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Utilizing Behavioral Monitoring to Detect Sickness or Injury in Dairy Cow and Calves

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To the Graduate Council:

I am submitting herewith a thesis written by Nicole Louise Eberhart entitled "Utilizing Behavioral Monitoring to Detect Sickness or Injury in Dairy Cow and Calves." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Peter D. Krawczel, Major Professor

We have read this thesis and recommend its acceptance:

Marc Caldwell, Gina M. Pighetti

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(Original signatures are on file with official student records.)

Utilizing Behavioral Monitoring to Detect Sickness or Injury in Dairy Cow and Calves

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Nicole Louise Eberhart

August 2016

DEDICATION

Here in this body of work is dedicated to all who offered support.

First of which, the feline Meridon, my main consort.

Secondly, my advisor, Dr. Krawczel, the wellspring of many great visions,

Also the source of my provisions.

The third cohort, a magnificent blonde,

Randi is her name, of which I have grown fond.

Fourthly, my younger sister, whose general enthusiasm

Has always given me gleeful spasms.

Finally, my mother, who has always been a great role model.

Who taught me independence and passion; full of patience even when in life I dawdled.

Many more have had a great role in all that I have accomplished in this time,

Unfortunately, the words to be said of such inspiration do not rhyme.

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ABSTRACT

Cattle experience behavioral changes during incidences of discomfort such as respiratory disease, hock injuries, and lameness. Visual evaluation of these conditions, particularly lameness and respiratory disease can be subjective and may lead to untreated animals, which reduces the overall well-being of the animals. In order to improve detection of affected cattle, continuous or combined monitoring systems should be used. These technologies may improve detection compared to visual assessment by reducing human bias. Behavioral changes in particular may be detected through these means, with changes indicating potential health abnormalities in the individual cattle. One such behavior that changes during incidences of discomfort is lying laterality, or the preference of lying on one side over the other. Calves infected with the bacterium, *Mannheimia haemolytica,* spent more time lying on their right side than their left side (7.8 ± 0.3 vs. 6.8 ± 0.3 h/d, $P = 0.01$) and experienced a mild respiratory infection as indicated by greater rectal temperatures 12 hours post inoculation compared to control calves (41.3 ± 0.3 °C vs 39.2 ± 0.3 °C; $P < 0.01$) and mean lung lesion scores (7.32 ± 0.39%). Lactating dairy cattle on Croatian dairy farms with unilateral hock injuries spent more time lying on their left side than their right compared to cows with bilateral injuries and uninjured cows ($P = 0.02$). Changes in lying behavior combined with presence of lung lesions or hock injuries indicate increased discomfort and decreased welfare in calves and cows.

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Behavior

Animal behavior changes during sickness (Dantzer and Kelley, 2007) and general discomfort (Bao and Giller, 1991, Fregonesi et al., 2007, Allen et al., 2013). Dairy cattle are no exception to this and may experience changes in lying times (Ito et al., 2010), lying side preference (Tucker et al., 2009), and feeding behavior (Buhman et al., 2000, Theurer et al., 2013) during incidences of discomfort.

Normal cattle behavior: lying behavior

Age influences the amount of time cattle spend lying down, resting (Hanninen et al., 2005). Resting time in young calves has been positively correlated to growth rates (Mogensen et al., 1997), where a decrease in lying times were associated with a decrease in growth rates. This suggests that a minimum resting time is necessary for adequate growth in calves. Adult lactating dairy cattle spend approximately 12 hours per day lying down (Ito et al., 2009) while pre-weaned calves will spend approximately 17 - 19 hours per day lying down (Hanninen et al., 2005, Bonk et al., 2013) and weaned calves will spend approximately 15 – 16 hours per day resting (Faerevik et al., 2008).

Although accelerometers are commonly used to record lying behaviors in calves and adult cows (Ledgerwood et al., 2010, Bonk et al., 2013), utilizing automatic technologies to determine the amount of time cattle spend sleeping is less common(Hokkanen et al., 2011). However, recent research has been focused on determining sleep characteristics in cattle, particularly identifying REM (rapid eye movement) and NREM (non-rapid eye movement) sleep in cows using EEG (Electroencephalographic) data (Hanninen et al., 2008). REM sleep is important for proper development in human infants (Cornwell and Feigenbaum, 2006). Research suggests a relationship between disrupted REM sleep and an increased risk for SIDS (sudden infant death syndrome) in infants (Cornwell and Feigenbaum, 2006). REM sleep is characterized by muscle atony, particularly in the neck muscles, which requires the head to be positioned on the ground, requiring that the animal be in a resting position (Hanninen et al., 2008). This further exemplifies the importance of adequate lying time in cattle, particularly in developing calves. The identification of inadequate lying time and therefore sleep time is imperative for mitigating the consequences of these conditions.

Normal cattle behavior: feeding behavior

Pre-weaned calves are commonly fed two or less quarts of milk per each feeding, twice daily until weaning, at 6 – 8 weeks of age (USDA, 2016). However, research has indicated improved growth rates in calves fed ad libitum access to milk (Jasper and Weary, 2002). Heifers have been found to spend approximately 3.5 to 4 hours at the grain bunk or hay feeder (Theurer et al., 2013). Healthy, adult lactating cows have been found to spend 2.5 to 3.5 hours at the feed bunk (Huzzey et al., 2007). Multiparous cows are often more aggressive at the feed bunk than primiparous cows (Huzzey et al., 2007).

Sickness behavior

Behavioral changes associated with illness are complex. Increased lethargy and decreased appetite are common symptoms in sick mammals, however, the cause and effect relationship of behavior and cytokine related illness (sickness causing an increase in pro-inflammatory cytokines) is complicated. Increased isolation and decreased appetite are thought to be a response to peripheral LPS (lipopolysaccharide; a component of gram negative bacteria) and IL-1 (interleukin 1; a pro-inflammatory cytokine involved in the immune and inflammatory response) (Konsman et al., 2002). The body responds to the presence of LPS by producing IL-1; production of IL-1 further propagates production of pro-inflammatory cytokines as well as increases production of anti-inflammatory cytokines to mitigate the inflammatory response (Konsman et al., 2002). Thus, the production of IL-1 through responding to the LPS associated with gram negative bacteria produces behaviors associated with sickness: increased depression and anorexia (Konsman et al., 2002).

Although these behaviors change during the cytokine response, research may suggest that sickness behavior is less noticeable during adverse conditions (Konsman et al., 2002, Borderas et al., 2009). As described by Konsman (2002), maternal behaviors (nest building and retrieval of dispersed pups) were evaluated in saline-injected and LPS-injected mice at ambient temperatures (22°C) and a colder environment (6 °C). When behaviors were evaluated at ambient temperatures, LPS-injected mice took longer to retrieve pups than saline-injected mice and did not build a nest. However, when the environmental temperature was at 6 °C, LPS-injected mice retrieved pups at the same speed as saline-injected mice and built a near-full nest (Konsman et al., 2002). In a study by Borderas (2009), calves were diagnosed as sick or healthy by a veterinarian; sick calves were categorized as having a gastrointestinal illness, respiratory illness, or both. Sick and healthy calves were assigned to either a low milk allowance diet (4 L/d) or a high milk allowance diet (12+ L/d). Calves fed a low milk allowance diet did not differ in milk intake despite health status. However, a difference in milk intake was significant between sick and healthy calves fed a high milk allowance diet (Borderas et al., 2009). In the case of the maternal mice behaviors, in adverse conditions (low ambient temperatures), maternal behaviors were similar to healthy mice. Similarly, sick calves fed restricted diets did not differ milk intake compared to healthy calves. This indicates that sickness behavior may go undetected during certain negative environmental conditions (increased cold temperatures and feed restriction).

