challenge administration were 73.7 kg ± 12.4 and 72.2 kg ± 9.1 for NUF and RES calves and 69.4 kg ± 4.6 and 69.9 kg ± 5.0 for BAN and CNTL groups respectively. Average daily gain (ADG) was calculated by subtracting the initial weight (d -14) from the final weight (d 9) and dividing by the number of days on feed at KSU (23 d). Model adjusted ADG for the main effects of FLUN or FLOR showed calves receiving FLUN either alone or in combination with FLOR had significantly ($P < 0.05$) lower ADG over the 23 d study period compared to those who did not receive FLUN. Calves receiving FLOR via Resflor Gold or Nuflor Gold had a significantly ($P < 0.05$) higher ADG than those not receiving FLOR (Figure 4.4).

Rectal temperature

The interaction of FLUN and study day was significant ($P < 0.05$) indicating the effect of FLUN on rectal temperature varied by study day (Figure 4.5). On d 0, prior to challenge administration, rectal temperatures ranged from 38.9 to 39.5 °C for all treatment groups. The

interaction of FLOR and study day was not significant, however; model estimated least squares mean temperatures over the 10 d study period were higher ($P < 0.05$) for calves that did not receive FLOR compared to those that did.

Tumor necrosis factor-α (TNF- α)

Evaluation of TNF-α values by the main effects (FLOR and FLUN) within study day revealed no differences ($P < 0.05$). Values of TNF-α were fairly similar among treatment groups within study days and there was an overall trend for treatments to have greater variability starting on day 5 until the end of the study compared with the first 5 days of the study (Figure 4.6).

Hematologic analysis

Results for levels of leukocytes, segmented neutrophils, basophils and sodium exhibited minimal variability (most readings were the same); therefore, models would not converge for these parameters. All remaining variables were tested for relationships with main effects (FLUN and FLOR) and potential interactions between main effects and study day. If the interaction was not significant, only main effects were kept in the models. Those variables that varied significantly ($P < 0.05$) by study day are listed in Table B.2. Briefly, the effect of FLUN on band cell concentration, mean corpuscular hemoglobin (MCH), plasma protein and fibrinogen all varied by study day. On d 1 (approximately 12 hours post treatment), band cells were higher ($P < 0.05$) for calves having received FLUN compared to those who did not (Figure 4.7).

The effect of FLUN on potassium, the sodium:potassium ratio and urea nitrogen varied by study day. Creatinine globulins, phosphorus, bicarbonate, alkaline phosphatase (ALP) and sorbitol dehydrogenase (SH) all varied by FLOR treatment status and study day. Glucose concentrations were lower ($P < 0.05$) in calves receiving FLUN compared to those who did not, although both values were above Kansas State University diagnostic lab published reference ranges. Albumin concentrations were higher ($P < 0.05$) in calves that received FLUN compared to calves that did not, although these values were still within the reference range.

Evaluation of the erythrogram values revealed calves receiving FLUN had lower red blood cell (RBC) concentrations, hemoglobin and hematocrit (calculated and spun %) values compared to calves who did not receive FLUN. Eosinophil concentrations were higher ($P < 0.05$) in calves receiving FLOR compared to those who did not and cortisol concentrations were lower ($P < 0.05$) in calves receiving FLOR compared to those who did not.

Behavior monitoring

Three hours of day three were excluded from analysis due to calves being placed in the wrong pens after morning processing and because behavior data is aggregated by day for analysis, d 3 was then excluded from the analysis due to the missing hours. Distance traveled and time spent within 1 foot (0.3m) of areas of interest (hay bunk, grain bunk and automatic waterer) were evaluated (Table 4.5). There were no significant ($P < 0.05$) differences found among treatment groups. A calf's baseline value for time spent daily at areas of interest and daily distance traveled was determined by obtaining the average value for each calf for days -13 through day -1. The percent change from baseline was evaluated for the main effects of FLUN and FLOR. Compared to baseline values, calves that received FLUN had a decrease in the daily amount of time spent at the water, while calves not receiving FLUN increased their time spent at the water (Table 4.5).

Discussion

This study evaluated how calves challenged with MH respond to early treatment with flunixin meglumine, alone or in combination with florfenicol. Several different physiologic parameters along with changes in calf behavior were evaluated during this trial. Most of the calves in the current study became clinically ill following MH challenge administration; however, the level of pulmonary consolidation detected at necropsy was minimal in many calves. The endoscopic challenge model resulted in lung lesions in all four treatment groups, with the majority of lesions occurring in the right apical cranial and caudal lobes. The variability in lung lesions in calves that were challenged and did not receive treatment (CON group) indicates calves responded differently to challenge administration. The challenge model resulted in clinical disease, but the variability and extent of pulmonary lesions limited the ability to draw broad inferences.

Bacterial pneumonia in cattle leads to pulmonary inflammation that can lead to matrix metalloproteinase (MMP) release. The release of these MMPs has been associated with lung tissue damage. (Starr et al., 2004) Others have proposed using concentrations of these MMPs bound to Hp as a test to discriminate between acute neutrophil activation and chronic inflammatory disease. (Bannikov et al., 2011) In the present study, a primary outcome was the measure of inflammatory response to BRD; however, the large variability in Hp-MMP 9

complexes and Hp revealed no differences in these inflammatory biomarkers among treatment groups within our study. Others have found differences in the concentrations of Hp and Hp-MMP 9 post MH challenge (Theurer et al., 2013); however, the importance of these differences is not well understood. The severity of challenge in our study may have influenced the levels of these inflammatory markers, and further studies are warranted to determine the usefulness of these serum biomarkers.

The challenge model resulted in a variable response among individual calves as measured by level of lung consolidation that ranged from 0.4% to 25.4% in all calves necropsied. In calves receiving no treatment (CON), 6/10 had 5% or less lung consolidation levels; therefore, the challenge may not have been robust enough in some calves to stimulate a strong inflammatory response regardless of treatment group. Previous studies using the same or very similar challenge methods have been successful in inducing pulmonary lesions; however, the overall volumes of inoculant and PBS were greater in their study compared to ours. Others have also noted that lesions resulting from experimental MH inoculation began to resolve on d 5 post-challenge. (Hanzlicek et al., 2010) In our study, lungs were scored d 9 post-challenge and it appears many of the lesions were still relatively active; therefore resolving lesions were not the most likely cause of the relatively low lung scores in our study.

There were no interactions identified between FLOR and FLUN related to Hp or Hp-MMP 9 values allowing the evaluation of all other variables with the benefits of the 2x2 design (NUF and RES evaluated as FLOR; BAN and RES evaluated as FLUN). Despite the mild challenge, some differences between treatment groups were identified in the physiological, hematologic, and behavioral parameters. Administration of FLUN either in combination with nuflor (Resflor Gold) or independently caused a decrease in rectal temperature one day post treatment administration, however this effect was transient and temperatures appeared to rebound the following day. The shorter-term effect is to be expected, as the half-life of flunixin is shorter than study duration. These results agree with previous studies evaluating pyrexia in naturally occurring BRD cases that were treated with an antibiotic or a combination of an antibiotic and NSAID. (Lockwood et al., 2003)

The effect of FLUN on band neutrophils, plasma protein, and fibrinogen varied by study day. On d 2, calves that received FLUN had higher ($P < 0.05$) concentrations of band cells compared to those that did not receive FLUN; however, differences were not observed on any

other study days. Others have observed a mild increases (within reference ranges) in band cell concentrations post challenge when no treatments were administered. (Hanzlicek et al., 2010)

Several of the measured chemistry values were also affected by study day and treatment status; however, there were no clear trends that indicated one treatment group was superior to the others. Overall the usefulness of CBC and chemistry values as reliable indicators of acute inflammation in our study was marginal and may have been limited by the mild challenge; however, others also have found that CBC and chemistry values are not reliable indicators of acute pulmonary inflammation. (Hanzlicek et al., 2010)

Calves will frequently change their behavior when ill and several methods to quantify these changes have been proposed. (Weary, 2009) Behavior of calves following experimental challenge with *Mycoplasma bovis* has been associated with severity of pulmonary disease. (White et al., 2012) In our study, calves receiving FLUN showed a decrease from baseline in the percent of time spent within 1m of the automatic waterer compared to calves not receiving FLUN. The significance of this finding is unknown since no other behavioral changes were noted among groups. Associations between distance traveled and extent of lung lesions at necropsy has been observed by previously. (White et al., 2012) The lack of distinction in behavioral changes observed in the current study may be related to the mild challenge.

Conclusion

The present study evaluated changes in physiologic, hematologic, and behavior of calves following challenge with MH and treatment with an antimicrobial or the combination of antimicrobial and NSAID. While most calves became clinically ill (CIS > 1) immediately following challenge the impact was transient as calves were clinically normal by d 2. Challenge resulted in lung lesions in all four-treatment groups; however, lesions were mild and therefore limited the ability to make inferences as to treatment success or failure and the reliability of serum biomarkers in relation to disease status.

Table 4.1 Timeline summary of study events

Event	Study day
Calf arrival at KSU	-15
Calf processing	-14
MH challenge	0
Treatment administration	0+8 hrs
Physiological and behavior monitoring	1 - 9
Euthanize and necropsy	9

Table 4.2 Trial design and number of calves by treatment group[1]

		florfenicol	
		+	-
flunixin	+	(n=10) RES	(n=10) BAN
	-	(n=10) NUF	(n=10) CNTL

Group E (n=5) will be used as replacements for any sick calves in groups A – D, prior to challenge administration

[1] BAN = flunixin meglumine, CNTL = Saline control, NUF = florfenicol, RES = florfenicol + flunixin meglumine

Table 4.3 Physiological monitoring sample collection timeline

Study day	-14	0	1	2	3	4	5	6	7	8	9
Event	■	❖ ■	❖ ■	○ ■	❖ ■	○ ■	❖ ■	○ ■	❖ ■	○ ■	❖ ■

■ Body weight recorded
❖ CBC, Chemistry (including Fibrinogen), TNF-α, cortisol, Hp-MMP 9, Hp, Rectal temp
○ Total Protein & Fibrinogen, TNF-α, cortisol, Hp-MMP 9, Hp, Rectal temp

Table 4.4 Descriptive full and cranio-ventral lung consolidation statistics by treatment group[1]

	Full % lung consolidation by treatment				Cranio-ventral % lung consolidation by treatment			
	BAN	NUF	RES	CNTL	BAN	NUF	RES	CNTL
Average lung score	8.8	7.4	5.7	7	23.7	20.8	15.6	19.4
Median	5.8	3.4	5.7	4.5	15	12.1	15.2	11.5
Standard deviation	9.8	8.8	3.1	7.5	25.7	22.8	9.2	18.8
Minimum	0.6	0.4	2.2	1.7	1.8	1.2	6.6	4.3
Maximum	24.7	20.9	9.6	25.3	65.3	53.1	30.2	61

[1] BAN = flunixin meglumine, CNTL = Saline control, NUF = florfenicol, RES = florfenicol + flunixin meglumine

Table 4.5 Model estimated least square means percent of day and percent change from baseline time calves spent within 1m of the hay bunk, grain bunk and water by treatment group

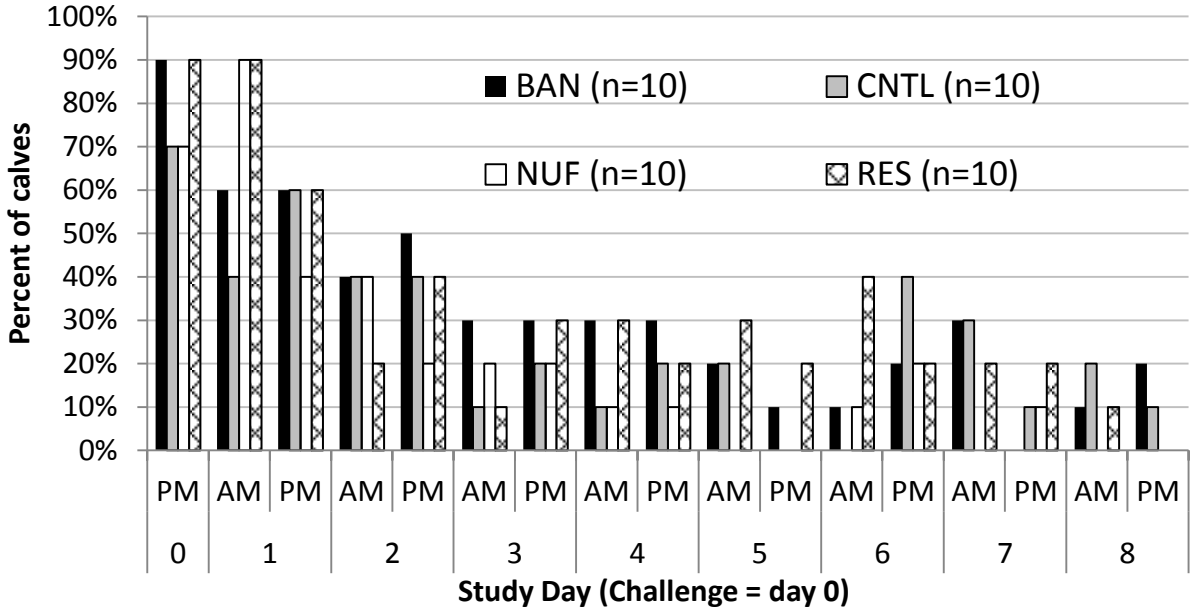
	Flunixin meglumine					Florfenicol				
% of day spent at	+	**SEM**	-	**SEM**	**p-Value**	+	**SEM**	-	**SEM**	**p-Value**
Hay (within 1 m)	12.35	0.82	11.89	0.82	0.49	11.55	0.82	12.68	0.81	0.10
Grain (within 1m)	3.52	0.37	3.50	0.37	0.95	3.29	0.37	3.73	0.37	0.13
Water (within 1m)	1.04	0.21	1.21	0.21	0.05	1.13	0.21	1.12	0.21	0.93
Distance traveled (m)	4725.23	380.37	4527.96	380.37	0.34	4672.93	380.89	4580.26	380.20	0.65

	Flunixin meglumine					Florfenicol				
% change from baseline[1]	+	**SEM**	-	**SEM**	**p-Value**	+	**SEM**	-	**SEM**	**p-Value**
Hay (within 1 m)	42.61	6.03	45.19	6.03	0.76	46.27	6.03	41.52	6.03	0.58
Grain (within 1m)	-55.34	2.72	-56.33	2.72	0.80	-57.16	2.72	-54.50	2.72	0.49
Water (within 1m)	-22.50	18.13	4.40	18.13	0.0077*	-7.64	18.15	-10.42	18.12	0.77
Distance traveled	3.24	4.61	-2.17	4.61	0.12	0.99	4.63	0.07	4.61	0.79

[1]baseline is the average value of days -13 to -1

* indicates significant at the 5% level

Figure 4.1 Percentage of calves receiving abnormal clinical illness scores (CIS > 1) for each study day between *Mannheimia haemolytica* challenge and study termination by treatment[1]



[1] BAN = flunixin meglumine, CNTL = Saline control, NUF = florfenicol, RES = florfenicol + flunixin meglumine

Figure 4.2 Number of calves by treatment status by histologic morphologic criteria