Respiratory disease: lying behaviors

Behavioral changes such as increased lethargy and decreased appetite are common characteristics of illness found in mammals (Bluthe et al., 1992, Kent et al., 1996, Ghai et al., 2015). These behavioral changes found in sick calves (Bunger et al., 1988) may be detectable through behavioral monitoring (Bonk et al., 2013, Theurer et al., 2013). Previous behavioral analysis of calves infected with *Mannheimia haemolytica* suggested infected calves will spend more time lying down compared to uninfected calves (Theurer et al., 2013). However, previous research evaluating the change in behavior following a low dose bacterial endotoxin challenge indicated no significant differences in lying time around the 2 hours of a fever spike (Borderas et al., 2008). While in the first study lying time did increase in sick calves, as would be expected due to increased lethargy associated with illness, the lack of differences in lying time in the second study may be due to the difference in observation times between the two studies. In the study by Borderas (2008) behavioral differences between the control group (inoculated with saline) and the infected group (treated with injections of LPS) were evaluated during the two hours prior to and the two hours following a spike in rectal body temperature (Borderas et al., 2008). Although rectal temperatures spiked to approximately 40.5 °C 4 hours after LPS injection, no differences between lying times were observed in the hours surrounding this peak (Borderas et al., 2008).

Respiratory disease: feeding behavior

The second signature characteristic of illness is anorexia or loss of appetite (Konsman et al., 2002). Sick calves arriving in feedlots have an increased duration and frequency of drinking water 4 to 5 days after arrival at the feedlot but experience a decrease in frequency and duration of eating 11 to 27 days after arrival (Buhman et al., 2000). Interestingly, sick calves had greater frequency and duration of eating 28 to 57 days following arrival than calves that were not sick (Buhman et al., 2000). Sick cattle may be less aggressive at the feed bunk as well (Sowell et al., 1998). Healthy (non-sick) cattle spend more time at the feed bunk in the first 15 minutes following feed delivery (Sowell et al., 1998).

Lameness and hock injuries: lying behavior

The risk for lameness and hock injuries can increase with abnormal lying behaviors, such as increased difficulty in standing up and lying down (Brenninkmeyer et al., 2013). Lame cows may spend more time lying down (Chapinal et al., 2010) than sound cows, which additionally increases the amount of time hocks are exposed to rough free stall surfaces; thus increasing the risk of developing hock injuries.

Lameness and hock injuries: feeding behavior

Lame cows will feed faster, but overall time spent eating silage decreases (Norring et al., 2014). Lame cows at farms with automatic milking systems also show a preference for location at the feed bunk, avoiding feed bunks that are further from the exit of the automatic milking system (Bach et al., 2007). The increase in feeding rate with increased locomotion scores and decreased overall feeding time (Norring et al., 2014) compliments the increased lying time of lame cows (Chapinal et al., 2010); lame cows are motivated to lay down longer than sound cows and therefore have less time to eat. Additionally, multiparous cows are more aggressive at the feed bunk than primiparous cows, which may exaggerate a decrease in overall feeding time in lame primiparous cows (Bach et al., 2007, Huzzey et al., 2007).

Lying behavior and laterality

Changes in lying laterality may occur when cows experience discomfort (Bao and Giller, 1991, Forsberg et al., 2008) but the full extent of uncomfortable situations that may cause a laterality preference is unknown. Changes in lying behavior may be linked to lameness (Ito et al., 2010). Because lameness is thought to potentially occur before hock injuries present themselves, through increased difficulty of lying and rising (Brenninkmeyer et al., 2013), it is possible that any behavioral changes that occur which may lead to hock injuries may be missed once hock injuries are assessed. Because severe hock injuries are primarily seen unilaterally instead of bilaterally (Potterton et al., 2011), investigation into the impact of severe unilateral hock injuries on lying side preference could further expound potential relationships.

Pain and Inflammation

Pain

Pain is the recognition by the central nervous system of actual or potential tissue damage. Noxious stimuli trigger pain receptors that send signals to the central nervous system via three main pathways: the neospinalthalamic tract, the paleospinalthalamic tract, and the archisponothalamic tract. The neospinalthalamic tract is the pathway in which pricking pain like cutaneous, superficial, or peripheral pain signals travel. The paleospinalthalamic and archisponothalamic tracts are pathways that deep or visceral pain signals travel. Pain sensations related to deep or visceral pain consist of burning or soreness from tissue damage in cutaneous, superficial, or peripheral areas of noxious stimuli activation (Neuroscience online, 2015). Utilizing these ideas of pain pathways, it is possible that cattle with respiratory disease might experience deep or visceral pain associated with lung lesions via the paleospinalthalamic and archisponothalamic tracts. Cattle with hock injuries would most likely experience pain through all three pathways: neospinalthalamic through surface abrasions and paleospinalthalamic and archisponothalamic tracts through inflammation. However, pain associated with respiratory disease in calves and hock injuries in cattle have yet to be quantified. Behavioral changes and physiological changes have been found to occur in respiratory infections (Schaefer et al., 2007, Miyamoto et al., 2010, Theurer et al., 2013) and hock injuries (Westin et al., 2016), and lameness (Ito et al., 2010) indicating that animals with these conditions experience some level of discomfort.

Pain receptors (nociceptors)

Four main pain receptors exist in mammalian organisms and differentiate based on what type of stimuli activate them and where they are found. These include skin, joint, visceral, and “silent” (Neuroscience online, 2015) nociceptors. Nociceptors are nerve cell endings that are unspecialized and initiate pain sensations. (Purves et al., 2001). Skin, joint, visceral, and “silent” (Neuroscience online, 2015) nociceptors can again be further categorized into high threshold mechanonociceptors, thermal nociceptors, chemical nociceptors, and polymodal nociceptors. High threshold mechanonociceptors on the skin respond only to intense mechanical stimulation such as pinching, cutting or stretching of the skin. Thermal nociceptors respond to mechanical stimulation as well as thermal stimuli. Chemical nociceptors respond only to chemical substances. Polymodal nociceptors are unmyelinated and respond to high intensity stimuli that can be mechanical, thermal, or chemical. Polymodal nociceptors tend to be sensitized by prolonged stimulation and then become sensitive to other sensations as well as the primary or initial mechanical, thermal, or chemical stimulus (Neuroscience online, 2015).

The process of pain perception is a complex system. Nociception is the detection of negative stimuli and transmission of signals resulting from this detection to the brain. This detection process occurs in normal tissues exposed to high intensity stimuli as well as in tissues that are inflamed or injured and are not directly exposed to external stimuli (Kidd and Urban, 2001).

Inflammation and immunology

Although clinical signs of inflammation, like those seen in hock injuries are characterized by redness and swelling locally, the trigger of inflammation in the specific region is caused by a neural process (Sternberg, 2006). In addition, the response to pathogens, like those causing bovine respiratory disease, also involves neural immune involvement (Snowder et al., 2006, Sternberg, 2006).

When pathogens cause an immune response, the first step is for the system to recognize pathogen-associated molecular patterns (PAMPS) thus creating a cellular and humoral response (Sternberg, 2006). Then the innate immune system releases cytokines and immune mediators that activate neural responses which amplify local immune responses which in turn clears pathogens. The goal of this process is to return the body to homeostasis (Sternberg, 2006).

Diagnosing ill health

Accuracy of visual detection and diagnosis

Detection of BRD lacks both sensitivity and specificity. Only 61.8% of sick calves are accurately diagnosed with BRD and only 62.8% of healthy calves are not treated for BRD (White and Renter, 2009). This suggests that approximately 40% of healthy calves are diagnosed with BRD and treated unnecessarily and approximately 40% of sick calves go untreated (White and Renter, 2009). Furthermore, a study by Thompson (2006) reported that 69.5% of calves with lung lesions at slaughter were never treated for BRD (Thompson et al., 2006). By not properly diagnosing and treating individual calves with respiratory disease, welfare of the animal decreases and costs increase. The cost of one single treatment of BRD was $23.60 in 2013 (USDA, 2013d). Eight-seven percent of the 16.2% of all cattle with signs of BRD were treated (USDA, 2013d). With an estimated 14 million cattle on feed in United States feedlots approximately 2 million of those cattle show signs of BRD and 1.8 million will be treated (USDA 2016).

Repeatability between multiple observers

As with many visual scoring systems, locomotion scoring may differ between multiple people, particularly cows with mild or subclinical lameness (Winckler and Willen, 2001). Inter-observer reliability of locomotion scoring is poor, especially in live scoring compared to video scoring (Schlageter-Tello et al., 2015). In the study by Winckler and Willen (2001), locomotion scores and claw health (sole and white line scores, heel horn scores, and digital dermatitis scores) data were collected. Locomotion scoring was primarily done by one person, but to measure inter-observer reliability, three observers on three farms collected locomotion data simultaneously (Winckler and Willen, 2001). All cows with severe claw disorders were lame, but not all clinically lame cows had a claw disorder; indicating that other factors were causing cows to be lame (Winckler and Willen, 2001). Individual observers agreed on locomotion scores 68% in of the cases, with the majority of the discrepancy (30%) being one unit of measure (Winckler and Willen, 2001).