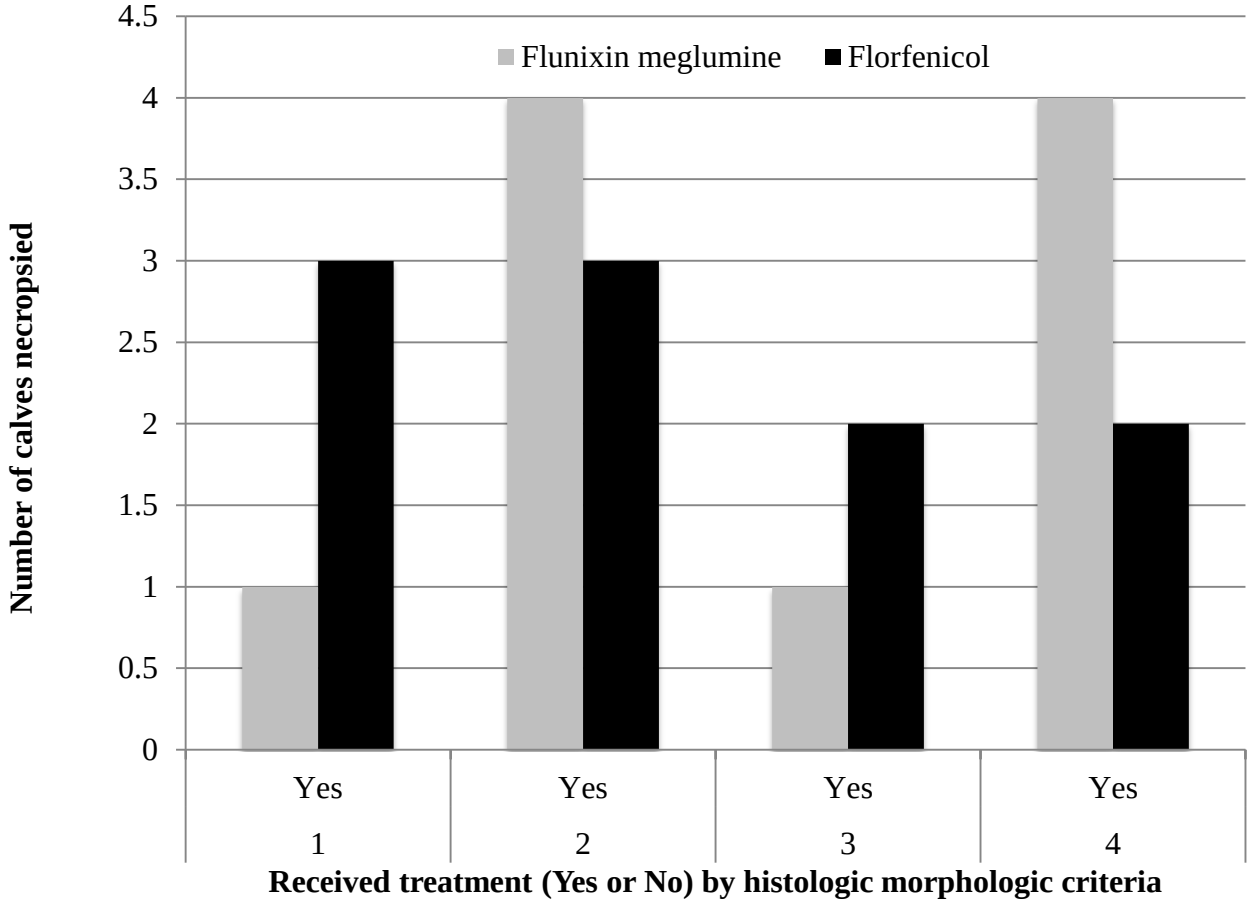
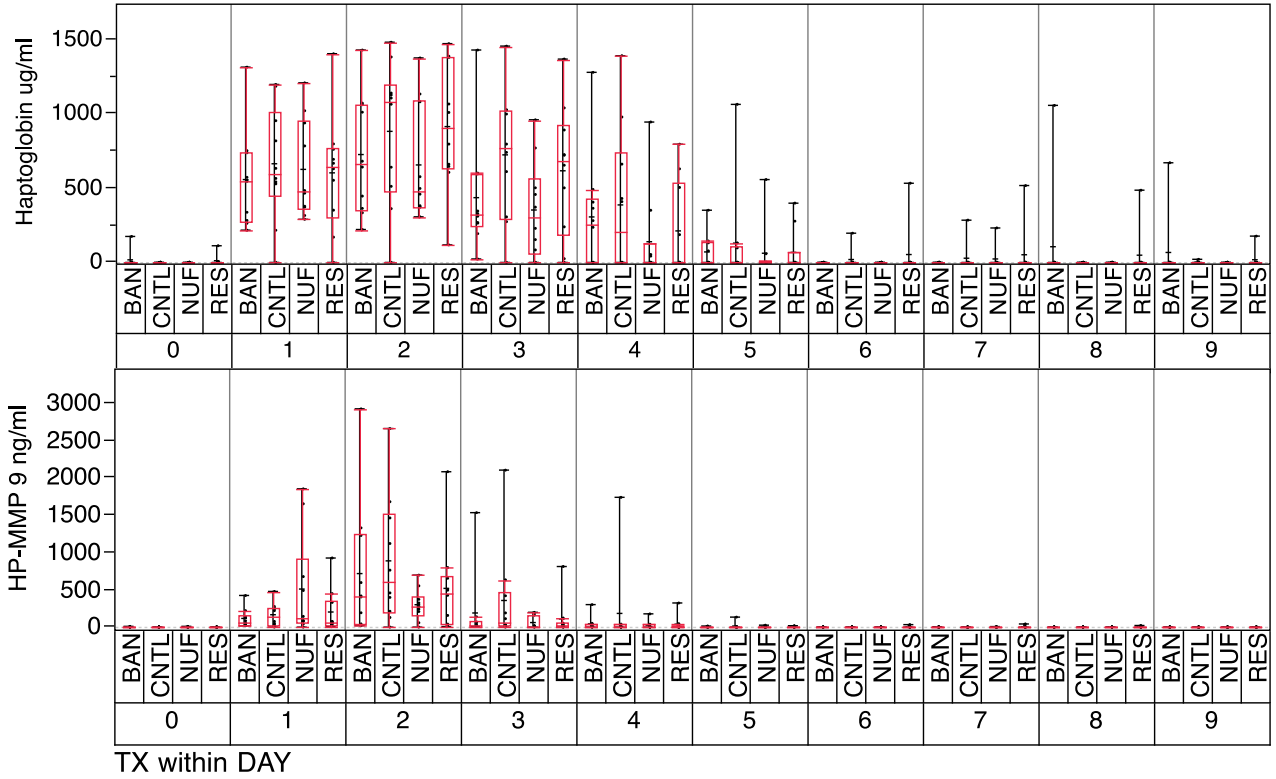


Figure 4.3 Variability of Hp and Hp-MMP 9 complexes by treatment group[1] within study day



[1] BAN = flunixin meglumine, CNTL = Saline control, NUF = florfenicol, RES = florfenicol + flunixin meglumine

Figure 4.4 Least square means average daily gain comparing main effects of calves receiving flunixin meglumine or florfenicol or not

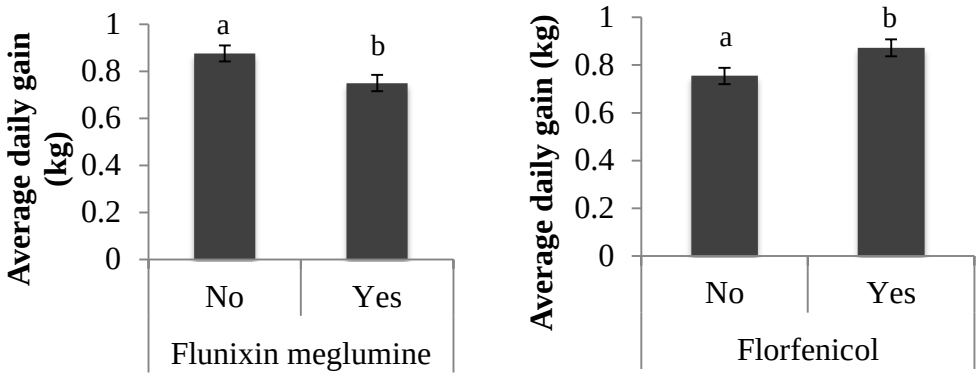


Figure 4.5 Least square means rectal temperatures in calves receiving flunixin meglumine compared to those that did not receive flunixin meglumine by study day

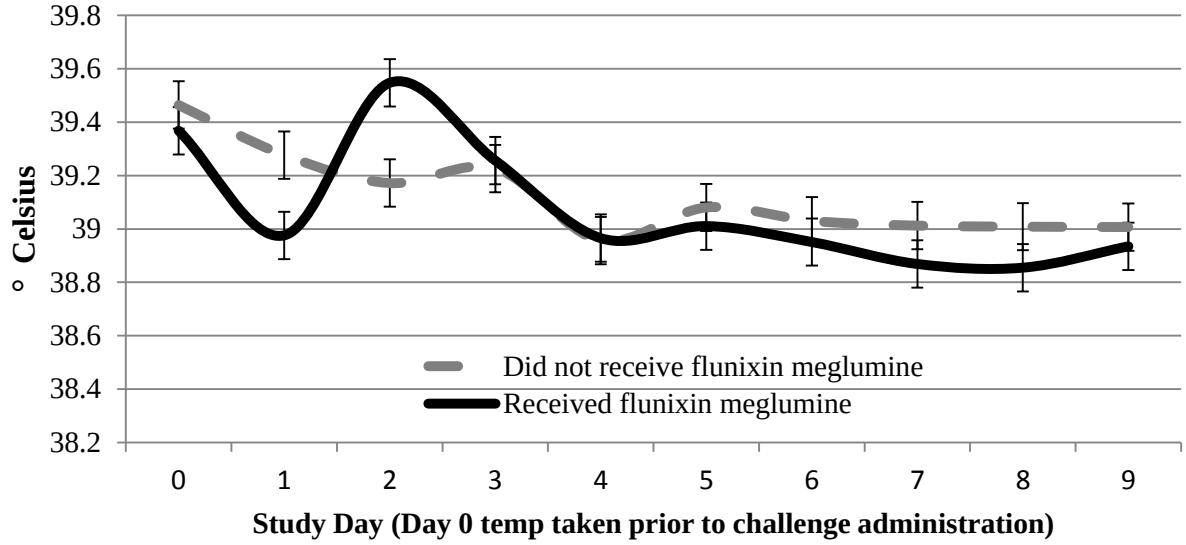
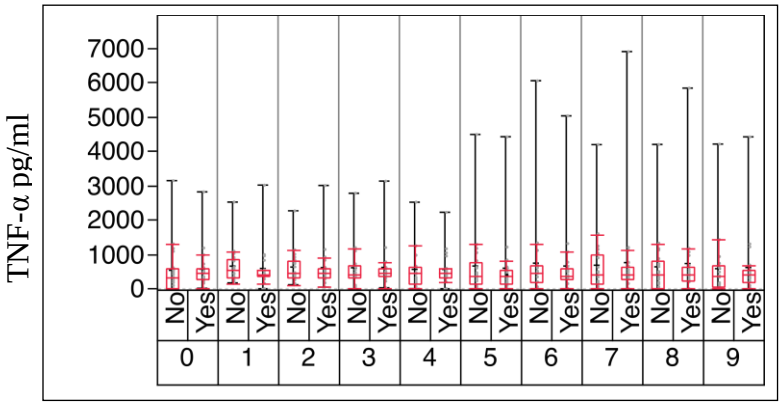
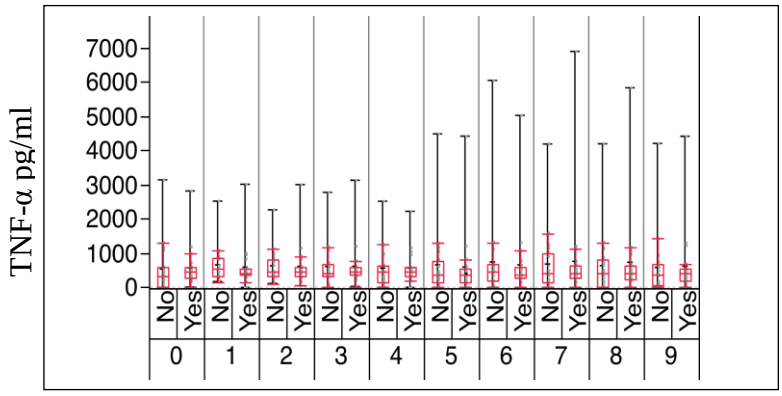


Figure 4.6 Variability of Tumor Necrosis Factor alpha (TNF- α) within treatment by study day

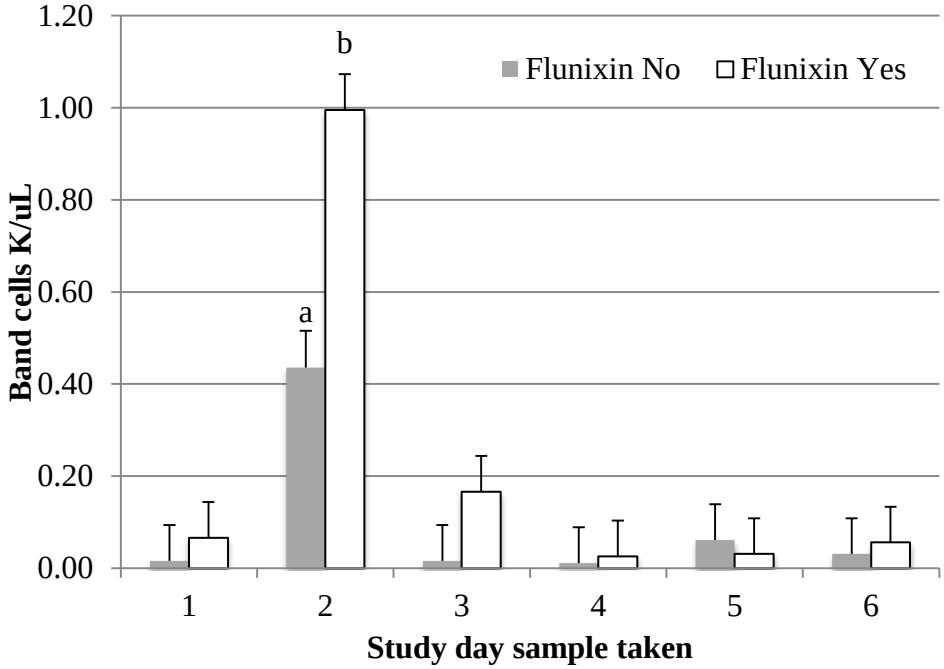


Flunixin meglumine treatment by study



Florfenicol treatment by study day

Figure 4.7 Least square means band cell concentrations by study day and treatment with flunixin meglumine



Appendix B - Lung lesion scores and hematologic analysis

Table B.1 Lung lesion scores as a percentage of each lobe and total lung volume[1]

Calf_ID	TX	LA1	LA2	LD	RA1	RA2	RC	I	RD	LS	CV-LS
1	BAN	80	90	10	80	90	40	0	0	24.7%	65.3%
9	BAN	0	0	0	7	90	0	0	0	5.8%	15.0%
16	BAN	0	0	0	7	0	20	3	0	1.8%	5.9%
19	BAN	0	0	0	90	80	10	0	0	11.1%	30.7%
21	BAN	0	0	0	10	0	0	0	0	0.6%	1.8%
10	NUF	0	0	0	10	0	0	0	0	0.6%	1.8%
14	NUF	50	60	10	90	70	30	10	0	20.9%	53.1%
17	NUF	0	20	0	0	0	40	0	0	3.4%	12.1%
32	NUF	0	0	0	5	2	0	0	0	0.4%	1.2%
40	NUF	3	7	0	30	60	80	20	0	11.7%	35.7%
2	RES	0	0	0	5	80	10	0	0	5.7%	15.2%
15	RES	0	0	0	50	0	0	0	0	3.2%	9.1%
34	RES	0	0	0	80	3	70	3	0	9.6%	30.2%
37	RES	10	7	0	7	7	7	0	0	2.2%	6.6%
39	RES	10	20	7	40	0	20	3	0	7.6%	17.0%
7	CNTL	3	30	0	0	70	5	0	0	6.1%	17.6%
8	CNTL	70	80	0	20	0	0	0	0	8.9%	28.8%
11	CNTL	10	40	0	0	15	10	0	0	4.0%	13.2%
22	CNTL	0	10	0	7	10	10	0	0	2.1%	6.7%
29	CNTL	3	3	0	10	10	0	0	0	1.5%	4.3%
31	CNTL	0	0	0	3	7	10	10	0	1.6%	4.9%
35	CNTL	70	90	10	50	80	60	0	7	25.4%	61.0%
38	CNTL	0	20	0	7	10	0	0	0	2.0%	6.4%
42	CNTL	0	40	7	3	3	7	0	0	5.0%	9.8%
45	CNTL	0	10	0	70	40	90	10	0	13.2%	40.9%

[1]Each score is the percent of lung lobe with lesions. Lobe Nomenclature: LA1 = left apical cranial, LA2 = left apical caudal, LD = left diaphragmatic, RA1 = right apical cranial, RA2 = right apical caudal, RC = right cardiac, RD = right diaphragmatic, I = intermediate (accessory)

Table B.2 Least square means CBC, chemistry and cortisol values by treatment administration

CBC Variable	Reference Values	Flunixin meglumine		p-Value	Florfenicol		p-Value
		+	-		+	-	
Band cells	0 - 2 K/uL	§	§	§	0.16	0.15	0.79
Lymphocytes	2.5 - 7.5 K/uL	4.55	4.67	0.63	4.78	4.43	0.19
Monocytes	0.025 - 0.85 K/uL	0.76	0.76	0.98	0.74	0.79	0.56
Eosinophils	0 - 1.6 K/uL	0.04	0.04	0.96	0.05	0.03	0.0466*
RBC's	6 - 12 M/uL	9.49	9.95	0.07	9.67	9.77	0.69
Platelets	100 - 800 K/uL	707.52	653.30	0.51	693.59	667.23	0.16
Hemoglobin	8 - 15 g/dL	10.69	11.25	0.0064*	10.87	11.06	0.34
HCT (calculated %)	24 - 46 %	30.82	32.26	0.0164*	31.20	31.88	0.25
Hematocrit (spun %)	26 - 42 %	33.25	34.97	0.0101*	33.68	34.54	0.19
MCV	40 - 65 fL	32.58	32.58	1.00	32.43	32.72	0.67
MCH	14 - 19 pg	§	§	N/A	11.34	11.41	0.76
MCHC	30 - 36 g/dL	34.85	34.92	0.71	34.92	34.85	0.74
Plasma protein	6 - 9 g/dL	§	§	N/A	7.18	7.07	0.39
Fibrinogen	300 - 700 mg/dL	§	§	N/A	622.33	650.50	0.44

Chemistry Variable	Reference Values	Flunixin meglumine		p-Value	Florfenicol		p-Value
		+	-		+	-	
Glucose	29 - 73 mg/dL	76.27	83.36	0.0067*	81.72	77.91	0.16
Urea Nitrogen	9 - 24 mg/dL	§	§	N/A	§	§	N/A
Creatinine	0.5 - 1.6 mg/dL	0.60	0.57	0.24	§	§	N/A
Protein	6 - 9 g/dL	6.44	6.56	0.45	§	§	N/A
Albumin	3.1 - 4.3 g/dL	3.11	3.26	0.0421*	3.22	3.16	0.40
Gloublin, calculated	No Ref Range	3.33	3.30	0.88	§	§	N/A
Phosphorus	4.9 - 9 mg/dL	6.25	6.90	0.12	§	§	N/A
Potassium	4.2 - 6.3 mmol/L	§	§	N/A	5.02	4.93	0.21
HCO_3	21 - 31 mmol/L	27.51	27.35	0.64	§	§	N/A
Anion GAP	No Ref Range	20.47	20.87	0.12	20.83	20.50	0.21
Na:K Ratio	No Ref Range	§	§	N/A	27.56	28.04	0.25
ALP	20 - 76 U/L	228.95	243.56	0.45	§	§	N/A
GGT	10 - 39 U/L	19.15	17.81	0.59	18.96	18.00	0.70
Sorbitol Dehydrogenase	6.1 - 18.4	31.03	31.48	0.97	§	§	N/A
CK	159 - 332 U/L	200.88	228.01	0.09	18.60	18.49	0.82
Cortisol	No Ref Range	27.42	27.37	0.62	24.85	28.95	0.0428*

* indicates significant at the 5% level

§ The interaction of variable and Study day was significant - see figures Appendix B

N/A = not applicable

Appendix C - Gross and histological alterations

Summary

Gross abnormalities in these calves ranged from mild to severe, and were characterized by atelectasis, parenchymal necrosis (sequestra), nodules/abscesses, and fibrosis. Table C.1 indicates the categories calves were placed in using broad morphologic criteria.

Table C.1 Lung lesion gross abnormalities by morphology criteria

Category*	Morphology	Calf numbers
1	-minimal scattered atelectasis -Mild fibrous adhesions	10, 22, 29, 32, 37, 38, 42
2	-A few small nodules comprising <10% of affected lobe -Variable atelectasis	8, 11, 16, 17, 21, 31, 34, 39
3	-Multiple nodules comprising >50% of affected lobe -Variable atelectasis	7, 14, 15
4	-Multiple nodules comprising >50% of affected lobe -Sequestra -Variable atelectasis	1, 2, 9, 19, 35, 40, 45

*Categories generally run from least severe (1) to most severe (4)

Two to three histological sections were viewed for each calf; each intended to be representative of the major gross lesion in the calf. Lack of a certain morphologic feature on the histological sections does not mean that the feature was absent from the calf. Most histological sections shared common features. All sections had variable amounts of normal tissue and atelectasis. There were peribronchial/ peribronchiolar lymphoid aggregates in all but 4 calves. There were abscesses or sequestra in 13/25 calves. Abscesses were typically smaller and oriented either in parenchyma or small airways. Sequestra were generally larger and retained recognizable remnants of pulmonary architecture. Fibrosis was prominent in 12/25 calves, and involved both the parenchyma and interlobular septae/pleura. Inflammation in all cases was characterized predominately by macrophages with fewer neutrophils. Bronchiolitis obliterans and

bronchiectasis were seen in 4 calves. Prominent histological features are categorized in Table C.2.

Table C.2 Lung histological morphological features and calves assigned to those categories

Morphological feature	Calf numbers
Minimal lymphoid aggregates	2, 9, 32, 37
Minimal fibrosis	8, 10, 11, 14, 17, 22, 29, 32, 34, 37, 38, 39, 42
No abscesses/Sequestra	10, 11, 14, 17, 22, 29, 32, 34, 37, 38, 39, 42
Bronchiolitis obliterans/bronchiectasis	8, 14, 40, 45

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Chapter 5 - Pulmonary lesions and clinical disease response to *Mannheimia haemolytica* challenge 10 days following administration of tildipirosin or tulathromycin.

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Abstract

This randomized, blinded, controlled clinical trial evaluated the impact of metaphylactic antimicrobial administration 10 days prior to experimental inoculation with *Mannheimia haemolytica* (MH) to mitigate pulmonary lesions. Thirty-three crossbreed heifers were procured as a single group and randomly allocated to one of three replicates and to treatment: tildipirosin (ZUP; 4 mg/kg) or tulathromycin (DRX; 2.5 mg/kg) or saline (SAL; 1 mL/45.5 kg) within replicate upon arrival to Kansas State University. All trial procedures were staggered by 7 d intervals for each replicate resulting in all animals within a replicate receiving treatment, challenge, and necropsy on the same dates. Calves within each replicate received an endoscopic MH challenge 10 d following treatment administration (d 0) and were housed in individual indoor stalls for 3 days post-challenge. Clinical illness scores (CIS), respiration quality scores, appetite scores and injection site reactions were recorded on all animals from d 0 through d 13. Rectal temperatures were measured once daily on all animals from d 8 through d 13. Calves were necropsied and lung lesions were evaluated on d 13. Lung lesion data were evaluated using nonparametric methods (Kruskall-Wallis) and generalized linear mixed models were used to evaluate the remaining variables. The pulmonary lesion scores (percentage of affected lung) ranged from 3.3% to 39.8% for all calves with 92% (11/12) of ZUP treated calves having < 10% lesions. Zuprevo treated calves had lower ($P < 0.05$) lung lesion scores when compared with DRX or SAL treated calves. Lung weight expressed as a percentage of BW was lower ($P < 0.05$) in ZUP calves compared to DRX and SAL treated calves. The probability of calves

receiving abnormal CIS, appetite scores and respiratory scores was lower ($P < 0.05$) in ZUP treated calves compared to DRX and SAL treated animals. This study showed that calves treated with tildipirosin 10 days prior to MH challenge have less pulmonary damage and fewer clinical signs of illness compared to calves treated with DRX or SAL.

Introduction

Bovine respiratory disease (**BRD**) causes significant economic and production losses in the cattle industry (Smith, 1998). *Mannheimia haemolytica* (**MH**) is considered the predominant bacterial pathogen associated with BRD and is often responsible for the acute inflammation, hemorrhage, and lysis of leukocytes and platelets that results in the fibrinous bronchopneumonia commonly associated with BRD mortalities (Griffin et al., 2010). Operations receiving calves considered at high risk to develop BRD frequently use immunizations and prophylactic antimicrobial therapy to manage this risk (Nickell and White, 2010; Thomson and White, 2006). Antimicrobials administered on arrival have shown positive impacts on animal health, performance, and economics (Booker et al., 2007; Booker et al., 2006; Corbin et al., 2009). However, relatively little research exists documenting the response of calves receiving metaphylaxis, and a subsequent challenge with MH ten days after metaphylactic treatment. Newer macrolide antimicrobials such as tulathromycin (Draxxin, Zoetis) and tildipirosin (Zuprevo, Merck Animal Health) have gained favor for metaphylaxis in part due to their ability to concentrate in pulmonary tissue, their spectrum of activity, and extended efficacy (Apley, 2006; Evans, 2005; Kilgore et al., 2005; Menge et al., 2012).

The primary objective of this study was to evaluate the long-term efficacy of two antimicrobials on mitigation of pulmonary lesions compared to negative (saline) controls in calves challenged with MH 10-days post-treatment. Secondary objectives were to evaluate the impacts of the two antimicrobials on measures of animal health (clinical disease, appetite, and respiratory scores) and performance.

Materials and methods

This trial was conducted at Kansas State University College of Veterinary Medicine Large Animal Research Center (**KSU-LARC**) in accordance with protocols approved by the Kansas State University Institutional Animal Care and Use Committee.

Experimental design

Thirty-eight crossbreed heifers (178 ± 19.7 kg) were acquired from a local sale barn and transported together to KSU-LARC 15 d prior to the initiation of the experiment. A summary study schedule that was followed for each of the 3 replicates is presented in Table 5.1. At processing, one day after arrival, all calves were assigned a random number using Excel's

random number generator (Microsoft Excel, Redmond, WA). The eleven calves with the lowest random numbers were assigned to replicate one, then next eleven to replicate two and the next eleven to replicate three. Calves within a replicate were randomly assigned to one of three treatment groups: Zuprevo (Tildipirosin, Merck Animal Health; **ZUP,** n=4), Draxxin (Tulathromycin, Pfizer Animal Health; **DRX**, n=4) and negative saline controls (0.9% NaCl, Baxter Healthcare Corporation, Deerfield, IL, **SAL** n=3). The five remaining calves were randomly assigned to a replicate and served as replacements for any calves that became ill prior to treatment administration within their assigned replicate (based on random number assignment). One day after arrival calves in all three replicates were processed; each calf was weighed, received 6.0 mL (200 mg/mL) ceftiofur crystalline free acid (Excede, Zoetis) SQ in the base of the ear and received a new ear identification tag (Allflex USA, Inc., Dallas, TX). All calves within each replicate were assigned to one of three open-air, dirt-floor pens with a total area of 297 m^2/pen. For each replicate, calves from all experimental treatment groups were housed in the same pen from arrival to experimental challenge (d 10 for each replicate). Following MH challenge, calves within each replicate were individually housed in stalls inside the KSU-LARC that prevented calf-to-calf contacts. Throughout the trial calves were fed a diet that met NRC requirements for their weight and age. All calves were started on a diet consisting of primarily cracked corn, soybean hulls and oat grain at 0.45 kg/(animal•d^{-1}) for the first three d and then increased to 2.27 kg per animal/day over the course of one week and remained at this rate for the remainder of the trial. Grass hay was made available ad libitum until study d 4, at which time chopped prairie hay 1.81 kg/(animal•d^{-1}) was mixed with the ration and ad libitum hay was discontinued. The transition to chopped hay was done to facilitate feeding and weigh backs once calves were moved inside to individual stalls post-challenge (d 10). Water was available ad libitum. Treatment, challenge administration and necropsy for each replicate occurred 7 d following that same event for the previous replicate.

Behavioral observations

During post-arrival processing all calves were equipped with ultra wide-band real time location system monitoring tags (Ubisense Series 7000 Compact Tag; Ubisense, Denver, CO) to monitor behavior and activity throughout the trial. The animal portion of the system consists of a dual-radio architecture tag that transmits ultra-wideband radio pulses, which are used to

determine the precise location in three dimensions within the containment area. Tags were affixed to a commercial ear tag button placed in the dorsal aspect of the right ear. Data were collected using methods described previously (White et al., 2012).

Calf observations

The same blinded veterinarian observed calves twice daily throughout the study (morning and evening) for any signs of illness. During the arrival-to-treatment administration period, any calf receiving an abnormal clinical illness score (CIS > 0) was individually evaluated by a veterinarian. If treatment was determined necessary prior to administration of the experimental treatments, the animal was treated and excluded from the trial. From d 0 to d 13 calves were observed twice daily by the same blinded veterinarian and assigned an individual CIS, appetite score, quality of respiration score, and monitored for injection site reactions. The CIS criteria were 0 = normal calf, 1 = mild signs of depression, 2 = moderate depression, 3 = severe depression and 4 = severe prostration/recumbence (Perino and Apley, 1998). Calf appetite at the time of feeding (twice daily) was assigned 0 for normal appetite, 1 for slightly restricted, 2 for restricted appetite and 3 for no appetite. The quality of a calf's respiration was visually observed from a distance and scored as 0 for normal, 1 for slight dyspnea, 2 for moderate dyspnea and 3 for severe dyspnea. Injection sites were also monitored twice daily and assigned 0 for no visible reaction or 1 for a noticeable reaction.

Study treatment administration

Study treatments were administered to each replicate (d 0 for each replicate) a minimum of 14 days after arrival processing. Calves were weighed and administered treatment based on previous group assignment by a veterinarian (RL) not participating in the calf observations. Calves within the ZUP group received a subcutaneous injection in the neck at the labeled dose of 4 mg tildipirosin/kg BW. Calves within the TUL group received a subcutaneous injection in the neck at the labeled dose of 2.5 mg tulathromycin/kg BW and calves within the SAL group, received a subcutaneous injection in the neck of 0.9% saline at 1 mL/45.5 kg BW. All calves were immediately returned to their assigned pen post treatment administration.

Challenge administration

Mannheimia haemolytica isolated from a field case of BRD was administered endoscopically to all calves within a replicate on study d 10. This strain was previously found susceptible to macrolides based on antibiotic sensitivity performed at Kansas State Veterinary Diagnostic Laboratory. Bacterial preparation procedures have been described previously (Hanzlicek et al., 2010). A 110-cm long, 5.9 mm endoscope with 2mm biopsy channel was introduced through the nasal passage and subsequently through the glottis into the trachea. The endoscope was passed into the smaller airways until the accessory bronchus was visually identified. A 195-μm diameter polyurethane catheter was passed through the endoscopic biopsy channel and an inoculum containing 10 ml of 1-5 x 10^9 per ml of MH was delivered into the accessory bronchus. The catheter was flushed with 60 ml of sterile PBS before removal from the bronchus of each calf. After challenge, all personnel who were in daily contact with the animals wore designated coveralls and boots that were only used in the facility.

Rectal temperatures

Rectal temperatures were recorded once daily on each animal at approximately the same time every day (8 to 9 am) from d 8 to d 13 for each calf within a replicate. This procedure was repeated for each replicate.

Feed intake

Each morning during the post-challenge phase, an individual calf's previous day's remaining feed was weighed and recorded. Feed consumed was calculated by subtracting that morning's feed remaining from the previous day's feed delivered. The percent of feed consumed based on each animal's body weight recorded on d 10 was calculated and used in analysis.

Necropsy

Three days post-challenge for each replicate (study d 13) calves were euthanized following the American Veterinary Medical Association (**AVMA**) euthanasia guidelines using a penetrating captive bolt (Accles & Shelvoke Ltd, CASH Dispatch Kit, England). Lungs were removed intact and the trachea was transected cranial to the right accessory bronchus. All remnants of the aorta, esophagus and connective tissue were removed from the lungs prior to obtaining individual lung weights. Lung lesions were evaluated and scored by a board certified

veterinary pathologist (DM) using a standardized system (Fajt et al., 2003). A modified scoring system was also developed that focused only on the cranioventral lung lobes, the most common site of the experimental lesion. The total cranioventral percentage lung consolidation was calculated as: (0.152 * cranial segment of left cranial lobe %) + (0.182 * caudal segment of left cranial lobe %) + (0.12 * accessory lobe %) + (0.212 * right middle lobe %) + (0.182 * cranial segment of right cranial lobe %) + (0.152 * caudal segment of right cranial lobe %). Representative lung samples were collected from each calf, immediately placed in a Whirl-Pak bag (Nasco, Fort Atkinson, Wisconsin) and submitted to the Kansas State Veterinary Diagnostic Lab for *Mannheimia* sp. isolation. A veterinarian performed a full gross necropsy on each calf to identify any additional abnormalities.

Statistical analyses

Trial data were imported into a commercial statistical software packages (JMP, SAS Institute, Cary, NC) for descriptive and statistical analyses. The experimental unit for all analysis was the individual animal. The distribution of the primary outcome variable (percent lung lesions) was tested for normality using the Shapiro-Wilk Goodness-of-Fit test and rejected ($P < 0.001$); therefore non-parametric tests (Kruskal-Wallis with the Steel-Dwass multiple comparison method) were used to evaluate potential differences among treatment groups. Standard least squares models were used to evaluate potential differences among treatment groups within time periods of interest (d 0 to 10 and d 10 to 13) for other continuous variables (lung weight as percent of BW, BW, rectal temperatures). For ordinal variables, the GLIMMIX procedure (SAS 9.3, SAS Institute, Cary, NC) with a logit link function was used to evaluate potential difference among treatment groups within time periods of interest (d 0 to 10 and d 10 to 13). All models accounted for repeated measures on individual animals and study days within replicates. The hierarchical structure of our data was accounted for by including random effects for the individual calves and the repeated measurements of calves within replicates. Each time period of interest (d 0 to 10 and d 10 to 13) was analyzed separately. The level of significance was $\alpha = 0.05$.

Results

At arrival, calves had mean body weights of 187.8 kg (range: 135 to 206 kg), 175.8 kg (range: 144 to 202), and 174.3 kg (range: 143 to 206 kg), for replicates 1, 2, and 3, respectively.

During the pre-treatment phase, 4 calves were removed from the study due to clinical signs of respiratory disease, including 2 in replicate 1 (d -4 and -1) and 2 in replicate 2 (d -1 and 0). Removed calves were replaced with one of the five extras that were assigned to that specific replicate. One calf (# 35) in replicate 1 (SAL group) exhibited signs of severe BRD (CIS = 4, non-ambulatory) on study day 12 and was humanely euthanized. Data from this animal was included in lung lesion and clinical observation analyses, and was removed from BW analyses for the d 10 to d 13 period.

Lung lesions and weight

Total lung lesion scores ranged from 3.3% to 39.8% for all calves (Table 5.2). Lesion scores in ZUP treated calves ranged from 3.3% to 39.1%, with 92% (11/12) of calves having < 10% lesions (Fig. 5.1). Lesion scores in DRX treated calves ranged from 3.8% to 27.8% with 50% (6/12) having < 10% lesions. Calves receiving only saline (SAL) had lesion scores ranging from 12.8% to 39.8% with 0% having lesions < 10% and 66% (6/9) having lesions > 15%. Total cranioventral lung scores (calculated using all lobes except the right and left dipraghmatic) ranged from 9.4% to 92.7% (Table 5.2). Median cranioventral lung scores ranged from 16.4% for the ZUP group to 61.2% for calves in the SAL group. Total lung lesion scores and cranioventral lesions were lower ($P < 0.05$) in ZUP calves compared to calves in both the DRX and SAL groups. Total lung lesion scores were lower ($P < 0.05$) for DRX treated calves compared to SAL calves; however, there was no difference ($P = 0.545$) between DRX and SAL treated calves for cranioventral lesion scores. Calves in the ZUP group had lower model estimated lung weights when analyzed as a percent of calf total BW (Fig. 5.2).