In the study by Schlageter-Tello (2015), inter-observer reliability of locomotion scoring was measured between 3 experienced individuals and two inexperienced individuals. Individual observer-1 was the most experienced observer, and was responsible for training the other observers (both experienced and inexperienced) one week prior to data collection (Schlageter-Tello et al., 2015). Training sessions were separated into 3 separate sessions; the first session introduced the scoring system to the individuals with videos of each score, the second session was a live scoring session, and the third session was a scoring session from video (Schlageter-Tello et al., 2015). In the live scoring sessions following the training, only observations between two experienced individuals (observer-1 and observer-3) reached the acceptable threshold of (κω = 0.48 – 0.53) agreement (Schlageter-Tello et al., 2015). However, during video scoring sessions, all observers (both experienced and inexperienced) reached the acceptable threshold for agreement (Schlageter-Tello et al., 2015). These studies suggest that reliability different individuals performing live locomotion scoring is not accurate, especially in cows with subclinical lameness.

Treatments: current practices

In the dairy industry, respiratory disease affects 18.1% of pre-weaned heifers (2.3% mortality rate), 11.2% of weaned heifers (1.3 % mortality rate), 1.2% of pregnant heifers (0.2 %

mortality rate), and 2.9% of cows (USDA, 2012). Approximately 82% of operations used some form of antibiotics to treat respiratory disease in weaned heifers, 87.1% of operations used medicated feed and approximately 98% of heifers diagnosed with respiratory disease were treated in 2010 (USDA, 2012). The two most common antibiotics used to treat respiratory disease in weaned heifers were macrolides and florfenicol (USDA, 2012).

In pre-weaned heifers, 88.6% of operations used a form of antibiotics to treat respiratory disease; the three primary antibiotics were macrolides, florfenicol, and fluoroquinolones (USDA, 2012). Approximately 90% of pre-weaned heifer calves that were affected by respiratory disease were treated with antibiotics (USDA, 2012).

Almost all (99.8%) pregnant heifers affected by respiratory disease were treated, but only 44.8% of operations treated pregnant heifers with antibiotics; 0.6% of operations did not treat affected heifers and the other 54.6% had no respiratory disease on farm (USDA, 2012). Primary antibiotics used to treat respiratory disease in pregnant heifers were florfenicol and macrolides (USDA, 2012).

Respiratory disease is less common in adult cows than heifer calves with only 2.9% of cows affected by respiratory disease in 2006 (USDA, 2008). Most of affected cows were treated (96.4 %), but due to the low incidence of respiratory disease in adult cows, only 2.8% of total adult cows were treated respiratory disease (USDA, 2008). Primary antibiotics used to treat respiratory disease in adult cows were cephalosporin and Beta-lactam (USDA, 2008).

In the beef industry in 2011, 16.2% of cattle on feed were diagnosed with respiratory disease and of those diagnosed, 87.5% were treated (USDA, 2013c). Ninety-six percent of feedlots vaccinated cattle during initial processing; however, despite these common vaccination practices, 95.6% of feedlots had cattle affected by respiratory disease (USDA, 2013b). Additionally, 59.3% of feedlots treated cattle metaphylactically (treating all cattle at one time to prevent potential outbreaks) through an injectable antibiotic (USDA, 2013c).

On beef cow-calf operations, 3.8% of calves, 3.2% of replacement heifers, and 0.4% of cows were affected by respiratory disease (USDA, 2010). Approximately 31% of deaths in calves 3 weeks of age or older were caused by respiratory disease, 8.2% of deaths were caused by respiratory disease in calves less than 3 weeks of age, and 3.4% of deaths in breeding cattle were caused by respiratory disease (USDA, 2010).

Prevalence of hock injuries can vary by management styles and region; previous research has reported injury rates of 57% in France (Veissier et al., 2004) and 50% in Germany and Austria (Brenninkmeyer et al., 2013). Greater prevalence of hock injuries have been found in the northeastern United States (81%) (von Keyserlingk et al., 2012) and southern British Columbia (73%) (Weary and Taszkun, 2000).

Hock injuries characterized solely by hair loss have been found to occur more often bilaterally (82.5%) than unilaterally (17.5%) where hock ulcerations have been found to occur more often unilaterally (70.9%) than bilaterally (29.1%).

Lameness has been associated with hock injuries (Klaas et al., 2003, Brenninkmeyer et al., 2013) but the timeline from hock injury to lameness or vice versa has not been determined. Cows with severe hock injuries are more likely to become lame (Klaas et al., 2003); however, minor hock injuries such as hair loss have been found to occur after a bout of lameness has occurred (Lim et al., 2013).

Lameness or injury was the 2nd most common health problem in mature dairy cows with 12.5 % of cows being affected (USDA, 2009). However, farmers reported 23.9% of mature cows being lame at least once in the past 12 months and 11.4 % of bred heifers being lame at least once in the past 12 months (USDA, 2009).

Lameness was caused by digital dermatitis in 49.1% of cows and 61.8% of heifers (USDA, 2009). Approximately 7% of cows were treated with antibiotics, with 56.5% of affected cows being treated (USDA, 2008). Lameness is also a common problem on feedlots; 92.8% of feedlots had lame cattle and 1.8% of cattle on feedlots were lame (USDA, 2013c). Older cows are more likely to have hock injuries (Rutherford et al., 2008) and have a higher prevalence of lameness (Haskell et al., 2006).

Detection methods

Certain diseases in dairy cattle, such as ketosis and metritis can be detected on farm with relative ease. Ketosis, a condition occurring most commonly in early lactation dairy cattle, is marked by a rise in body ketone body levels (β-hydroxybutyrate, acetone, and acetoacetate) caused by a significant increase in fat mobilization (Goff and Horst, 1997) that occurs shortly after calving. Behavioral changes associated with ketosis include decreased feed intake, decreased milk production, and increased lethargy (Herdt, 2014). Diagnosis of ketosis is based on cow characteristics that may be risk factors, such as stage of lactation as well as clinical signs and the presence of ketone bodies in the milk or blood serum (Herdt, 2014). Currently, cow-side detection methods have been proven effective for diagnosis, such as ketone urine strips (Nielen et al., 1994). Regular automatic testing of β-hydroxybutyrate in milk samples can also be used to detect cases of subclinical ketosis (DeLaval).

Clinical metritis can be detected through visual signs; cows with metritis display foul smelling uterine discharge (Burnell). Cows with metritis also spend less time at the feed bunk than healthy cows after parturition and cows with acute metritis spend less time feeding pre-parturition than other cows (Urton et al., 2005). Cows with severe metritis will consume less dry matter than healthy cows (Huzzey et al., 2007). These studies indicate that feeding behavior can be used to determine cows at risk for metritis. However, as decreased feeding time and feed intake decreases in most illness cases, feeding behavior may be more useful as a single tool in a multitude of strategies for diagnosis of various diseases.

Novel detection and diagnostic methods

Visual detection of ill health in cattle can be subjective and lack accuracy (Winckler and Willen, 2001, White and Renter, 2009), therefore, research has turned to automatic detection technologies that can remove human error in detecting activity, behavioral, and physiological changes in individual animals.

One of the biggest challenges researchers, producers, and veterinarians face is measuring and quantifying the amount of pain animals experience in disease or ill health conditions. The use of algometry has been able to determine pain sensitivity in calves being dehorned (Heinrich et al., 2010) and cows that are lame (Tadich et al., 2013). Mechanical nociceptive threshold (MNT) is the measurement of pain sensitivity through applying pressure to an area on the animal and measuring how much pressure is required to elicit a response from the animal. This method can measure levels of sensitivity in different animals, i.e. a calf that has been dehorned and treated with a pain mitigation drug compared to a calf that was not (Heinrich et al., 2010). Previous research has been able to detect pain tolerance differences between groups of calves being dehorned (Heinrich et al., 2010), providing measurable data to the extent of discomfort experienced by calves following dehorning. Further, research suggests that calves may still be sensitive to pain 24h after dehorning, as pain sensitivity did not return to baseline levels at that time (Stock et al., 2015). However, measurements between individuals may vary and may lack consistency over time when measuring pain sensitivity in cows with claw lesions (Raundal et al., 2014).