Body weights

Calves were weighed during processing, and on days 0, 10 and 13. The mean (± SD) weight at processing was 178 ± 19.7 kg for all calves. There were no differences ($P > 0.50$) in weights by treatment group at processing, d 0, d 10, or d 13. All calves lost weight from days 0-13, calves in the SAL group lost more than ZUP or DRX treated calves (Table 5.3).

Clinical illness scores

All calves included in the study had CIS = 0 at each observation prior to study treatment administration (day 0) and during the treatment to challenge period (days 0 to 10). Calves were

observed twice daily from days 0-10. The distribution of calves receiving abnormal CIS (CIS > 0) post challenge is presented in Fig. 5.3. One hundred percent (9/9) of calves in the SAL group received abnormal CIS on study day 12. Calf #35 in the SAL treatment group of replicate 1, received a CIS = 4 on the PM observation of study day 12 and was humanely euthanized. No other calves in the study received a CIS > 3. For the post-challenge period, the probability (± SE) of calves receiving an abnormal CIS was higher ($P < 0.05$) in SAL treated calves (63% ± 8.8) compared to calves in the DRX group (38% ± 8.8) and both groups had a higher probability than the ZUP calves (22% ± 6.9). There were no differences in probability of an abnormal CIS during the study treatment-to-challenge period.

Appetite scores

The distribution of abnormal appetite scores (score > 0) by study day is shown in figure 5.4. There were no differences detected in the probability of receiving an abnormal appetite score among treatment groups during the study treatment-to-challenge period. For the post-challenge period, the probability (± SE) of calves receiving an abnormal appetite score was higher ($P < 0.05$) in both SAL calves (53% ± 7.1) and DRX calves (36% ± 6.0) compared to calves in the ZUP group (9% ± 3.6). There was no difference in probability of abnormal appetite scores between SAL and DRX treated calves ($P = 0.08$).

Respiration scores

All calves received normal respiration scores (score = 0) during the post treatment period (prior to challenge administration). Figure 5.5 shows the percent of calves that received abnormal respiration scores following challenge administration. For the post-challenge period, the probability (± SE) of calves receiving an abnormal respiration score was higher ($P < 0.05$) in SAL calves (22% ± 7.6) compared to calves in the ZUP group (2.2% ± 1.7) and the DRX group (8% ± 3.8). Probabilities were not different ($P = 0.12$) between ZUP and DRX treated calves.

Injection site reactions

Injection sites were monitored for all calves from the day of treatment until study termination. No calf, in any treatment group had visible signs of injection site reaction at any period during the study.

Rectal temperatures

Rectal temperatures were measured daily on all calves within a replicate from study d 8 (2 days prior to challenge administration) through study termination (study d 13). Although the interaction of treatment and study day was not significant ($P > 0.10$), temperatures varied by study day ($P < 0.01$) and treatment ($P < 0.01$) during both the study treatment-to-challenge period (d 8-10) and post-challenge administration (d 11-13) (Fig. 5.6).

Feed intake

There was no difference ($P > 0.10$) in the amount of feed consumed as a percent of body weight between ZUP and DRX treated groups, however, ZUP treated calves consumed more ($P < 0.05$) than SAL calves. There was no difference ($P > 0.10$) between DRX and SAL treated calves (Fig. 5.7). The interaction of treatment and study day was found to be non-significant ($P > 0.10$).

Bacteriology

Mannheimia sp. was recovered in 100% of both DRX and SAL treated calves and 25% (3/12) of ZUP treated calves.

Behavior monitoring

Distance traveled and time spent at areas of interest were evaluated among treatment groups during the treatment-to-challenge time period. While all variables measured were modified by study day, there was no effect of treatment on distance traveled and time spent at areas of interest as presented in Table 5.4.

Discussion

Results of this study indicate that calves receiving metaphylactic treatment with ZUP 10 d before experimental MH challenge had lower pulmonary lesion scores compared to DRX or SAL treated calves. Pulmonary lesions following induced or naturally occurring BRD are commonly used to evaluate disease progression and the efficacy of therapeutic or biologic interventions (Amrine et al., 2013; Fajt et al., 2003; Hanzlicek et al., 2010; White et al., 2012). Previous studies have evaluated the dynamics of tildipirosin in bovine lungs and bronchial fluid (Menge et al., 2012). However, to our knowledge this is the first study evaluating the impact of

tildipirosin administered 10 days prior to challenge on pulmonary lesions, a situation analogous to a pen outbreak in animals that were metaphylactically treated on arrival. The characteristics of commingling, transport, and diet changes commonly associated with beef cattle raised in the U.S. make it highly probable that animals will be exposed to many of the various BRD-associated pathogens and provide many opportunities for these pathogens to invade the lower respiratory tract (Nickell and White, 2010). Metaphylactic antimicrobial administration upon feedlot arrival is frequently used to manage the risk of BRD within populations of cattle, and has been associated with reduced morbidity (Frank et al., 2002; Step et al., 2007). Researchers classified temporal patterns of BRD cases during the first 100 days on feed for over 7000 cohorts of cattle representing over 1.2 million individual feeder cattle and determined there were 7 common temporal patterns of BRD among these animals. In 4 of the 7 temporal distribution patterns, the cumulative percentage of BRD cases was < 25% at d 10 (Babcock et al., 2010). These data indicate it is not uncommon for 75% of the total BRD-associated morbidity within a cohort of cattle to occur after 10 days on feed. Cattle treated with a long-acting antimicrobial on arrival can still experience a pen-outbreak of BRD as the antimicrobial concentration decreases over time.

The impact of BRD on individual animals' lungs can vary based on the severity of infection and the host immune response. The presence of bacteria in the pulmonary tissue causes recruitment of pro-inflammatory cells and mediators that lead to tissue damage, accumulation of fluid, and in later stages, consolidation and tissue necrosis (Griffin et al., 2010). These BRD associated alterations in the pulmonary parenchyma result in damaged lungs that weigh more than those from healthy cattle. We evaluated lung weight as a percent of the animals BW and found ZUP animals had lower weights in comparison to DRX or SAL animals. This difference may be associated with the ability of tildipirosin to concentrate in lung tissue and bronchial fluid (Menge et al., 2012). However, calves administered tulathromycin have also been shown to have high drug concentrations in pulmonary tissues (Evans, 2005). One limitation of the method we used is the ability to standardize each set of lungs by transecting the trachea at the same location and removal of all extraneous tissue. In our study the trachea was transected proximal to the right tracheal bronchus and every effort was made to remove all extra tissues, thus leaving only the lungs and associated airways to measure. By having the same blinded veterinarian prepare and weigh lungs from all calves we attempted to minimize any bias associated with the process.

In general, lung weight as a percent of BW appears to provide a quantitative method to analyze the impact of BRD in groups of cattle.

Visual appraisal of cattle is the primary diagnostic method used to identify animals suffering from respiratory disease. Depression, nasal discharge, coughing, decreased rumen fill and elevated respiratory rates have all been used to evaluate animals for respiratory disease (Perino and Apley, 1998; Thomson and White, 2006). Our study evaluated clinical signs of illness, appetite, quality of respiration, and injection site reactions for all animals from treatment administration (d 0) through necropsy (d 13). The probability of abnormal clinical illness scores, quality of respiration scores, and appetite scores was consistently lower for ZUP calves compared to DRX and SAL calves. While others found clinical signs of illness to be lower in calves treated with Draxxin compared to negative saline controls (Godinho et al., 2005), the differences in their study populations (dairy calves vs. beef heifers) and experimental challenge agent (*Mycoplasma bovis* vs. MH) limit potential comparisons among the two studies. In our study, post-challenge administration, ZUP calves had lower ($P < 0.05$) probabilities of receiving abnormal scores in all categories, which may indicate those animals felt better post- challenge and were more likely to consume more feed than sick herd mates. Caution should be used when using subjective measures of animal health as studies have shown these evaluations are not always accurate indicators of health status (Amrine et al., 2013; White and Renter, 2009; Wittum et al., 1996).

Once animals identified as potentially suffering from BRD are identified, the decision to treat is commonly based on the animal's rectal temperature in relation to a predefined cutoff. Calves in the present study had rectal temperatures recorded once daily from d 8 through d 13 and temperatures varied by study day and treatment. Following challenge administration, ZUP calves had lower average rectal temperatures compared to all other calves. No differences in rectal temperatures measured post-challenge were identified between DRX and SAL calves. In a previous multi-site natural BRD exposure study, calves treated with Draxxin had lower ($P < 0.05$) rectal temperatures 2 d post treatment compared to saline treated negative controls (Kilgore et al., 2005). Since rectal temperature is frequently used along with clinical appraisal as an indicator of disease, the ability of an antimicrobial to decrease pyrexia is commonly associated with increased efficacy. In this study, Zuprevo was able to decrease pyrexia associated with BRD when compared to Draxxin and saline.

Cattle suffering from BRD frequently have reduced feed intake and the number of treatments for BRD have been associated with decreased ADG (Cernicchiaro et al., 2013). Our study evaluated feed intake for the 3 d post-challenge period, and found no difference between ZUP and DRX calves and no difference between DRX and SAL calves. ZUP calves however, did consume more than SAL calves during these 3 study days. During the treatment-to-challenge phase (d 0 to d 10) animals within a replicate were housed and fed together in a common grain bunk. Following challenge administration the acclimation of animals to individual stalls and feeding may have resulted in alterations in individual animal consumption; therefore, results of differences in feed intake should be evaluated within these constraints.

The objective of our study was to evaluate pulmonary lesions between calves administered metaphylactic treatment 10 d prior to experimental induction of MH pneumonia. The experimental challenge with MH was successful in inducing pneumonia as evident from the pulmonary changes, clinical, respiratory and appetite scores of the saline treated calves compared to the other two groups (ZUP and DRX). ZUP calves 10 d prior to challenge administration had lower pulmonary lesion scores, lung weight as a percent of BW, and lower probabilities of abnormal clinical, appetite and respiratory scores compared to DRX and SAL calves.

Table 5.1 Timeline summary of study events for each replicate

Event[1]	Replicate 1	Replicate 2	Replicate 3	Month of year
Processing	-14	-21	-28	September
Treatment administration	0	0	0	October
Challenge administration	10	10	10	Oct. – Nov.
Necropsy	13	13	13	Oct. – Nov.

[1] All calves arrived at KSU on the same day and were all processed one day following arrival. Events within each replicate were 7 d following that same event for the previous replicate.

Table 5.2 Descriptive full and cranioventral lung lesion statistics by treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL)

	Full % lung lesions			Cranioventral % lung lesions		
Pulmonary lesion score	ZUP	DRX	SAL	ZUP	DRX	SAL
Mean	8.7	13.1	25.5	21.4	33.4	57.4
Median	5.9	9.9	25.1	16.4	27.9	61.2
Standard deviation	9.8	8.6	11.2	18.4	18.9	23.8
Minimum	3.3	3.8	12.8	9.4	10.9	25.2
Maximum	39.1	27.8	39.8	77.8	68.6	92.7

Table 5.3 Model estimated mean (SEM) daily gain (kg) by study period and treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL)

	Treatment-to-challenge (d 0 -10)		Post-challenge (d 11-13)	
Treatment	Mean	SEM	Mean	SEM
ZUP (n=12)	-0.27[a]	0.148	-1.53[a]	0.752
DRX (n=12)	-0.43[a]	0.148	-1.4[a]	0.752
SAL (n=9)	-1.12[b]	0.180	-1.69[a]	0.821

[a,b] Within a column, means with different superscripts are different ($P < 0.05$)

Table 5.4 Model estimated mean percent of day calves spent within 1 m of the hay bunk, grain bunk, automatic waterer and average daily distance traveled by treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL)

	ZUP (n=12)		DRX (n=12)		SAL (n=9)		
% of day spent with 1m	LSM	SEM	LSM	SEM	LSM	SEM	*P*-value[1]
Hay bunk	30.1	3.3	20.6	3.4	29	3.9	0.114
Grain bunk	7.9	1.1	9.2	1.1	8.1	1.3	0.687
Water	2.1	0.3	2.5	0.3	1.9	0.4	0.379
Distance traveled (m)	5521	366	5412	366	5890	423	0.682

[1] Probability corresponding to the hypothesis of no difference among treatment groups

Figure 5.1 Box and whisker plots showing the variability of full percent lung lesions by treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL). Boxes represent the 25th and 75th quartiles and whiskers extend from the ends of the box to the outermost data point that falls within 1.5 times the interquartile range.

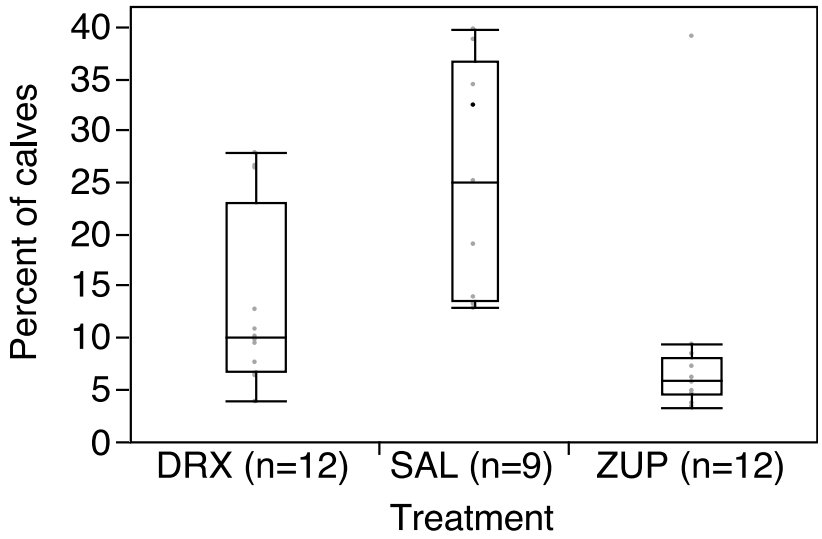
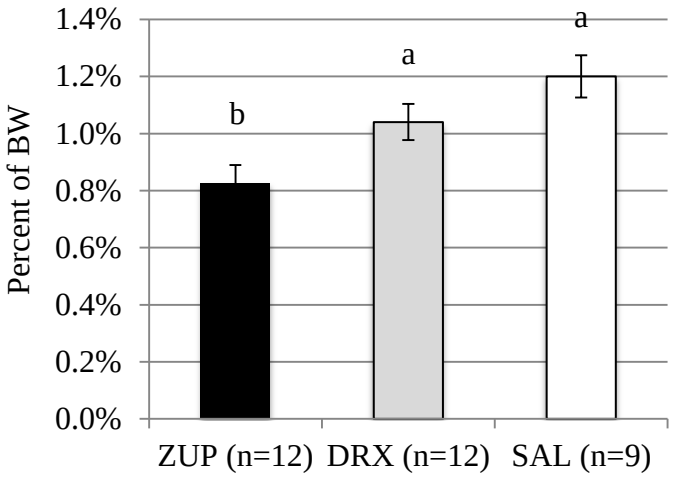


Figure 5.2 Model estimated mean lung percent of BW[1] by treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL)



[1] Body weight as measured immediately prior to challenge administration (d 10)

[a,b] Least squares means with unlike letters differ ($P < 0.05$)

Figure 5.3 Distribution of calves with abnormal Clinical Illness Scores (CIS > 0) from all replicates by study day and treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL). No abnormal scores (d 0 to 9) therefore displaying only (d 10 to 13)

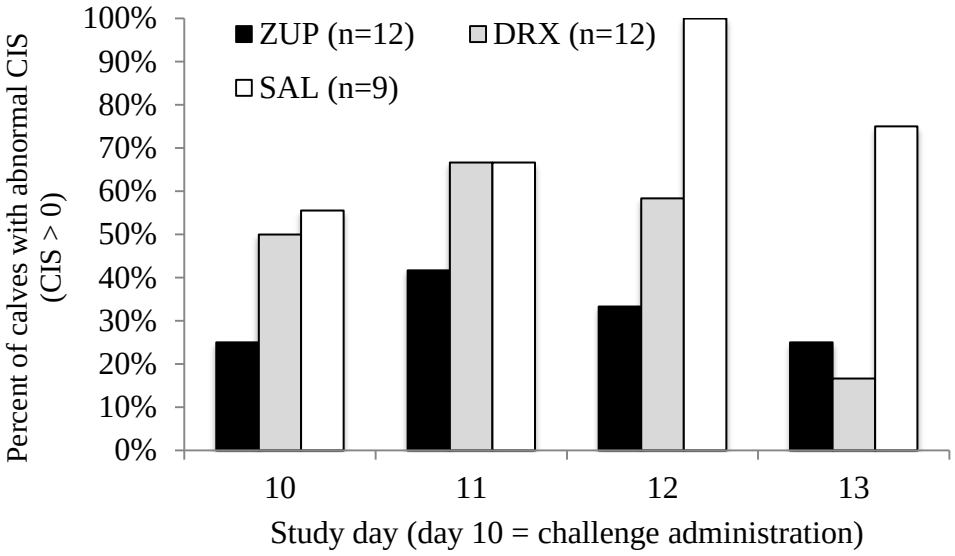


Figure 5.4 Distribution of abnormal appetite scores by study day and treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL)

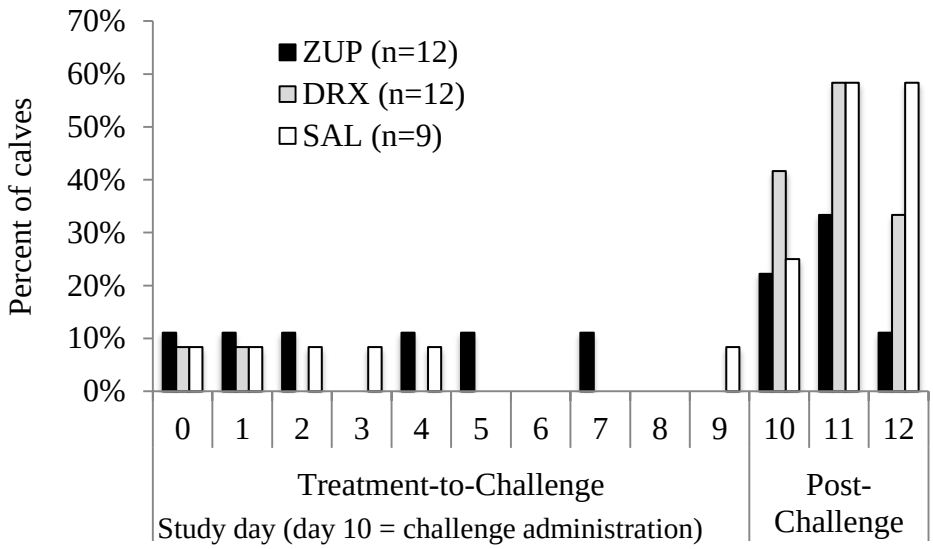


Figure 5.5 Distribution of calves receiving abnormal respiration scores by study day and treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL). No abnormal scores (d 0 to 9) therefore displaying only (d 10 to 13)

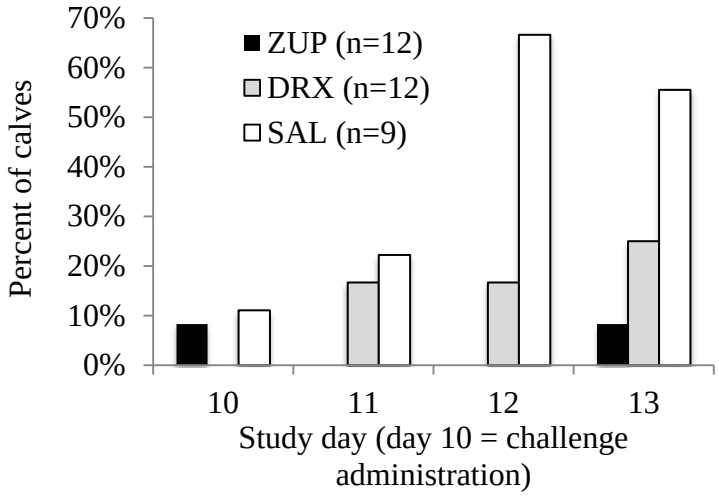
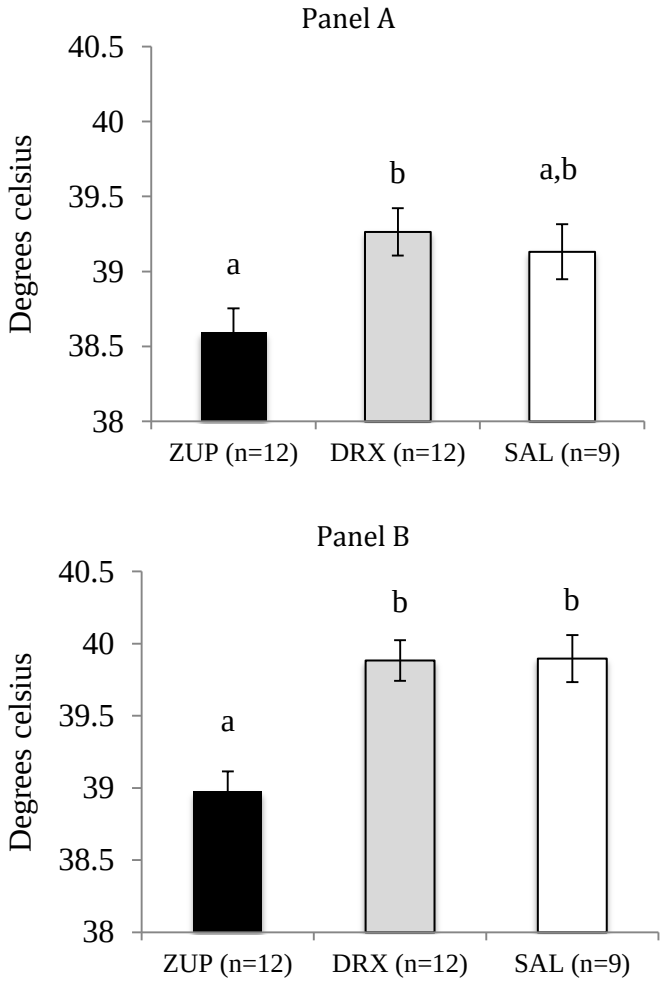
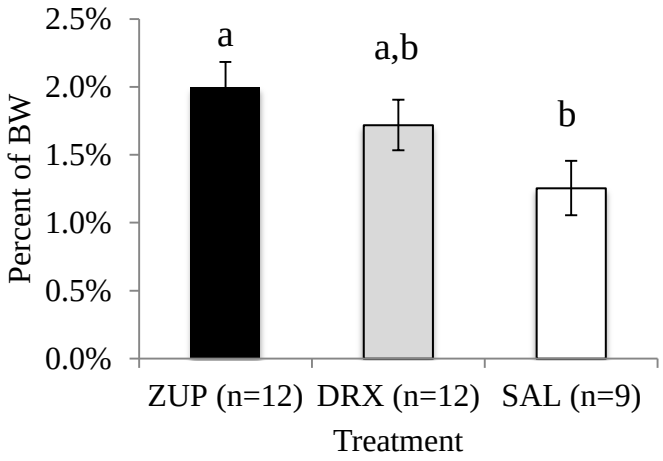


Figure 5.6 Model estimated mean rectal temperature (°C) by study period and treatment; Zuprevo (ZUP), Draxxin (DRX), Saline (SAL). Panel A – Pre-challenge (study days 8 – 10). Panel B – Post-challenge (study days 11 – 13).



[a,b] Least squares means with unlike letters differ ($P < 0.05$)

Figure 5.7 Model estimated mean total feed consumed as a percent of BW[1]



[1] Body weight as measured immediately prior to challenge administration (d 10)

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Chapter 6 - Comparison of classification algorithms to predict outcomes of feedyard cattle identified and treated for Bovine Respiratory Disease

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Abstract

Bovine respiratory disease (BRD) continues to be the primary cause of morbidity and mortality in feedyard cattle. Accurate identification of those animals that will not finish the production cycle normally following initial treatment for BRD would provide feedyard managers with opportunities to more effectively manage those animals. Our objectives were to assess the ability of different classification algorithms to accurately predict an individual calf's outcome based on data available at first identification of and treatment for BRD and also to identify characteristics of calves where predictive models performed well as gauged by accuracy.

Data from 23 feedyards in multiple geographic locations within the U.S. from 2000 to 2009 representing over one million animals were analyzed to identify animals clinically diagnosed with BRD and treated with an antimicrobial. These data were analyzed both as a single dataset and as multiple datasets based on individual feedyards and partitioned into training, testing, and validation datasets. Classifiers were trained and optimized to identify calves that did not finish the production cycle with their cohort. Following classifier training, accuracy was evaluated using validation data. Analysis was also done to identify sub-groups of calves within populations where classifiers performed better compared to other sub-groups.

Accuracy of individual classifiers varied by dataset. The accuracy of the best performing classifier by dataset ranged from a low of 63% in one dataset up to 95% in a different dataset. Sub-groups of calves were identified within some datasets where accuracy of a classifiers were greater than 98%; however these accuracies must be interpreted in relation to the prevalence of

the class of interest within those populations. We found that by pairing the correct classifier with the data available, accurate predictions could be made that would provide feedyard managers with valuable information.

Introduction

Bovine respiratory disease continues to be the most important syndrome affecting post-weaned cattle and is associated with approximately 75% of the morbidity and 50% of the mortality in feedyards. (Smith, 1998) The overall incidence of BRD has was reported as 14.4% in 1999 and 16.2% in 2011 and the estimated cost of treating a single case of BRD has nearly doubled. (USDA, 1999, 2013) Feedyards collect large amounts of individual and cohort level data; however, most of these data are used retrospectively in analysis of trends and to provide guidance for future management practices. Data is frequently collected on individual animals at the time of health events such as treatment for BRD. Previous authors have advocated the use of dynamic feedyard data in the prediction of overall outcomes. (Babcock et al., 2013a) However, there is no literature that uses both individual and cohort level dynamic data to make predictions regarding an individual animal's response to treatment. The ability to use dynamic information to predict an individual animal's response at the time of respiratory disease treatment would provide tremendous advantages and offer the ability to tailor treatment programs for individual animals.

Our primary objective was to assess the ability of different classification algorithms to accurately predict an individual calf's post-treatment outcome based on data available at first identification of and treatment for BRD. Our secondary objective was identification of calf characteristics or situations where predictive models performed well as gauged by accuracy.

Materials and methods

The research strategy involved evaluation of predictive ability of several classification algorithms and data management methodologies both within and across multiple feedyards; therefore, project goals were achieved through an iterative process using multiple datasets. Creation, revision, and evaluation of predictive algorithms based on existing and generated data from multiple sources were accomplished in a stepwise fashion (Fig 6.1). Multiple datasets were used to create an independent series of classification algorithms (based on training and test data) and to allow comparison of predictive accuracy (validation data).

Data source

Individual and cohort-level data from 23 feedyards in multiple geographic locations throughout the US were collected on cattle that arrived from 2000 to 2009. A population of

cattle (or lot) that were purchased, managed and marketed in a similar fashion was defined as a cohort, although the entire cohort may or may not have been housed in the same pen throughout the production phase. Cohort-level data included demographic characteristics known about the population at feedyard arrival (e.g. arrival date, arrival weight). Individual animal data were collected at the time a calf was treated for any disease and included characteristics of the individual at that time point (e.g. treatment date, diagnosis, rectal temperature).

Data preparation

Individual and cohort data were combined into an original dataset containing 1,400,437 event records from 804,631 individual calves within 35,737 cohorts. This dataset contained 27 unique variables and several combination, derived, and redundant variables. The 27 variables consisted of cohort level data and individual information recorded when an animal was pulled for any event such as suspected illness. Cohort level variables available for most animals were date they arrived at the feedyard, total number of head within that cohort and average arrival weight (weight of all animals within the cohort divided by total head in that cohort). Some feedyards also recorded the gender of the lot (male, female, mix, or designated the lot as Holstein if they were dairy breeds) and the risk code assigned to that lot (high, medium, low) which represents the feedyard's perceived risk of those animals developing respiratory disease. Some variables such as individual animal weight, and rectal temperature at the time of treatment were not consistently recorded by all feedyards. The goal of this project was to develop and compare the accuracy of models for predicting our outcome of interest, animals that had been treated for BRD with an antimicrobial and did not finish (DNF) the production cycle with their cohort. Our case definition for DNF was similar to previous work describing feedyard mortality (Babcock et al., 2013b) and included any animal that died following BRD treatment or any animal that was removed from the feeding phase prior to cohort harvest following initial treatment for BRD. A binary variable (DNF) was created and populated with values of 0 and 1 (finished the production cycle normally and did not finish normally, respectively). Our study population dataset (SPD) was a subset of the original data and included all calves identified as being treated for BRD with an antimicrobial. This subset included 468,734 animals of the total 1,400,437 events. Calves could have been diagnosed and/or treated for other conditions prior to or after the initial

diagnosis and treatment for BRD. In the SPD, 8.5% (39,699 / 468,734) of the calves did not finish the production cycle normally.

Variable creation

New variables were derived in an effort to capture predictive characteristics relative to an individual animal's outcome of DNF and thereby enhance predictive models accuracy. Cohort level variables were created that identified trends in the incidence of calves identified as diseased and treated within a cohort over the course of time on feed. For each day a cohort was in the feedyard, variables were created that calculated the daily incidence proportion of calves diagnosed and treated for BRD. The daily incidence proportion of calves identified as ill for any reason within a lot was also calculated. As changes in BRD morbidity over time can be important to understanding an outbreak, cumulative proportions of BRD incidence were calculated for the previous 2, 5, 15, 20, and 30 days for each day a cohort was in the feedyard. Cumulative proportion variables using the same structure were created representing the incidence of all disease within the cohort, not just BRD incidence.

Variables were created using the previously created cohort incidence proportion variables, querying only those animals within those variables having rectal temperatures at the time of treatment greater than or equal to common industry breakpoints of 39.4, 39.7 and 40 degrees Celsius. Cohort level variables involving the proportion of animals within a lot meeting certain criteria such as dying or having been identified as diseased for any reason were also created. Table 6.1 displays cohort-level variables and specific definitions used to create each variable.

To capture temporal information associated with changes in cohort-level incidence of BRD, variables were created calculating differences in incidence proportions from one time point to another. The change from the previous calendar day's incidence proportion of BRD was calculated as well as changes in cumulative incidence proportions from the previous 3, 5, 10, 15, 20, and 30 days. Variables representing changes for incidence rates associated with diseases of all causes within a cohort, were also created.

Derived variables were also created using individual animal information. The type(s) of antimicrobial that calves received were classified into one of seven categories: cephalosporins, tetracyclines, fluoroquinolones, macrolides, mixed (animal was treated with more than one class

of antimicrobial), ampicillins, or older drugs (e.g. penicillins). A binary variable (0 = no, 1 = yes) was created for all animals indicating if they had received a non-steroidal anti-inflammatory (NSAIDYN). Variables capturing information relating to the time of year, month, and week when an animal was treated were also created. Table 6.2 lists all individual animal level variables.

The variable building process resulted in a dataset combining the original and the newly created variables. Each record in the final SPD dataset contained the 27 original variables, 126 newly created cohort-level variables, and 13 newly created individual animal or event variables. Data were not consistent across feedyards and if data were not available to calculate one of the new variables, the resulting fields were treated as null or 0 depending on the variable structure.

Prior to predictive model building, a pair-wise correlation analysis was performed on all variables within the dataset using the linear correlation node within KNIME. (Michael R. Berthold, 2008) If the value of the correlation statistic between any two variables was |0.9| or higher, only one of the variables was selected and included in any subsequent predictive classifiers. Variables identifying a specific feedyard or containing information pertaining to specific dates (i.e. year of feedyard entry, or treatment date) were not used when training classifiers. The goal was to train classifiers that could be used on new data and not be tied to only the original datasets.

Data partitioning

The SPD dataset was randomly partitioned into training, testing and validation datasets representing 40%, 30%, and 30% of the full dataset, respectively. Within each partitioned dataset, 23 subset datasets each representing only data from an individual feedyard were created. All training, testing and validation steps were performed 24 times, once using the dataset with all feedyards combined (COMBO) and once for each of the individual feedyard datasets (1 thru 23). The validation datasets were saved and evaluated with the final trained algorithms only once to evaluate classifier accuracy.

Classification algorithms

All classification algorithms were implemented using The Waikato Environment for Knowledge Analysis (WEKA)(Hall, 2009) nodes available as extensions within KNIME.

Decision trees

Decision tree classification is a learning process that recursively partitions a training dataset and is then used to determine the appropriate class for each example within a test dataset.. (Zhang, 2012) Each node or branch within a tree splits the data into two or more categories usually based on a single attribute. Each leaf of a node is then assigned to a class that represents the most appropriate target value and calculates a probability that an individual belongs to that node. (Rokach, 2005) We evaluated two variations of decision tree classification algorithms, Random forests (RF) and Decision stump (DS). Random forests build several individual classification trees using random samples of the data (i.e. bagging) and then vote for the most popular class. (Breiman, 2001) Decision stump finds a single attribute that provides the best discrimination between the classes and then bases future predictions on this attribute. (Iba, 1992)

Bayesian networks

In general, Bayesian classifiers estimate the conditional probability distributions of each attribute within the training dataset and then assign cases within the test datasets to the class with the highest posterior probability using Bayes' Theorem. (Sebastiani, 2005) We used bayesnet (BN) with a K2 search algorithm and Naïve bayes (NB) with the default settings as supplied by Knime for those nodes. Bayesian network classifiers use directed acrylic graphs where each node in the graph represents a random variable and the edges represent probabilistic dependencies among those random variables. Naïve bayes classifiers analyze the relationship between each variable and the class of interest to then determine a conditional probability for the relationship. (Williams, 2006)

Meta-classifiers

Boosting is a type of meta-learning that classifies subsets of the initial training dataset. As each subset is used to train the classifier, the algorithm attempts to use information from the previous subset cases that were incorrectly classified. (Vilalta, 2005) Multiboost (MB) and logitboost (LB) are algorithms that are constructed by multiplying the individual conditional probabilities from each feature to get the total probability of a class. (Webb, 2000) The class with the highest probability is then selected as the winner. In our application, the base learner for MB was the random tree algorithm and DS was used as the base learner for LB. The filtered

classifier (FC) algorithm used a J48 tree algorithm after the data was passed through a discretization filter.