Infrared thermography has been used to measure inflammation associated with respiratory disease in calves (Schaefer et al., 2007), subclinical mastitis in cows (Pampariene et al., 2016), and lameness in cows associated with lesions (Wood et al., 2015). In calves with respiratory disease, the use of infrared imaging is able to detect calves in the early stages of the disease (Schaefer et al., 2007) and in cows, increased inflammation associated with subclinical mastitis can be observed using this technology (Pampariene et al., 2016). The usefulness of this technology is particularly apparent in its ability to detect disease at the early stages. Further, the ability to definitively determine the absence or presence of the condition, as demonstrated in the use of infrared thermography in detection of foot lesions (Wood et al., 2015), establishes the practicality of this tool for on farm use.

Accelerometers can be used to measure activity of cattle, which can provide important information on the health status of individual animals. Accelerometers have been established in the use of detecting activity differences in calves with respiratory disease (Theurer et al., 2013), normal and abnormal lying behaviors in adult cattle (Ito et al., 2009, Ito et al., 2010, Ledgerwood et al., 2010), as well quantifying normal lying behaviors in calves (Bonk et al., 2013). Utilizing automatic activity and behavior data loggers allows for an elimination of human bias in behavioral observation.

Surfactant proteins A (SP-A) and D (SP-D) are a part of the protein fraction of pulmonary surfactant. Surfactant protein A and SP-D are primarily involved in the innate defense through assisting resident phagocytic cells in the clearing of pathogens. These proteins are upregulated during infection and thus have the potential of entering circulation systemically through an increase in lymphatic drainage or leakage through damaged alveolar basement membranes, which occurs during respiratory disease (Honda et al., 1995, Miyamoto et al., 2010). Research has demonstrated an increase in SP-D concentrations in lungs of lambs with respiratory disease (Grubor et al., 2004), in calves experimentally inoculated with *Mannheimia haemolytica* (Miyamoto et al., 2010), and SP-D concentrations in blood serum of horses experimentally infected with *Streptococcus zooepidemicus* (Hobo et al., 2007).

CHAPTER ONE

Behavioral and Physiological Changes in Dairy Steer Calves Infected with *Mannheimia haemolytica*

ABSTRACT

The objective of this study was to determine the extent of behavioral changes in Holstein dairy steer calves in response to a *Mannheimia haemolytica* infection. Behavior data were collected over 7d from 12 steer calves aged 4 - 5 mo with mean body weight of 166.5 ± 13.0 kg. On d 0, treatment calves (MH; n = 6) were inoculated endoscopically in the tracheal bronchus with 3 - 5 x 10^9 cfu of *M. haemolytica* suspended in 5 ml of PBS followed by 60 ml wash PBS. Control calves (n = 6) were sham inoculated with 5 ml sterile PBS and subsequent 60 ml PBS. Mixed grass hay and water were available ad libitum. Approximately 2.7 kg of pelleted feed per calf was provided daily. Control and MH calves were group housed in an open-sided covered barn with dirt floor pens. Treatment pens were separated by 10 m. Both pens measured 20 × 20 m with 66.7 m^2 per calf. All calves were acclimated to pens 7 d prior to inoculation and monitored for disease 2× daily. Prior to inoculation, calves randomly sorted into treatment groups and blocked by body weight. Data loggers were attached 2 d prior to inoculation. Data, recorded at 1-min intervals, were summarized into daily lying times (h/d) and lying time laterality (h/d). Rectal temperatures (RT), respiration rates (RR), and clinical illness scores (CIS; 1 = normal; 4 = severe illness) were recorded 2× daily following inoculation. RT and RR were collected while calves were restrained in the headlocks. CIS were assessed from outside of the pen on unrestrained calves. A mixed model was used to determine effect of treatment, date and their interaction on total lying time, RT, RR, and CIS. Calf within treatment was treated as a random variable and observations were repeated by day. Laterality was analyzed using the above model plus split-plot effects of lying side and its interactions. Compared to control calves, MH calves lay down longer on d 0 (16.4 ± 0.5 vs. 14.4 ± 0.5 h/d, $P < 0.01$). MH calves spent more time lying on their right side than their left side (7.8 ± 0.3 vs. 6.8 ± 0.3 h/d, $P = 0.01$). No lateral preference was evident from control calves ($P = 0.22$). Mean RT of MH calves 12 hr post inoculation (41.3 ± 0.3 °C) exceeded 40 °C and was greater than control calves (39.2 ± 0.3 °C; $P < 0.001$). Mean RR of MH calves was greater over the study period ($P = 0.03$). Mean CIS of MH calves (2.2 ± 0.1) was greater than control calves (1.0 ± 0.1) 12 hr post inoculation ($P < 0.001$). Infection of *M. haemolytica* likely increased lethargy and shifted lying side preference. As a result, continuous behavioural monitoring may improve disease detection in calves.

Introduction

Bovine respiratory disease (BRD) is the most common cause of illness and mortality in cattle feedlots in the United States (USDA, 2013c). The last USDA-NAHMS (2013) feedlot survey reported 16.2% of cattle in feedlots displayed signs of respiratory illness and 87.5% of those with clinical symptoms were treated with antimicrobials. With 14.0 million cattle on feed in January 2011,(USDA, 2013a) this suggests that approximately 2 million feedlot cattle experienced symptoms and 1.8 million were treated for BRD (USDA 2016). The average cost of one treatment for BRD is $23.60 (USDA, 2013c); almost double the cost in 1999 (USDA, 2000). Therefore, the total cost of treating feedlot cattle diagnosed with clinical BRD may approach $40 million annually. However, this number may likely be an underestimation of the total costs of BRD; mild and subclinical cases of BRD were not included in the estimation of treated cattle (USDA, 2013c). Further, meat quality and yield also decreases in calves diagnosed and treated for BRD (Duff and Galyean, 2007). In addition to beef cattle in feedlots, respiratory disease was the most common illness found in weaned dairy heifers (11.2%) and the second most common illness in pre-weaned dairy heifers (18.1%), further demonstrating the overall impacts of BRD in US cattle production (USDA, 2012).

Early detection and treatment of BRD is important to ensure positive clinical outcomes (Wittum et al., 1996). Visual assessment of calves at a distance and the appraisal of subtle clinical signs often initiates the diagnosis and treatment of BRD. However, the instinctive response of ill cattle is to alter their behavior in order to appear fit, especially in the context of human interaction (Weary et al., 2009). Recently, the sensitivity and specificity of visual assessment and clinical illness scoring in the detection of calves with lung lesions was reported to be 61.8% and 62.8%, respectively (White and Renter, 2009, Leruste et al., 2012, Buczinski et al., 2014).

Behavioral changes such as increased lethargy and decreased appetite are common characteristics of illness found in mammals (Bluthe et al., 1992, Kent et al., 1996, Ghai et al., 2015). These behavioral changes found in sick calves (Bunger et al., 1988) may be detectable through behavioral monitoring (Bonk et al., 2013, Theurer et al., 2013). Previous behavioral analysis of calves infected with *Mannheimia haemolytica* suggested infected calves will spend more time lying down compared to uninfected calves (Theurer et al., 2013). Utilizing an objective measurement of activity and lying time could aid in detection of *M. haemolytica* infection or general BRD.

Surfactant proteins are a promising biomarker for the assessment of pulmonary inflammation. The protein fraction of pulmonary surfactant consists of four proteins; surfactant proteins A, B, C, and D. Surfactant proteins B and C are hydrophobic in nature and function primarily to reduce the surface tension at the air-liquid interface. Surfactant A (SP-A) and D (SP-

D) are predominantly involved in innate defense by assisting resident phagocytic cells in the clearance of pathogens. SP-A and SP-D are upregulated during infection and have the potential of entering systemic circulation through increased lymphatic drainage or leakage through damaged alveolar basement membranes.(Honda et al., 1995, Miyamoto et al., 2010) Previous studies have demonstrated an increase in SP-D concentrations in the lungs of lambs and calves experimentally infected with *M. haemolytica* and horses experimentally infected with *Streptococcus zooepidemicus* (Grubor et al., 2004, Hobo et al., 2007, Miyamoto et al., 2010).

Alternative strategies that provide greater discernment in early or subclinical cases are necessary for the industry to address judicial use of antimicrobial treatment in cattle with BRD. Utilizing physiological biological markers, such as SP-A or SP-D (Grubor et al., 2004, Hobo et al., 2007) and changes in lying behaviors (Theurer et al., 2013) could be promising tools in the identification of sick calves (Ackermann and Brogden, 2000, Theurer et al., 2013). Although previous research has identified these physiological and behavioral changes in calves with BRD, research has yet to determine the relationship between changes in lying behaviors and surfactant protein concentrations in calves with BRD.

The purpose of this study was a parallel investigation to determine (1) if accelerometers could predicatively quantify changes in behavior of infected calves and (2) if an experimental bacterial infection altered the concentration of SP-A and SP-D in the bronchoalveolar lavage fluid and serum of calves.

Materials and Methods

Animals

All procedures described here in this chapter were approved by the University of Tennessee Institutional Animal Care and Use Committee. Twelve, male Holstein steer calves (4 – 5 months old) from the University of Tennessee Little River Dairy Unit (Walland, TN) were enrolled in the study. Calves in the study were moved from a group pasture to the research pens. Prior to enrollment, calves were screened for *M. haemolytica* infection via aerobic bacterial culture of nasopharyngeal swabs and antibodies against *M. haemolytica* via a whole cell lysate ELISA of serum to ensure appropriate immune-naïve status. Prior to inoculation, treatments were randomly assigned to create groups balanced by body weight: sham inoculated control group (control, n = 6) and principal induced pneumonia group (MH, n=6).

Calves were group housed in approximately 20 m × 20 m covered pens according to treatment with 10 m physical separation between pens. Each treatment pen had independent feed and water sources. After arrival to the research pens calves were permitted a 10 day acclimation period. On acclimation d 3, calves were separated by prospective treatment in order for the calves to adjust to fellow pen mates. Throughout the acclimation period calves were monitored twice daily for the development of disease.

Each animal was provided approximately 2.7 kg of a complete starter/grower ration formulated for dairy calves provided twice daily. The pelleted ration contained 18 % crude protein and 2 % crude fat. In addition, each group had ad libitum access to mixed grass hay and water. Prior to enrollment in the study, calves were managed with the same ration and feeding regimen described above.

Pneumonia Induction

Bacteria

Mannheimia haemolytica was prepared for inoculation as previously described (Mosier et al., 1995). Briefly, lyophilized *M. haemolytica* serotype 1, (kindly provided by Dr. D. Mosier, Kansas State University) was reconstituted in sterile water and grown on brain heart infusion (BHI) agar supplemented with 5% bovine blood in a CO_2 incubator at 37 °C for 18-22 hours. Colonies were selected and suspended in BHI broth, and incubated for 7 hours at 37 °C. Bacteria were pelleted by centrifugation, washed 3 times in sterile phosphate-buffered saline (PBS), and aliquoted and suspended in PBS. Optical density (650 nm) was used to approximate a concentration of 1×10^9 colony forming units (cfu) per ml based on a standard curve of cfu vs. optical density. Plate counts were conducted on the bacterial suspensions to confirm the bacterial concentration.

Bacteria administration

Calves were restrained in a head lock and haltered with their heads elevated and stabilized. The external nasal area was cleaned and a 5.9 mm endoscope with 2 mm biopsy channel was introduced into the right nasal passage. The endoscope was passed through the nasopharyngeal region and, subsequently, through the laryngeal folds into the trachea and progressed until the tracheal bronchus was identified. A sterile, 190 cm long, 1.8-mm diameter tracheal wash catheter was passed through the endoscopic biopsy channel and into the right accessory lung lobe, similar to previously described (Hanzlicek et al., 2010). *M. haemolytica* calves received an inoculum containing approximately 3 - 5 x 10^9 cfu of *M. haemolytica* suspended in approximately 5 ml of PBS delivered through the catheter followed by 60 ml wash

PBS. Control calves were administered 5 ml + 60 ml of PBS solution. New sterile catheters were used for each calf. Challenge was initiated at 0600 h on study d 0.

Sample Collection

Blood samples (40 ml total from each calf) were collected via jugular venipuncture on all calves, beginning immediately prior to challenge (study d 0) and then daily until the end of the study period (study d 7). Samples were collected directly into either potassium EDTA or serum separator tubes and centrifuged for 10 minutes at 3000 x *g* at 4 °C. Plasma and serum were aliquoted into 2 ml cryovials and stored at – 80 °C until analyzed. Broncho alveolar lavage (BAL) fluid samples were collected via endoscopic lavage of the tracheal bronchus and the right accessory lung lobe. BAL was collected from each calf beginning immediately prior to administration of the challenge (study day 0) and on days 1, 3, 5, and 7. A sterile tracheal wash catheter was advanced into the tracheal bronchus and up to 120 ml of sterile 0.9% saline delivered. Immediately following administration of the saline, aspiration was applied until a minimum of 5 ml of BAL fluid was collected from each calf. BAL samples were transferred into potassium EDTA tubes and aliquoted, unfiltered into 2 ml cryovials and stored at – 80 °C until analyzed.

Surfactant Protein Analysis

Total SP-A and SP-D concentration in serum and BAL fluid were determined using a commercially available ELISA kit specific for Bovine SP-A and SP-D, respectively (MyBioSource, San Diego, CA). Each assay was previously validated by the manufacturer and used according to the instructions.

Behavioral and clinical observations

Behavioral activity data was collected using data loggers (Pendant G data loggers, Onset Computer Corp., Bourne, MA) as previously validated (Bonk et al., 2013). Two days prior to *M. haemolytica* or sham inoculation, data loggers were wrapped in and attached to the lateral aspect of the left metatarsus of each calf with a cohesive bandage (VetWrap, 3M Products, St. Paul, MN). Data loggers were removed from control calves after sample collection on study day 8. Data loggers were removed from infected calves following euthanasia.

Calves were observed twice daily from the beginning of the acclimation period by consistent experienced personnel (n = 5). At each observation, CIS (Table 1) was assigned to each calf based on a modified system from Perino and Apley (Perino and Apley, 1998). If clinical respiratory disease progressed and the calf was assigned a CIS of 4 (severe illness), the calf was exited from the study, humanely euthanized and a full necropsy performed. Clinical scoring was conducted prior to the collection of samples and while calves were unrestrained in the pens. All observations were made from outside of the pen. Following scoring, calves were fed and restrained in headlocks for collection of samples, rectal temperatures, and respiration rates. In addition, body weights were collected daily each morning at approximately 0630 h. All cattle handling and sample collection were conducted first in the control group followed by the MH group. Clean personal protective equipment and gloves were donned prior to entry in each pen and a separate supply of materials was maintained for each group.

Post-Mortem Analysis

MH infected calves were euthanized via barbiturate overdose and lungs collected and evaluated by an internal medicine specialist experienced in lung lesion scoring. A lung scoring system was adapted from that previously described previously (Fajt et al., 2003). Briefly, the total lung consolidation was calculated with the following formula: (0.06 × right caudal apical/100) + (0.063 × right cranial apical/100) + (0.053 × left cranial apical/100) + (0.049 × left caudal apical/100) + (0.319 × left diaphragmatic/100) + (0.043 × intermediate/100) + (0.352 × right diaphragmatic/100) + (0.061 × accessory/100) = total lung score.

In addition, tissue sections were collected for aerobic bacteriologic culture and histopathology to verify the presence of the challenge strain in active lesions and confirm that lesions demonstrated inflammation and disruption of alveolar architecture.

Data Analysis

Calf lying behaviors collected with the loggers were summarized with a SAS code (AWP, 2013). Summarized lying behaviors included total lying time, total lying bouts, total bout duration, and left and right side time, bouts, and bout duration. A mixed model was used to determine effect of treatment, date and their interaction on total lying time, RT, CIS, and respiration rate. Calf within treatment was treated as a random variable and observations were repeated by day. Laterality was analyzed using the above model plus split-plot effects of lying side and its interactions.

Linear regression models were used to determine potential relationships between maximum CIS, maximum RT, and changes in lying time during the first 24 hours following inoculation. The change in lying time was determined by subtracting the baseline lying time for each individual calf (the mean lying time of the 48 hours prior to inoculation) from the mean total lying time for each calf in the 24 hour period following inoculation. Maximum RT for each calf was the greatest rectal temperature and maximum CIS was the greatest CIS recorded in the 24 hours following inoculation.