Functions/Neural networks

The VotedPerceptron (VP) classifier is based on the perceptron algorithm as described by Feund and Schapire in 1999. Neural networks predict outcomes based on relationships between variables that may be complex and multidimensional and are well suited to our data structure, as they do not require *a priori* assumptions about the underlying data structure. (Zhang, 2005) The VP takes advantage of data that are linearly separable with large margins. (Freund and Schapire, 1999) The VP classifier we used was configured to run with the default settings as supplied by Knime for that particular node

Statistical methods

Logistic regression models were developed and prediction equations were used to evaluate the test and validation datasets. Only variables significantly associated ($P < 0.05$) with our outcome of interest (DNF) in a univariable screening were included when training our logistic classifiers. No further attempt was made to create a more parsimonious model, as the goal was to evaluate logistic regression classification in a similar manner to the other classifiers we had trained.

Classifier selection

Approximately 25 to 30 potential classifiers were trained using their default settings on the COMBO training dataset and initial accuracies were evaluated after classifying the test COMBO data. Classifiers with the lowest accuracies and or lowest sensitivity (Se) or specificity (Sp) values were eliminated. To further eliminate classifiers providing similar information, pairwise correlation coefficients between predicted probabilities from each classifier were calculated using the linear correlation node in KNIME. Classifiers with correlation statistics of > |0.5| were removed leaving twelve classification algorithms. From these 12 algorithms, nine were selected that were representative algorithms belonging to one of five general groups of classifiers evaluated in this study (Decision trees, Bayesian Methods, Meta-classifiers, Functions/Neural Networks, and Statistical). Where possible, individual parameters for each of the nine classifiers were modified, retrained and evaluated using the COMBO test data. The

accuracy of the classifier was compared with those from the same classifier using different settings. This procedure was repeated for each classifier until optimal accuracy for each classifier had been achieved using the test dataset.

Sampling of rare events

Determining the optimal training dataset distribution for the class of interest in respect to classifier performance can be challenging. Previous research has determined that classifier performance varies based on the data structure and classifiers used; however, general conclusions favored balanced (equal number of events and non-events) training datasets to optimize the performance of classification algorithms. (Japkowicz, 2000b; Weiss G.M., 2003) Overall, calves meeting our case definition (DNF = 1) represented a low proportion (8.5%) of the total number of animals. To evaluate the impact of an un-balanced training dataset on overall classifier accuracy, two different types of balanced training datasets were created; over-sampled and under-sampled. The over-sampled training dataset was created by selecting all of the calves in our dataset belonging to the minority class (DNF = 1) and creating exact duplicates of them until the distribution of DNF = 1 in the oversampled training data was approximately 50%, or equal to the distribution of our majority class (DNF = 0). The under-sampled training dataset preserves all of the minority class rows and was created by randomly removing rows belonging to the majority class until there are an equal number of rows belonging to both the majority and minority classes within the final dataset. (Japkowicz, 2000b) Each classification algorithm was created using the three different training datasets, and accuracy of each classification algorithm was determined with the same test data. Analysis of the variance among dataset sampling techniques was performed on Area Under the receiver operating Curves (AUC) using the Kruskal-Wallis test allowing for multiple comparisons using Steel-Dewass methods in JMP (JMP, SAS Inc.) The sampling technique resulting in the highest AUCs was selected as the method to train all classifiers.

Classifier accuracy

Classifier accuracy was determined by allowing each algorithm to classify the validation datasets. Classifier predicted probabilities of DNF = 0 and 1 were created for each calf for each classifier. Using these probabilities, receiver-operating characteristic curves (ROC) were created using the LOGISTIC procedure in SAS (SAS 9.3, SAS Institute. Inc.). The ROC curve allows

for evaluation of the trade-off between correctly identifying animals that meet the case-definition for DNF (DNF = 1) and falsely identifying DNF = 0 calves as DNF = 1 (false positives) for each classification algorithm. (Gardner and Greiner, 2006) As the optimal cutoff varies based on the application of the diagnostic test (classifiers), we elected to identify the point on the ROC curve where Se and Sp are maximized by calculating Youden's index(Youden, 1950) and using the corresponding classifier generated probability as the cutoff between DNF = 1 and DNF = 0. Youden's index (J) ranges between 0 and 1, with a value of 1 indicating a test with perfect sensitivity and specificity. The J_{max} is the point on the ROC curve that has the greatest vertical distance from the diagonal or chance line. (Schisterman et al., 2005)

Logisitic regression models for each classifier were fit in SAS using the LOGISTIC procedure with the animal's true status (DNF) as the dependent variable and the predicted probabilities out of KNIME as the independent variable of interest. The OUTROC statement was included in the MODEL statement to output a dataset for each classifier with all distinct predicted probabilities and their corresponding sensitivity and specificity values. Youden's index (J) = Se + (Sp - 1) was calculated for each possible Se, Sp combination and the maximum J (J_{max}) was identified along with its corresponding probability (P). The probability P represents the cutoff that maximizes Se and Sp for an individual classifier. Final predicted classification for each calf (predicted DNF = 1 or 0) was based on their predicted probability from that specific classifier in relation to P. Calves with predicted probabilities greater than or equal to P were classified as DNF = 1 and all others were assigned DNF = 0. Classifier diagnostic performance was then assessed using the final predicted DNF status to calculate true positives (TP), false positives (FP), true negatives (TN), false negatives (FN), Se, Sp, and accuracy = (TP+TN)/(TP+TN+FP+FN) for each classifier.

Accuracy among sub-groups within populations

Logistic regression models were employed to evaluate potential changes in classifier accuracy based on sub-groups within each dataset population. For each dataset the classifier that provided the highest overall accuracy after identifying the cutoff yielding the highest combined Se and Sp was selected. A binary variable (CORR) for each calf within a classified dataset was created and populated with a value of 1 if the classifier predicted DNF status agreed with the true value of DNF for that calf, otherwise the value was 0. For example: if the Naïve Bayes classifier

provided the highest overall accuracy for the COMBO dataset, then a CORR variable for each calf was created and populated with a value of 1 where that calf's true status for DNF agreed with the Naïve Bayes prediction, otherwise the variable was populated with 0. Independent variables of interest were gender, arrival weight (WTIN), rectal temperature at treatment (TEMP), days on feed at treatment (TDOF), and the interaction of all variables with TDOF. Gender was categorized into four categories representing all genders of calves in our population with gender information provided; males (MAL), females (FEM), mixed (MIX), and Holsteins (HOL). Arrival weight was categorized into five categories; less than 181kg (400lbs), 181 to 226 kg (400 to 500 lbs), 227 to 272 kg (501 to 600 lbs), 273 to 318 (601 to 700 lbs) and greater than 318 kg (700lbs). Rectal temperature was also categorized into 4 categories; less than 39.1, 39.1 to 39.4, 39.41 to 40 and greater than 40 degrees Celsius. Days on feed at treatment was categorized into animals less than 15 days on feed, 15 to 30 days on feed, 30 to 45 days on feed and greater than 45 days on feed. The true status of each calf, DNF was also offered to each model as an independent variable of interest. The dependent variable of interest was predictive model accuracy or agreement between the model prediction for DNF and true calf status (CORR).

A multi-variable logistic regression model was fit using the GENMOD procedure in SAS with a binary distribution and logit link function. Manual backwards elimination was performed keeping only those variables associated to the outcome at a 5% significance level ($P< 0.05$). Main effects remained in the model regardless of significance if their corresponding interaction terms were significantly associated with the outcome. Least squares means for each predictor remaining in the model were calculated and then transformed back to probabilities using the formula: P = exp(logit)/(1+exp(logit). Probabilities for a given predictor represented the agreement/accuracy of calves within that group of the population represented by that variable. The true status of the animals DNF if significant ($P< 0.05$) in the model represented the model adjusted positive predictive value (PPV) for each DNF category.

Results

Descriptive statistics

A total of 468,734 individual calves from 23 different feedyards representing multiple geographic locations in the United States were included in our study population. The mean

number of individual animals per feedyard meeting our case definition of having been treated with an antimicrobial for BRD was 20,379 (SE = 3168) with a median of 17,823. The prevalence of DNF = 1 within the COMBO dataset was 8.5 % with a range among feedyards of 0.5% to 14.5% and averaging 9.1% (SE = 0.7%) and a median of 9.9%.

Comparison of dataset balancing techniques

To determine the optimal training dataset balancing technique for our data, the AUC from each of the nine classifiers were analyzed using the native, under-sampled, and over-sampled test datasets. Variance of the nine classifier AUCs was compared among datasets. There were no differences ($P > 0.05$) between the native dataset and the over-sampled datasets as well as between the over-sampled and under-sampled datasets. The AUC's using under-sampled data were higher ($P < 0.05$) when compare to those using the native dataset (Fig. 6.2). The under-sampled COMBO training dataset contained 15,821 animals in each DNF category and was used to perform all evaluations of classifier accuracy.

Classification accuracy

The accuracy of the nine classification algorithms was evaluated using the validation data sets. Predictions were generated using the COMBO dataset and individual data sets for each feedyard. Accuracies were based on predictions from each classifier following the use of Youden's index to determine the cutoff that maximizes Se and Sp. Variation in accuracies of each classifier and each dataset are displayed in Fig 6.3. Accuracies of the nine classifiers using the COMBO dataset ranged from 52% to 77% for BN and NB, respectively. Classifier accuracy using dataset 23 ranged from 6% (DS) to 79% (LB). While classification of the dataset 23 using the DS algorithm resulted in a sensitivity of 99% (not displayed) the algorithm predicted almost every calf as DNF = 1 (4097/4156). The prevalence of DNF = 1 in dataset 23 was 4.7% (197/4156). The FC algorithm achieved the highest accuracy of 95% when applied to dataset five; however the prevalence of DNF = 1 in this dataset was less than 1% (21/3957) (Table 6.3). Of the 24 datasets analyzed, six achieved accuracies greater than 80% using one of the nine classification algorithms with 50% (3/6) of those algorithms using classifiers with Bayesian network architecture. Logistic regression was the highest performing classifier in only one dataset and overall accuracy was 73% in a population where the prevalence of DNF = 1 was high (14.5%).

Sub-group analysis

Logistic regression was employed to evaluate potential sub-groups within each dataset where classification accuracy was better than overall accuracy. The classifiers with the highest overall accuracy by dataset were analyzed to identify these potential sub-groups. For the COMBO dataset, the main effects of gender, WTIN, TEMP, TDOF and DNF were significantly associated ($P < 0.05$) with classifier accuracy as well as all interactions with TDOF (Table 6.4). However, for dataset 15, DNF was the only effect associated ($P < 0.05$) with agreement. Overall, TDOF or the interaction of WTIN and TDOF were associated ($P < 0.05$) with agreement/accuracy in 22 of the 24 models. Rectal temperature recorded at treatment was associated ($P < 0.05$) with agreement/accuracy in 16 of the 24 models.

Prevalence of calves DNF = 1 in our study was low, 8.5% overall and varied by dataset. Sub-groups within dataset populations with model adjusted significant ($P < 0.05$) accuracies greater than 1-prevalence represent calves within that population where classifiers performed better than guessing all animals would finish the production cycle normally. Using the BN classifier on calves in dataset 1, the accuracy of predicting DNF for lightweight calves on arrival (less than 181 kg) that were 15 to 30 days on feed at their initial treatment for BRD was 95 ± 4 % while the overall prevalence in this population was 12%. The prevalence of DNF = 1 in dataset five was less than 1%; however, accuracy using the FC algorithm was near 100% in all categories of gender, TEMP, WTIN, and TDOF (Table 6.5). For calves in dataset 19 that were greater than 45 TDOF and had rectal temperatures greater than 40 °C the MB classifier was over 97 ± 2 % accurate in identifying calves DNF = 1 (Table 6.5).

Discussion

Bovine respiratory disease continues to adversely impact cattle health with an estimated 16.2% of all cattle placed in feedyards showing signs of respiratory disease at some point during the feeding period. (USDA, 2013) Accurately predicting health outcomes is an important component in increasing performance within feedyards. (Babcock et al., 2013a; Corbin and Griffin, 2006) Characteristics of cohorts and individual animals upon arrival and individual animal treatment records are frequently recorded. These data have previously been analyzed for risk factors associated with developing BRD;(Babcock et al., 2009; Step et al., 2008) however, to our knowledge, using this information to make prognostic predictions for an individual animal at

the time of first treatment for BRD has not been reported. In this study we evaluated the ability of several classification algorithms to accurately predict calves within cohorts that would not finish the production cycle normally. Accuracy of classification algorithms was relatively low using combined data from all feedyards; however, when applied to datasets representing individual feedyards, accuracy of some classifiers improved. This is not surprising given the inconsistency in data recorded among feedyards. There were sub-groups of calves within individual datasets where classifier accuracy was quite good considering the prevalence of animals meeting our case definition was relative low in most datasets.

When learning from imbalanced datasets, some classifiers can learn to provide adequate distinction between FPs and FNs while others simply learn to predict the majority class. (Maloof, 2003) Sampling techniques have been developed to minimize the impact of learning with imbalanced data by changing the distributions within the training sets. (Maalouf and Trafalis, 2011) Several methods have been proposed to handle imbalanced datasets and results differ based on the classification algorithm chosen;(Japkowicz, 2000b) however, with large datasets it is generally accepted that a balanced class distribution performs better than un-balanced. (Weiss G.M., 2003) Two frequently used techniques involve under-sampling and over-sampling on the class of interest. While sampling techniques may provide benefits in accuracy they can also introduce bias due to choice based sampling. However, if this choice based sampling introduces bias that impacts a classifiers ability to accurately predict minority events, then overall accuracy should be negatively impacted when validation data (naturally imbalanced) is classified. There were minor differences in classification accuracy from test to validation data (data not shown) indicating the choice based sampling method used to train classifiers was not introducing an important source of bias. In our study, following validation, only those classifiers with the highest within-dataset accuracies were used for subsequent sub-group analyses.

Previous research has discovered that training classifiers on imbalanced data frequently produces classifiers that favor the majority class. (Weiss G.M., 2003) In our study, the prevalence of calves that received treatment for BRD and then DNF was relatively low (8.5%) resulting in an imbalanced dataset. We evaluated the AUC for each of the classifiers using 3 versions of the COMBO dataset (native, under-sampled, over-sampled). Area under the curve, unlike accuracy, provides a measurement of a classifiers abilities using all possible cutoffs in Se and Sp and has previously been used to distinguish among sampling methods. (Maloof, 2003)

While no difference was found in AUCs between the native and over-sampled datasets, we found AUC's were significantly higher ($P < 0.05$) using the under-sampled data in relation to our native distribution. Under-sampling and over-sampling techniques are both appropriate methods to balance the class distribution; however, some have noted that over-sampling can lead to over-fitting due to making exact copies of the minority class records. (Weiss G.M., 2003) This potential of over-fitting was avoided by using under-sampled data to train all classifiers in our study.

Area under the ROC curve does summarize the ROC curve and provides an overall method to discriminate among potential classifiers; however, it does not directly supply a classifiers predictive ability (accuracy) given a specific trade-off in Se and Sp (Greiner et al., 2000). Given the imbalanced nature of datasets and the complex nature of BRD within feedyards, the cost of a FP is likely not the same as that of a FN. The impact of predicting a calf would not finish the production cycle normally could involve changes in management procedures for that animal that minimize further expenses. False positive calves represent those where significant economic loss could be realized if these animals are not kept in the herd while FN animals managed normally could result in increased expenses of feed and treatments that will provide negative returns on investment. We attempted to minimize FPs and FNs by altering the decision threshold for each classifier by using the point on the ROC curve furthest from the line of chance. (Fluss et al., 2005) While this decision threshold may not be the most appropriate for every situation (i.e. the cost difference in a FN in relation to FP), by selecting the same threshold across all classifiers we were able to compare predictive ability among classifiers using overall accuracy.

We evaluated the accuracies of multiple classifiers to predict our outcome of interest (DNF = 1). Accuracies varied by classifier within a dataset and among datasets. Within datasets, the variation in classifier accuracies ranged from 15% up to 75%; however, in some datasets where variation in accuracy was relatively low (15%), the accuracy of the best classifier was only 63%, indicating in this dataset, classifiers lacked the appropriate training to provide useful predictions. Accuracy of individual classifiers varied by greater than 49% considering all the datasets. Filtered classifier for example, achieved the highest overall accuracy of classifiers evaluated when using dataset five and was only 5% accurate using dataset 23. Bayesian network and meta-classifiers each achieved the highest within dataset accuracies in 33% (8/24) of

datasets but no one type of classifier outperformed all the others. These large variations in accuracies within and among datasets as well as differences in individual classifier accuracies across datasets are likely due to differences in variables recorded at each feedyard (represented by different data available among datasets) as well as differences in management practices at each feedyard that were not represented within these data. The complex epidemiology of BRD within feedyard production systems makes it highly plausible that management of BRD varies by feedyard. (Taylor et al., 2010) Differences in accuracies discovered here highlight the importance of pairing the classifier that works bests given the data available.