Results

Surfactant Proteins

No differences were found between treatment groups for SP-A or SP-D concentrations in blood serum (*P* = 0.40 and *P* = 0.41, respectively; Figures 1.8 and 1.9) or BAL fluid (*P* = 0.38 and *P* = 0.13, respectively; Figures 1.10 and 1.11).

Behavior

M. haemolytica calves spent more time lying down in the first 24 hours following inoculation compared to control calves (*P* < 0.01). No other lying time difference was found between groups over the period of the study (Figure 1.1). Infected calves spent more time lying on their right side than their left side (*P* = 0.01; Figure 1.2). No lateral preference was evident from control calves (*P* = 0.22). No differences between groups were observed for total lying bouts (*P* = 0.42), total lying duration (*P* = 0.36), right side lying bouts (*P* = 0.71), right side lying bout duration (*P* = 0.22), left side lying time (*P*= 0.13), left side lying bouts (*P* = 0.25), or left side lying bout duration (*P* = 0.66).

Clinical Evaluations

M. haemolytica calves had greater mean rectal temperatures 12 hours post infection (41.3 ± 0.3 °C vs. 39.2 ± 0.3 °C, respectively; *P* < 0.001; Figure 3). CIS for any given calf throughout the period did not exceed 3, therefore challenged calves were not euthanized until necropsy at the end of the study. Mean CIS of infected calves (2.2 ± 0.1) were greater 12 hours post inoculation than control calves (1.0 ± 0.1; *P* < 0.001; Figure 1.6). Infected calves had greater mean respiratory rates than control calves over the study period (*P* = 0.03; Figure 1.7). There were no time (*P* = 0.06) or treatment*time (*P* = 0.16) effects on respiratory rates.

There was a slight increase in lying time with increased CIS (R^2 = 0.35; Figure 1.3) but no relationship was found between change in lying time and maximum RT (R^2 = 0.1; Figure 1.4).

Post-Mortem Analysis

Upon necropsy, all calves challenged with *M. haemolytica* demonstrated gross lesions consistent with bronchopneumonia. In addition to generalized congestion, the most consistent changes included acute focal pneumonia affecting the right cranial lung lobes. These areas were characterized as having mild to moderate fibrinous consolidation and edema. No other areas of the lungs demonstrated evidence of infection. The mean lung lesion score ± SE was 7.32 ± 0.39%. Total lung scores ranged from 6.12 to 8.33%. The median score was 7.69%.

Histopathologic examination revealed infiltration of neutrophils, macrophages and deposition of proteinaceous debris in affected alveoli as well extensive edema and widening of adjacent interstitial spaces and damage to type I pneumocytes and underlying basement membranes. All challenged calves yielded a positive culture for *M. haemolytica* in each of 3 tissue samples collected from the margins of active lesions.

Discussion

Behavior

Infected calves in the present study spent more time lying down than control calves on the day of inoculation, similar to previous studies (Hanzlicek et al., 2010, Theurer et al., 2013). This was the only day of the study that lying times differed between treatment groups. On study d 0 (day of inoculation) control calves spent 14.4 ± 0.5 hours lying down where infected calves spent 16.4 ± 0.5 hours lying down. Comparatively, in a previous study, (Theurer et al., 2013) control calves spent approximately 40% (~9.6 hours) of the time lying down and infected calves spent approximately 55% (~13.2 hours) of the time lying down on study d 1 following inoculation (Theurer et al., 2013). While the previous study observed differences in lying times between groups throughout the study period, the present study only found differences on study d 0.

Both the present study and a previous study found an increase in lying time 24 hours post inoculation in infected calves, while a different study did not find changes in lying behaviors during a low dose of bacterial endotoxin (Borderas et al., 2008, Theurer et al., 2013). However, that particular study (Borderas et al., 2008) looked only at behaviors 2 hours surrounding peak body temperatures, while the present study used continuous behavior data collection. The differences in increased lying time observed between the present study and the Borderas (2009) study could be caused by the increased behavior data collected in the present study. It is possible that calves in both studies experienced increased lethargy, as would be expected during sickness (Konsman et al., 2002), however, overall changes in lying behavior may be more noticeable when collected over a longer period of time.

Infected calves in the present study spent more time lying on their right side than their left side. Previous research in dairy cows suggests that cows will spend approximately the same amount of time lying on their left side as their right (Tucker et al., 2009), with more variation occurring during incidences of discomfort such as late pregnancy (Forsberg et al., 2008) or cannulation (Grant et al., 1990). To the authors' knowledge, no previous research has examined lying laterality in calves, particularly illustrating the relationships between lying laterality preference and BRD in calves. In the present study, calves were inoculated in their right lung, and only experienced lesions in the right lung. It is possible that MH calves experienced increased discomfort, particularly in their damaged lung, causing a shift in lying laterality.

Clinical Evaluations

Diagnosing respiratory disease by trained observers can lack precision (Amrine et al., 2013) and accuracy (Wittum et al., 1996, Thompson et al., 2006). In the present study, CIS differed between treatment groups 12 hours post inoculation, with MH calves averaging a score of 2.2 and healthy calves averaging a CIS of 1. It is important to note that due to the necessity of collecting samples from the healthy calves before entering the MH pen, persons performing CIS were not blinded. Therefore, it is possible that biases were present when performing CIS on MH calves. However, behavioral changes found through the accelerometers indicate that MH calves may have been experiencing ill health, particularly on the day of inoculation. Further, the presence of lung lesions indicate potential discomfort.

In incidences of infection, rectal temperatures and respiratory frequency are expected to increase (Husler and Blum, 2001, Borderas et al., 2008). In the current study, rectal temperatures were greater in MH calves 12 hours post inoculation compared to control calves and respiratory rates differed between treatment groups over the study period but not on a specific day. A previous study (Theurer et al., 2013) found lung lesions in all calves infected with *M. haemolytica*. Similar to the present study, rectal temperatures were higher in infected calves on days 0 and 1 of that study, however respiration rates did not differ between study groups. Previous research suggests that rectal temperatures will return to normal 1 – 3 days post infection (Ames et al., 1985, Vestweber et al., 1990), which could explain the lack of difference between treatment groups on later study days. Previous research has reported increased respiration rates from a controlled baseline following infection with *M. haemolytica* (Hanzlicek et al., 2010). In this previous study all calves were infected with the pathogen and compared to their own baseline. Differences in respiration rates were found on days 5 – 8 following inoculation compared to the baseline (Hanzlicek et al., 2010). Comparatively, in the present study, there were no effects of time or treatment*time on respiration rates, indicating that respiration rates remained relatively the same over the course of the study and only differed between treatment groups.

In a previous study (Theurer et al., 2013) as well as the current study, CIS, RT, and lying time was greater in infected calves than healthy calves in the 24 hours following inoculation with *M. haemolytica.* Previous research has also reported decreased activity and increased CIS following inoculation with this pathogen (Hanzlicek et al., 2010). However, neither reported data on an individual calf basis nor any potential relationships between these variables. In the present study, a slight relationship between CIS and changes in lying time was determined. It is possible that the visual lethargy or physical signs of respiratory disease quantified by CIS was also reflected in the increased lying time following inoculation, particularly by the only calf having a CIS of 3.

Surfactant Proteins

Previous studies have found increased concentrations of surfactant protein D in the lungs of lambs infected with *M. haemolytica* (Grubor et al., 2004) and horses infected with *S. zooepidemicus* (Hobo et al., 2007). However, in the present study, no significant differences were found between concentrations of SP-A or SP-D in serum or BAL fluid in the control and infected groups. SP-D serum concentrations have been found to parallel clinical conditions of observed horses with respiratory illness; when CIS indicated a horse may be sick, SP-D serum concentrations also reflected that indication (Hobo et al., 2007). In the present study, in addition to a lack of difference between control and infected calf SP-D or SP-A serum or BAL fluid concentrations, there were no significant differences in CIS between control and infected groups outside of the first 12 hours post inoculation. It is possible that infected calves experienced a milder than expected respiratory infection, which did not cause an exhibition of detectable visual clinical signs or sufficient damage to lung tissue to cause leakage of SP-A or SP- D into alveolar fluid or serum.