Evaluating accuracy alone can lead to misleading results when the class of interest within the dataset is imbalanced. (Chawla, 2005) In our study, the overall prevalence of DNF =1 in the COMBO dataset was 8.5% and ranged from < 1 % to 14.5 % in individual datasets. As mentioned previously, the FC algorithm achieved an accuracy of 95% within one dataset (5) and appeared to be useful in identifying calves meeting our case definition. However, further analysis reveals the prevalence of calves within dataset five of DNF = 1, was less than 1%. A default strategy of guessing every calf in this population will finish the production cycle normally would have resulted in a predictive accuracy of greater than 99%. Therefore, in this population, to achieve performance better than guessing a classifier would need to be greater than 99% accurate.

Overall accuracies of individual classifiers and for individual datasets within our study were relatively low; however, we identified sub-groups of calves within some datasets where classification accuracy was considered good (greater than 1- prevalence of DNF = 1). The characteristics that we used to discriminate among sub-groups within dataset (gender, WTIN, TEMP, TDOF) would be known at time of first pull and could be used to guide selection of the appropriate classifier given characteristics of that specific animal. Using a Bayesnet algorithm on dataset 1, accuracies were greater than 1-prevalence for all categories of WTIN although they were modified by the TDOF. This makes sense, as we would expect animals visually identified as suffering from BRD would express different clinical signs based on the amount of time they have been in the feedyard. We also found that in some datasets using an animal's TEMP and TDOF provided accuracies that were better than (1-prev of DNF =1) for that yard. While these four sub-groups provided insight into instances where classifiers performed well, there are likely other sub-groups that could be analyzed and included in sub-group analysis because several risk

factors have previously been associated with the risk of developing BRD. (Babcock et al., 2010; Cernicchiaro et al., 2012) By understanding sub-groups of cattle where classification algorithms are known to perform well, one could tailor classifier selection at the time of treatment based upon the characteristics of the population. Further research is needed to more clearly define these populations and specific classifiers that would optimize prediction performance.

Conclusion

The objective of this study was to evaluate the ability of several different classification algorithms to identify individual calves that would not finish the production cycle normally. As with many real-world classification problems, these data with respect to our class of interest were highly imbalanced. Under-sampling dataset balancing was performed prior to classifier training to give algorithms the best opportunity to learn the class of interest. We compared the ability of these classifiers by using accuracy after adjusting the decision threshold for each classifier by maximizing Se and Sp in relation to each other and found it varied not only by classifier but also by the data analyzed. We identified sub-groups within each population where specific classifiers performed well considering the prevalence of our class of interest. These sub-groups of calves were based on demographic characteristics available at the time an animal was pulled and treated for BRD indicating there are specific characteristics that could be used to tailor classification accuracy.

Information recorded among feedyards was not always consistent as was partially evident in the ranges of accuracy for individual classifiers among datasets. The predictive accuracy of a classifier is directly related to the data provided when training. If information provided during training does not help distinguish the class of interest then results of classification using validation data will not be useful. Methodology used here has provided insight into the capability of predictive models to be used in a production setting and the importance of pairing the data available with the correct classifier can lead to accurate predictions of calves of interest.

Table 6.1 Cohort animal level variables

Variable	Description
arrivalmonth	Month of lot arrival (1,2,3,4,5,6,7,8,9,10,11,12)
arrivalquarter	Quarter of the year of lot arrival (1,2,3,4)
arrivalyear	Year of lot arrival
brdcasestothispoint	Sum of animals diagnosed with BRD and administered an antimicrobial as of the previous treatdate
distbrdcasestothispoint	sum of distinct animals diagnosed with BRD and administered an antimicrobial as of the previous treatdate
treatment failure #1 (txfailure#1)	1 = any animal requiring re-treatment for BRD or didnotfinish after receiving antimicrobial treatment for BRD
treatment failure #2 (txfailure#2)	1 = any animal requiring treatment with an antimicrobial for any reason after an initial treatment for BRD or didnotfinish after receiving antimicrobial treatment for BRD
treatment failure #3 (txfailure#3)	1 = any animal being pulled for any event after their initial antimicrobial treatment for BRD
treatment failure #4 (txfailure#4)	1 = same as txfailure#3, but includes animals on the same event day
1st treatment success rate #1 (1ssttxsuccessrate_p1)	((distbrdcasestothispoint -sum of txfailure#1) / distbrdcasestothispoint) * 100
1st treatment success rate #2 (1ssttxsuccessrate_p2)	((distbrdcasestothispoint -sum of txfailure#2) / distbrdcasestothispoint) * 100
1st treatment success rate #3 (1ssttxsuccessrate_p3)	((distbrdcasestothispoint -sum of txfailure#3) / distbrdcasestothispoint) * 100
1st treatment success rate #4 (1ssttxsuccessrate_p4)	((distbrdcasestothispoint -sum of txfailure#4) / distbrdcasestothispoint) * 100
propbrdcasestothispoint	Proportion of BRD cases to this point = (brdcasestothispoint/headin)*100
propdistcaestothispoint	Proportion of distinct BRD cases to this point = (distbrdcasestothispoint/headin)*100
propbrdcasestothispoint (time and temperature cutoffs)	(brdcasestothispoint/headin)* 100; New variable created for each combination of day (2,3,5,10,15,20,30) and temperature cutoffs ($\geq 103, \geq 103.5, \geq 104$)
propdistcaestothispoint	(distbrdcasestothispoint/headin)* 100; New variable created for each combination of day

(time and temperature cutoffs)	(2,3,5,10,15,20,30) and temperature cutoffs (≥ 103, ≥ 103.5, ≥ 104)
deathstothispoint	Sum of deaths to this point
propdeathstothispoint	Proportion of deaths to this point = (deathstothispoint / headin) * 100
propdeathstothispoint (time cutoffs)	(deathstothispoint / headin) * 100 New variable created for each combination of the previous days (2,3,5,10,15,20,30)
propdailybrdpulls	Proportion of lot pulled for BRD on this event day = (Dailybrdpulls/headin) *100
propdailyallpulls	Proportion of lot pulled for any reason on this event day = (Dailyallpulls/headin) * 100
deltapropdailybrdpulls	Change in propdailybrdpulls from previous calendar day
deltapropdailyallpulls	Change in propdailyallpulls from previous calenday day
deltapropdailybrdpulls (time cutoffs)	Change in propdailybrdpulls for the previous (2,3,4,10,15,20,30 days)
deltapropdailyallpulls (time cutoffs)	Change in propdailyallpulls for the previous (2,3,4,10,15,20,30 days)
dailyyardpopulation	Total number of head on feed for that calendar day
propryardbrdpulls	Proportion of yard pulled for BRD on this event day (totalbrdpullsforyard/dailyyardpopulation)*100
deltapropyardbrdpulls	Change in propyardburdpulls from previous calendar day
deltapropyardbrdpulls (time cutoffs)	Change in propyardburdpulls for the previous (2,3,4,10,15,20,30 days)
Exponential moving averages (EMA)	EMAs were calculated for the average daily BRD pulls for the lot for 3, 5,10,15,20, and 30 days
Moving average convergence divergence (MACD)	MACDs were calculated for differences in all EMA combinations

Table 6.2 Individual animal level variables

Variable	Description
tagno	Individual calf's tag number
treatdate	Date calf was treated
eventno	Running count of events per calf
diag	Diagnosis assigned to calf for this event
dxcode	Diagnosis code assigned to calf for this event
dieddxcode	Diagnosis code for calf's death
antimicrobial	1 = antimicrobial was administered at this event, 0 = no antimicrobial administered
deaddate	Date animal died if known and recorded
didnotfinish	1= calf did not finish production cycle, 0 = finished production cycle
treatdof	Days on feed at treatment for this animal
wt	Individual animal weight
temp	Rectal temperature
overallclass	Class of antibiotic administered (CEPH, TET, FLU, MAC, MIX, AMP, OLD)
nsaidyn	1 = NSAID administered during this event, 0 = no NSAID administered
brdabxyn	1 = calf diagnosed with BRD and administered an antimicrobial (denominator of CFR)
treatnobrdabxyn	Count of the times this calf has been diagnosed with BRD and treated with an antimicrobial
abxtreatno	Running total of the number of times calf treated with antimicrobial for any reason
eventspriortobrdyn	1 = calf had events recorded prior to brdabxyn = 1, 0 = no events recorded prior to brdabxyn = 1
nbreventspriortobrd	Sum of eventspriortobrdyn
pulldayofweek	The day of the week for this event (M,Tu, We, Thr, F, Sa, Su)
pullweekdayYN	1 = animal pulled on a weekday, 0 = pulled on a weekend
pullonmondayYN	1 = animal pulled on a Monday, 0 = pulled any other day
pullmonth	Month of the year for this event (1,2,3,4,5,6,7,8,9,10,11,12)
pullquarter	Quarter of the year for this event (1,2,3,4)
pullyear	Year the calf was pulled

Table 6.3 Diagnostic performance of classifiers[a] achieving the highest accuracy by dataset

Dataset	Classifier	TP[b]	FP[b]	TN[b]	FN[b]	Sensitivity	Specificity	Accuracy	Prev DNF=1[c]
0	NB	3240	24345	104322	8714	27.1%	81.1%	76.5%	8.5%
1	BN	315	1512	6234	770	29.0%	80.5%	74.2%	12.3%
10	NB	155	1039	1768	170	47.7%	63.0%	61.4%	10.4%
11	BN	7	33	994	102	6.4%	96.8%	88.1%	9.6%
12	BN	73	275	2466	206	26.2%	90.0%	84.1%	9.2%
16	NB	235	1786	4875	575	29.0%	73.2%	68.4%	10.8%
18	NB	55	211	3192	388	12.4%	93.8%	84.4%	11.5%
21	NB	297	1780	7474	655	31.2%	80.8%	76.1%	9.3%
7	VP	103	745	4034	459	18.3%	84.4%	77.5%	10.5%
17	VP	312	2033	6637	664	32.0%	76.6%	72.0%	10.1%
4	LB	221	1149	1568	135	62.1%	57.7%	58.2%	11.6%
5	FC	18	191	3745	3	85.7%	95.1%	95.1%	0.5%
13	FC	329	2604	6900	407	44.7%	72.6%	70.6%	7.2%
14	LB	68	578	2152	46	59.6%	78.8%	78.1%	4.0%
15	LB	6	19	154	23	20.7%	89.0%	79.2%	0.6%
19	MB	400	4576	15508	640	38.5%	77.2%	75.3%	4.9%
22	LB	379	2772	4776	271	58.3%	63.3%	62.9%	7.9%
23	LB	62	724	3235	135	31.5%	81.7%	79.3%	4.7%
8	LR	109	394	1521	216	33.5%	79.4%	72.8%	14.5%
2	DS	38	141	4997	603	5.9%	97.3%	87.1%	11.1%
3	RF	337	2104	3824	329	50.6%	64.5%	63.1%	10.1%
6	DS	90	291	1330	106	45.9%	82.0%	78.2%	10.8%
9	DS	85	297	6364	725	10.5%	95.5%	86.3%	10.8%
20	DS	257	1532	8453	707	26.7%	84.7%	79.6%	8.8%

[a] BN = Bayesnet, DS = Decisionstump, FC = Filteredclassifier, LB = Lobitboost, LR = Logistic Regression, MB = Multiboost, NB = Naïve Bayes, RF = Random forest, VP = VotedPerceptron

[b] TP = true positives, FP = false positive, TN = true negatives, FN = false negatives

[c] Prevalence of calves within each dataset that did not finish (DNF) the production cycle normally

Table 6.4 Type 3 fixed effects results of logistic regression models evaluating the associations between algorithm accuracy and gender[a], temperature category (temp_cat)[b], arrival weight category (wtin_cat)[c], days on feed at first treatment category (tdof_cat)[d] and selected interactions of gender, temp_cat, wtin_cat all with tdof_cat.

Dataset	FYYDNO	Fixed effects offered to each model							
		DNF	gender	temp_cat	wtin_cat	tdof_cat	gender*tdof	temp_cat*tdof_cat	wtin_cat*tdof_cat
COMBO	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.02	< 0.01
1	NA	-	-	< 0.01	0.03	0.04	-	-	0.02
2	NA	< 0.01	-	-	-	< 0.01	-	-	-
3	NA	< 0.01	0.04	-	< 0.01	< 0.01	-	-	< 0.01
4	NA	< 0.01	-	< 0.01	< 0.01	< 0.01	-	-	-
5	NA	-	0.01	0.01	< 0.01	< 0.01	-	-	-
6	NA	< 0.01	0.01	< 0.01	0.80	0.94	-	-	< 0.01
7	NA	< 0.01	< 0.01	-	< 0.01	0.27	-	-	< 0.01
8	NA	< 0.01	-	< 0.01	-	< 0.01	-	-	-
9	NA	< 0.01	< 0.01	-	< 0.01	0.08	-	-	< 0.01
10	NA	< 0.01	-	0.02	< 0.01	< 0.01	-	-	< 0.01
11	NA	-	-	< 0.01	-	-	-	-	-
12	NA	< 0.01	< 0.01	0.04	-	< 0.01	-	-	-
13	NA	< 0.01	0.06	< 0.01	0.75	0.05	0.02	0.01	0.02
14	NA	< 0.01	-	< 0.01	< 0.01	< 0.01	-	-	0.01
15	NA	< 0.01	-	-	-	-	-	-	-
16	NA	< 0.01	-	-	< 0.01	< 0.01	-	-	0.02
17	NA	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-	-	-
18	NA	< 0.01	-	-		< 0.01	-	-	-
19	NA	< 0.01	-	0.270	< 0.01	< 0.01	-	< 0.01	-
20	NA	< 0.01	-	< 0.01		0.012	-	-	-

21	NA	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-	-	-
22	NA	0.03	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	-	< 0.01
23	NA	< 0.01	-	< 0.01	< 0.01	< 0.01	-	-	-

(-) Indicates P > 0.05

[a] Gender was categorized into: MAL = males, FEM = females, MIX = mix of males and females, HOL = Holstein

[b] Rectal temperature (temp_cat) was categorized into: < 39.1, 39.1 to 39.4, 39.41 to 40, and > 40 degrees Celsius.

[c] Arrival weight was categorized into 5 categories: < 181kg (400lbs), 181 to 226 kg (400 to 500 lbs), 227 to 272 kg (501 to 600 lbs), 273 to 318 (601 to 700 lbs), and > 318 kg (700lbs).

[d] Days on feed at treatment was categorized into animals less than 15 days on feed, 15 to 30 days on feed, 30 to 45 days on feed and greater than 45 days on feed.

Table 6.5 Results of logistic regression models of associations between classifier accuracy and gender[a], temperature category (temp_cat)[b], arrival weight category (wtin_cat)[c], days on feed at first treatment category (tdof_cat)[d] and known status of animal finishing the production cycle (DNF).

dataset	Variable	DNF	gender	temp_cat (°C)	wtin_cat (lbs)	tdof_cat	n	Agreement	SE
1	temp_cat			39.1 to 39.4			456	0.922	0.014
1	temp_cat			39.41 to 40.0			2221	0.897	0.010
1	wtin_cat				227 to 272		1843	0.886	0.009
1	wtin_cat				273 to 318		2956	0.901	0.007
1	wtin_cat				>700		2992	0.909	0.007
1	tdof_cat					< 15 d	3535	0.902	0.012
1	tdof_cat					15 to 30 d	2397	0.916	0.017
1	wtin_cat*tdof_cat				<181	< 15 d	38	0.926	0.041
1	wtin_cat*tdof_cat				<181	15 to 30 d	21	0.955	0.044
1	wtin_cat*tdof_cat				181 to 226	< 15 d	328	0.886	0.018
1	wtin_cat*tdof_cat				181 to 226	> 45 d	160	0.890	0.023
1	wtin_cat*tdof_cat				227 to 272	15 to 30 d	564	0.891	0.013
1	wtin_cat*tdof_cat				227 to 272	30 to 45 d	225	0.904	0.019
1	wtin_cat*tdof_cat				227 to 272	> 45 d	314	0.891	0.017
1	wtin_cat*tdof_cat				273 to 318	< 15 d	1278	0.900	0.009
1	wtin_cat*tdof_cat				273 to 318	15 to 30 d	743	0.912	0.011
1	wtin_cat*tdof_cat				273 to 318	30 to 45 d	315	0.902	0.017
1	wtin_cat*tdof_cat				273 to 318	> 45 d	620	0.891	0.013
1	wtin_cat*tdof_cat				>318	< 15 d	1140	0.926	0.008
1	wtin_cat*tdof_cat				>318	15 to 30 d	741	0.925	0.010
1	wtin_cat*tdof_cat				>318	30 to 45 d	270	0.896	0.018
1	wtin_cat*tdof_cat				>318	> 45 d	841	0.883	0.012
2	didnotfinish	0					5138	0.983	0.002