Post-Mortem Analysis

Lung lesions found during necropsy indicated a mild infection experienced by the challenged calves. Previous research (Fajt et al., 2003) utilizing the same scoring system used in the present study observed a mean lung consolidation score of 18.1 % for calves not treated for their infection. Comparatively, mean lung consolidation score in the present study was 7.32 ± 0.39%. This indicates that calves in the previous study had greater damaged lung tissue than in the present study.

Conclusions

Mild respiratory infections, as indicated by lung consolidation scores, may go undetected via serum and BAL fluid SP-A and SP-D concentrations. However, changes in lying behaviors such as increased lying time and a shift in laterality may be detected utilizing behavioral monitoring systems combined with other clinical evaluations. Observing and detecting these changes in behavior may improve earlier detection and treatment of sick calves which could improve calf welfare.

Appendix

Table 1.1. Clinical Illness Scoring. Description, clinical appearance and corresponding score used to determine CIS.

Clinical Illness Score	Description	Clinical Appearance
1	Normal	No abnormalities noted
2	Slightly Ill	Mild depression, gaunt, +/- cough
3	Moderate Illness	Severe depression, labored breathing, ocular / nasal discharge, +/- cough
4	Severe Illness	Moribund, near death, little response to touch

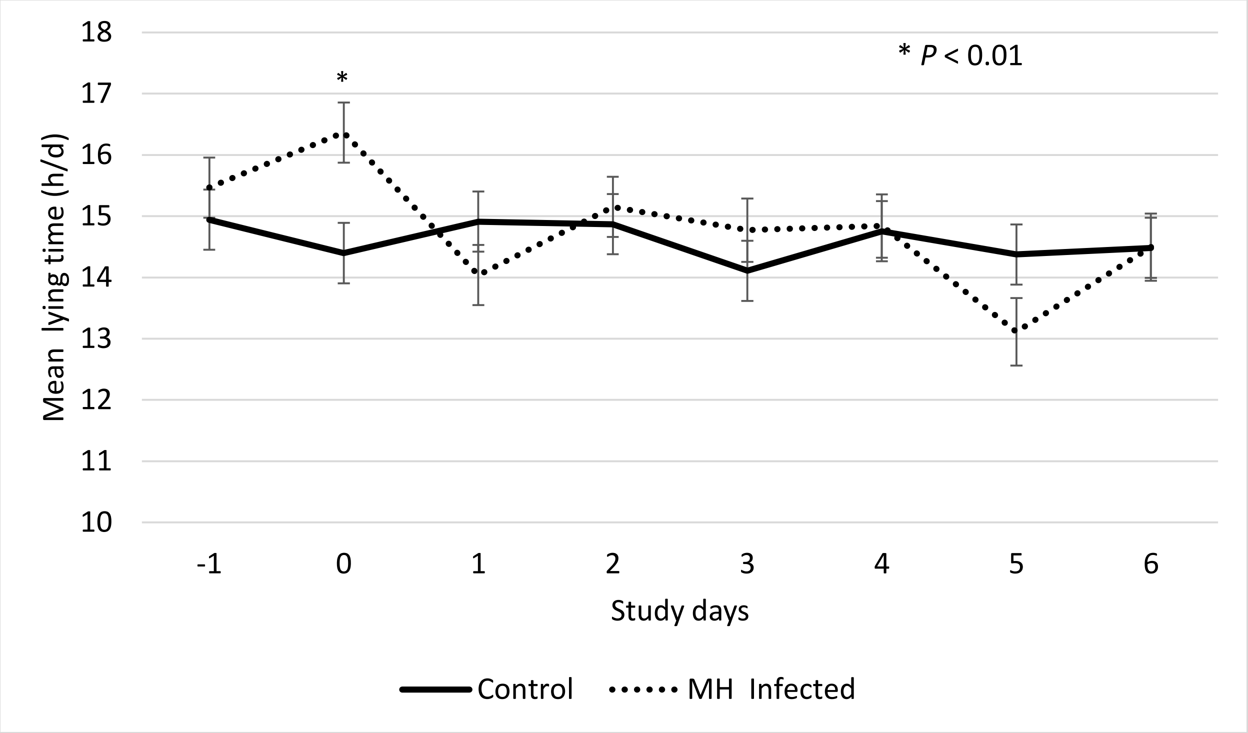

Figure 1.1. Mean lying time. Least square means (± SE) of daily lying time by study day and treatment groups. Day 0 indicates day of inoculation. Calves infected with *M. haemolytica* spent more time lying down on study d 1 than control calves ($P < 0.01$).

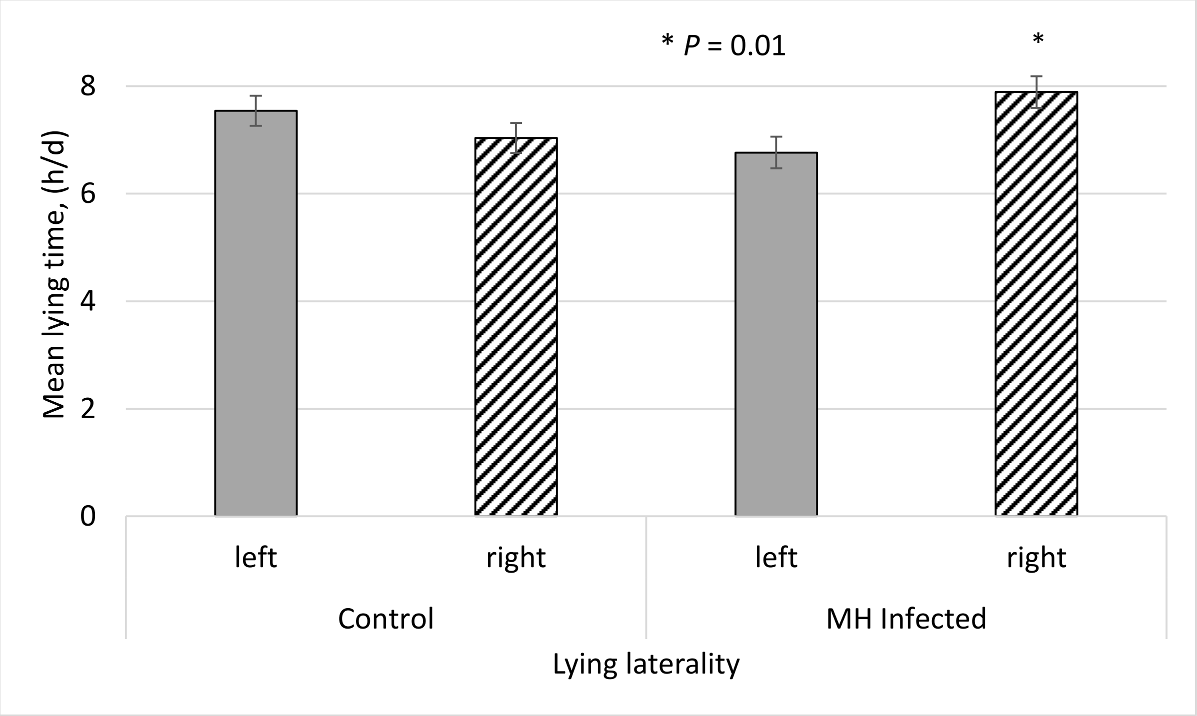

Figure 1.2. Mean lying laterality. Least square means (± SE) of left and right side lying time across the study period between treatment groups. Day 0 indicates day of inoculation. Calves infected with *M. haemolytica* spent more time lying on their right side (7.9 h/d) than their left side (6.8 h/d) ($P = 0.01$).