5	gender		HOL				122	0.970	0.018
5	gender		MIX				55	1.000	0.000
5	temp_cat			< 39.1			188	1.000	0.000
5	temp_cat			39.1 to 39.4			175	1.000	0.000
5	temp_cat			39.41 to 40.0			1381	1.000	0.000
5	temp_cat			> 40.0			2087	1.000	0.000
5	wtin_cat				<181		218	1.000	0.000
5	wtin_cat				181 to 226		1276	1.000	0.000
5	wtin_cat				227 to 272		1200	1.000	0.000
5	wtin_cat				273 to 318		718	1.000	0.000
5	wtin_cat				>318		544	0.999	0.000
5	tdof_cat					< 15 days	1704	1.000	0.000
5	tdof_cat					15 to 30 days	898	1.000	0.000
5	tdof_cat					30 to 45 days	374	1.000	0.000
5	tdof_cat					> 45 days	981	1.000	0.000
6	didnotfinish	0					1621	0.930	0.012
6	temp_cat			39.41 to 40.0			377	0.924	0.023
6	wtin_cat*tdof_cat				<181	< 15 d	13	0.905	0.079
6	wtin_cat*tdof_cat				227 to 272	15 to 30 d	53	0.945	0.027
7	didnotfinish	0					4779	0.927	0.026
7	gender		MIX				20	0.923	0.081
7	wtin_cat*tdof_cat				<181	30 to 45 d	15	0.902	0.080
8	didnofinish	0						0.861	0.012
9	didnotfinish	0					6661	0.970	0.006
9	wtin_cat*tdof_cat				<181	15 to 30 days	81	0.911	0.056
11	temp_cat			< 39.1			14	0.929	0.069
11	temp_cat			39.1 to 39.4			22	0.955	0.044
11	temp_cat			39.41 to 40.0			48	0.958	0.029
12	didnotfinish	0					2741	0.936	0.007

13	temp_cat*tdof_cat		<39.1		15 to 30 days	93	0.928	0.035
13	temp_cat*tdof_cat		39.1 to 39.4		15 to 30 days	86	0.941	0.033
13	temp_cat*tdof_cat		39.1 to 39.4		> 45 days	167	0.937	0.025
13	temp_cat*tdof_cat		39.41 to 40.0		15 to 30 days	560	0.930	0.015
13	temp_cat*tdof_cat		39.41 to 40.0		30 to 45 days	348	0.935	0.019
14	didnotfinish	0				2730	0.983	35.754
14	temp_cat		< 39.1			74	0.966	68.032
14	temp_cat		39.1 to 39.4			77	0.979	43.554
14	temp_cat		39.41 to 40.0			356	0.970	61.386
14	wtin_cat			227 to 272		189	0.999	5.021
14	tdof_cat				30 to 45 days	388	1.000	1.543
14	wtin_cat*tdof_cat			227 to 272	30 to 45 days	22	1.000	0.000
18	didnotfinish	0				3403	0.950	0.006
19	temp_cat*tdof_cat		≥ 104		> 45 d	227	0.973	0.021
20	didnotfinish	0				9985	0.952	0.004
22	wtin_cat*tdof_cat			>318	15 to 30 d	1522	0.924	0.008
22	wtin_cat*tdof_cat			>318	30 to 45 d	749	0.936	0.009

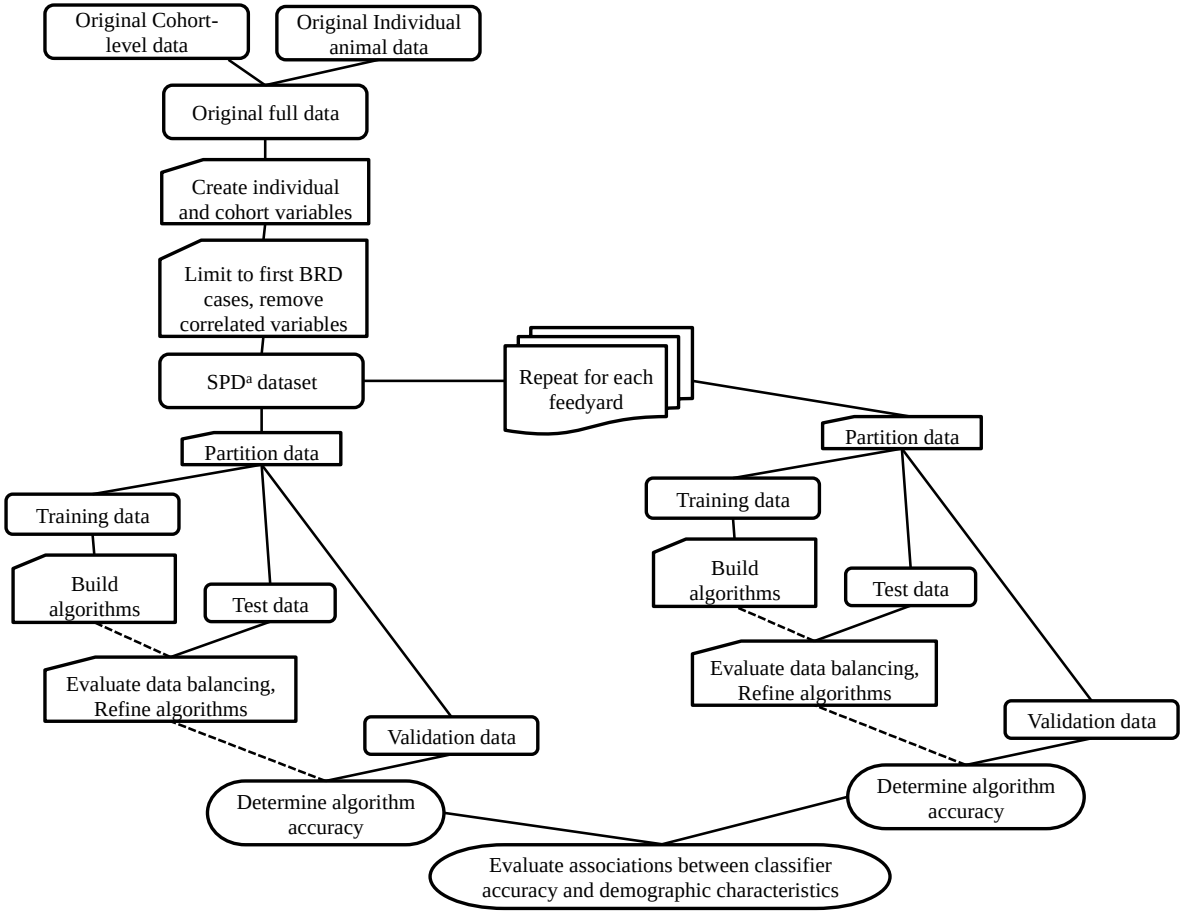
[a]Gender was categorized into: MAL = males, FEM = females, MIX = mix of males and females, HOL = Holstein

[b] Rectal temperature (temp_cat) was categorized into: < 39.1, 39.1 to 39.4, 39.41 to 40, and > 40 degrees Celsius.

[c] Arrival weight was categorized into 5 categories: < 181kg (400lbs), 181 to 226 kg (400 to 500 lbs), 227 to 272 kg (501 to 600 lbs), 273 to 318 (601 to 700 lbs), and > 318 kg (700lbs).

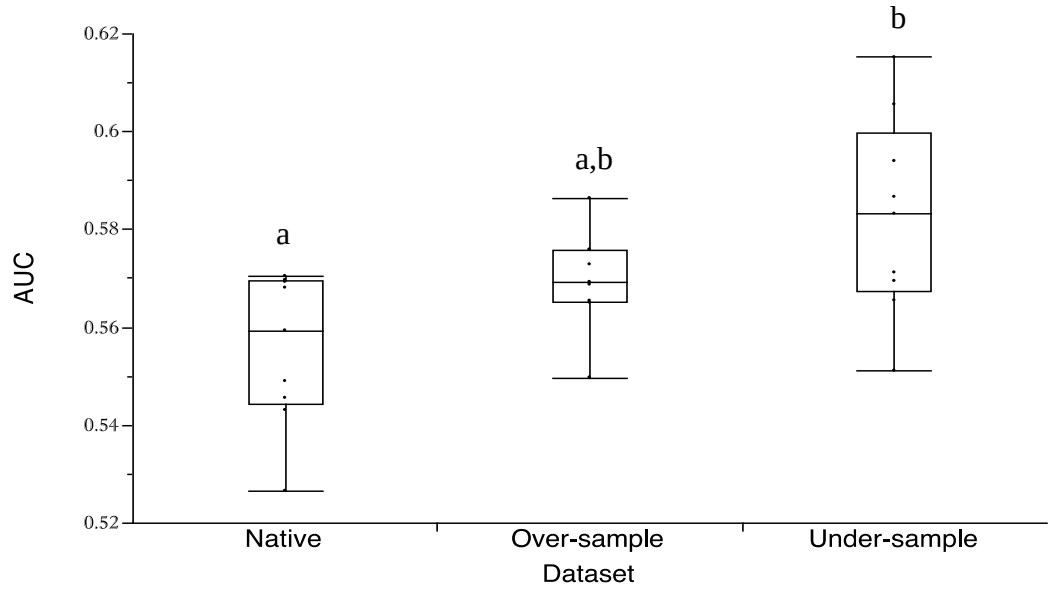
[d] Days on feed at treatment was categorized into animals less than 15 days on feed, 15 to 30 days on feed, 30 to 45 days on feed and greater than 45 days on feed.

Figure 6.1 Schematic flow of data refinement, partitioning and classification algorithm evaluation



[a] Study Population dataset

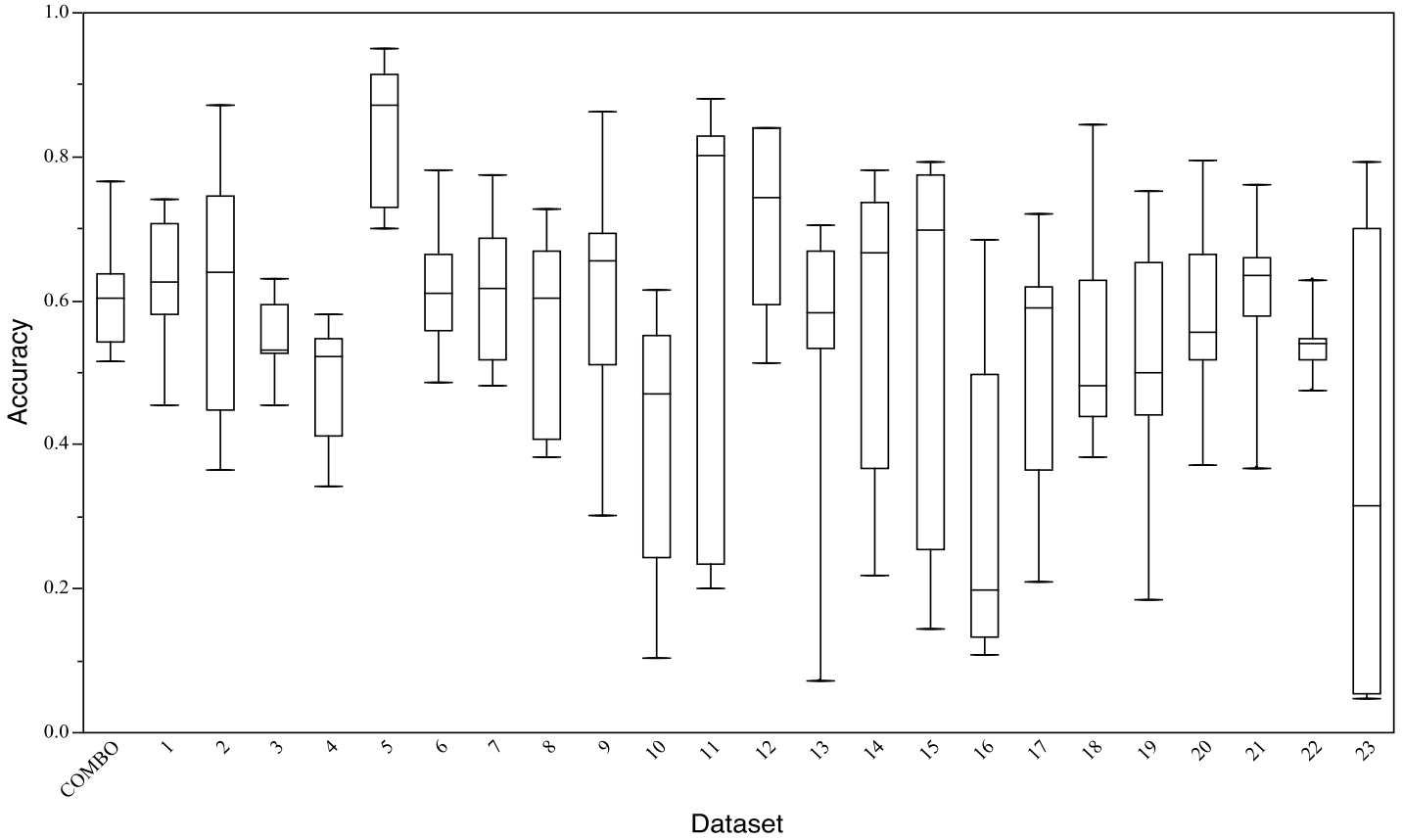
Figure 6.2 Box and whisker plots of classifier[a] Area under the receiver operating characteristic curve from three different versions of the full dataset each with different distributions of the classification variable of interest (percent of calves that did not finish feeding period within cohorts). Native (raw, observed data; 8.5% rate of calves not finishing the production cycle normally; total n= 187,493), Over-sample (duplicates of calves within the minority class until distributions are balanced; 50% calves did not finish; total n= 374,986, Under-sample (removal of records from the majority class until distributions are balanced; 50% calves did not finish; total n= 31,642).



[a] BN = Bayesnet, DS = Decisionstump, FC = Filteredclassifier, LB = Lobitboost, LR = Logistic Regression, MB = Multiboost, NB = Naïve Bayes, RF = Random forest, VP = VotedPerceptron

Datasets with different letter superscripts represent differences ($P < 0.05$) determined using Kruskal-Wallis analysis of variance accounting for multiple comparisons using the Steel-Dewass method.

Figure 6.3 Box and whiskers plots of accuracies for nine classification algorithms[a] by dataset[b]. Boxes represent the 25th and 75th quartiles and whiskers span from minimum to maximum accuracy values.



[a] BN = Bayesnet, DS = Decisionstump, FC = Filteredclassifier, LB = Lobitboost, LR = Logistic Regression, MB = Multiboost, NB = Naïve Bayes, RF = Random forest, VP = VotedPerceptron

[b] COMBO represents combined dataset with all feedyards. Individual numbers represent datasets containing one feedyard

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Chapter 7 - Dissertation conclusions

Respiratory disease in cattle continues to impact animal health and losses due to decreased performance and mortality make it the most costly disease syndrome in the beef industry. The epidemiology of BRD is multifactorial and complex and although research has uncovered some determinants of disease, quite likely there are many more yet to discover. The purpose of this dissertation research was to evaluate current methods of identifying cattle suffering from BRD, investigate methods of treating BRD, and to lay the foundation for new methods of predicting individual animal outcomes based on available data.

Identification of those animals suffering from BRD continues to be a significant challenge in adequately managing disease within a population. We found a lack of consistency among observers when using clinical signs of disease to identify morbid animals. These data indicate that visual appraisal of animals' health status lacks precision. The usefulness of a diagnostic test with poor precision is questionable at best. The accuracy of using clinical signs of illness in relation to pulmonary lesions was also evaluated. As there is no consensus to the amount of pulmonary lesions that constitute an ill animal, we evaluated several cutpoints in total pulmonary consolidation. We found that in general, as pulmonary lesions increased, observers were more likely to identify ill animals. Conversely, observers were also more likely to falsely identify animals as ill when higher thresholds of disease were evaluated. While there are limitations in this study that impact external validity, these data are still relevant to those using clinical signs of illness to identify morbid animals and provide novel information that should be considered when selecting clinical sings of illness as a diagnostic test.

Inflammation associated with BRD can cause significant lung damage leading to decreased pulmonary function as well as decreased animal performance and carcass characteristics. The acute phase of inflammation is thought to be a critical control point in eliminating some of these detrimental impacts. We conducted a study to evaluate how calves with experimental bacterial pneumonia respond to early treatment with flunixin meglumine, alone or in combination with the antimicrobial florfenicol. Results from this study were less conclusive than hoped due to a highly variable response to experimental challenge by study calves. None of the serum biomarkers or CBC and chemistry values measured provided significant associations with the magnitude of pulmonary lesions observed at necropsy.

Upon feedyard arrival, limited tools are available to manage the risk of BRD. Metaphylaxis is frequently administered to those animals considered to be at an increased risk of BRD. We evaluated the long-term efficacy of two macrolide antimicrobials on mitigation of pulmonary lesions compared to negative (saline) controls in calves challenged with *Mannheimia haemolytica* 10-days post treatment. The goal of this study was to simulate an outbreak of BRD 10 days after calves had received metaphylactic therapy. These data suggest that calves treated with tildipirosin 10 days prior to MH challenge have less pulmonary damage and fewer clinical signs of illness compared to calves treated with tulathromycin or saline. More research is needed to determine the response of calves treated with tildipirosin in relation to natural disease challenge.

Within a feedyard production system, the ability to accurately predict health outcomes of individual animals would provide tremendous benefits to more efficiently manage animals. Research on BRD has evaluated retrospective data for risk factors associated with developing disease, but literature does not exist evaluating the ability to predict individual animal health outcomes based on routinely collected feedyard data. We evaluated the ability of several different classification algorithms to identify calves that would not finish the production cycle normally. Results from this study revealed the importance of using balancing techniques when the class of interest is imbalanced or rare, and pairing the appropriate classification algorithm with the data available. While overall classifier accuracy of combined and individual feedyards were relatively low, we identified sub-groups of calves within populations where classifiers performed extremely well. The amounts of data available within feedyard production systems continue to increase. These methodologies have laid a framework that can be used to develop more accurate predictions related to individual calf outcomes.