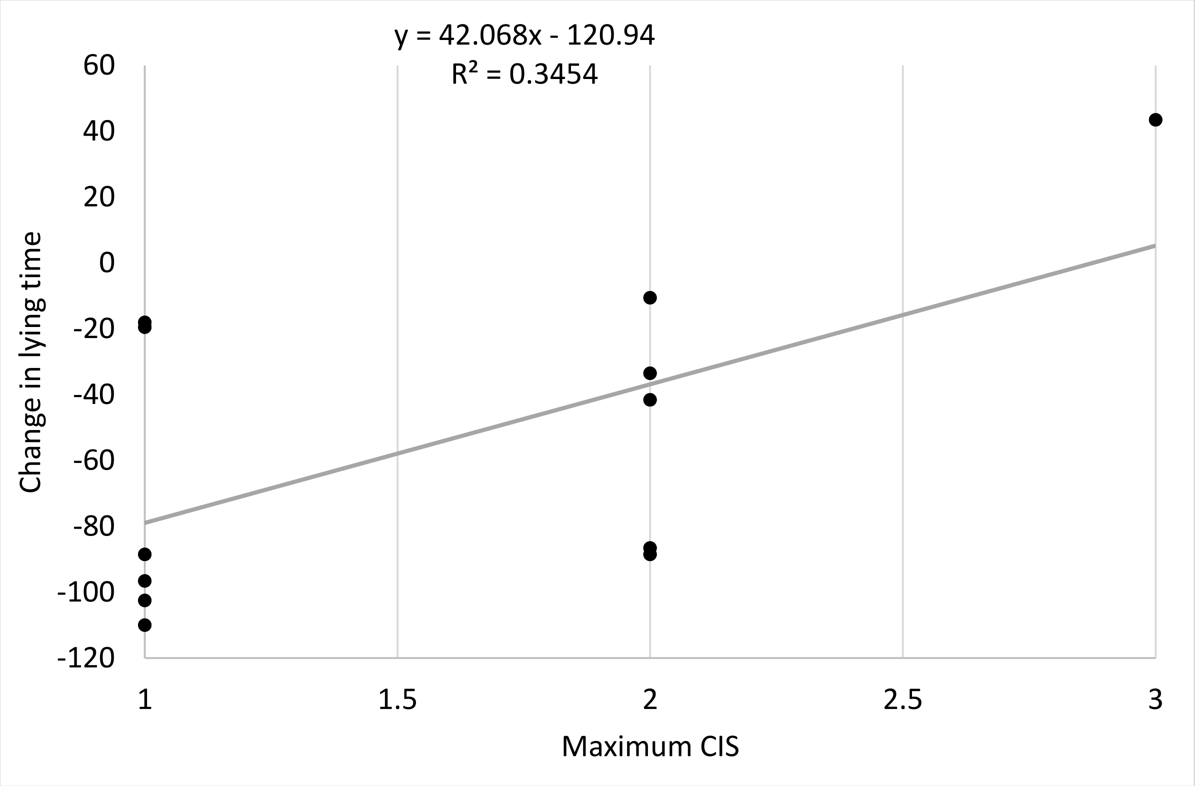

Figure 1.3. Change in lying time by maximum clinical illness score. Each marker indicates an individual calf. Baseline behavior was determined by the mean overall lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the overall lying time on day 0 (inoculation day). Maximum clinical illness score was the greatest score given to each calf in the 24 hours following inoculation. As clinical illness score increased, calves slightly increased lying time.

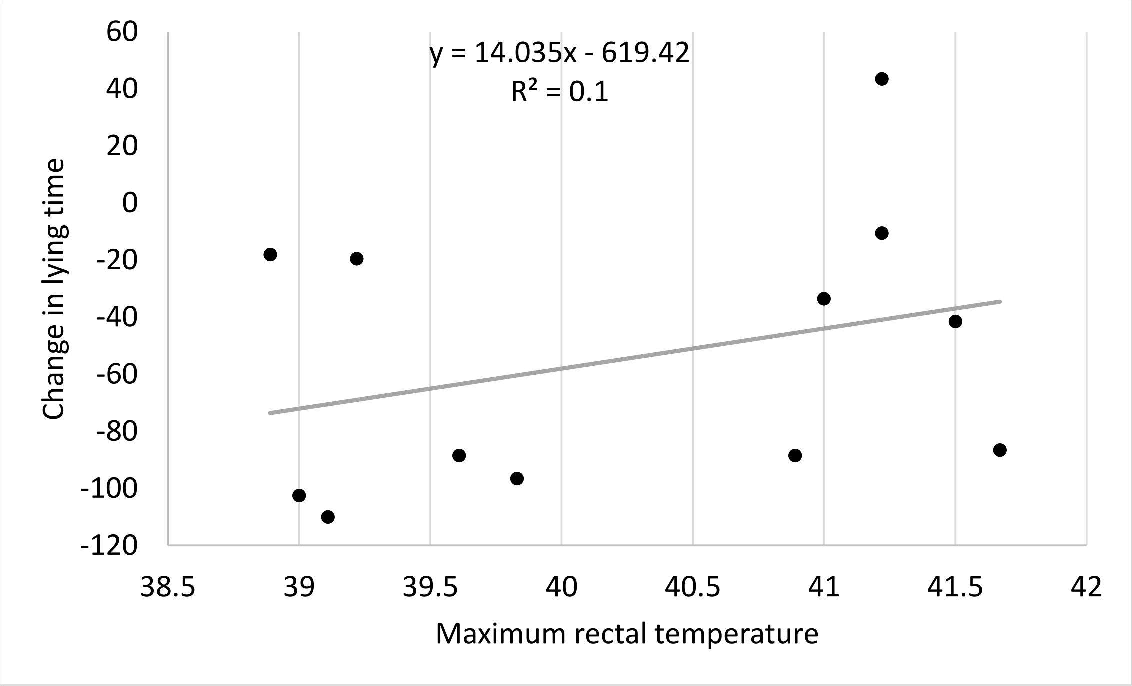

Figure 1.4. Change in lying time by maximum rectal temperatures. Each marker indicates an individual calf. Baseline behavior was determined by the mean overall lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the overall lying time on day 0 (inoculation day). Maximum rectal temperature was the greatest rectal temperature recorded from each calf in the 24 hours following inoculation. There was no significant relationship between maximum rectal temperature and change in lying time.

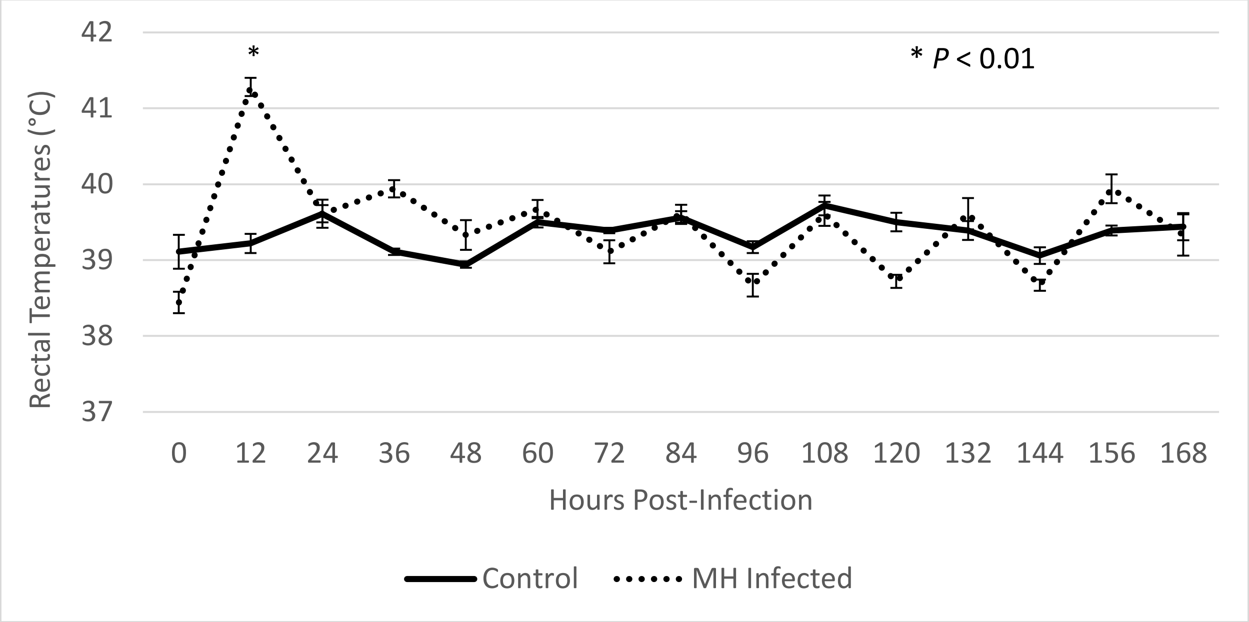

Figure 1.5. Mean rectal temperatures. Mean rectal temperatures by study day and treatment groups. Hour 0 indicates time of inoculation. Calves infected with *M. haemolytica* had greater mean rectal temperatures 12 hours post inoculation than control calves (P < 0.01).

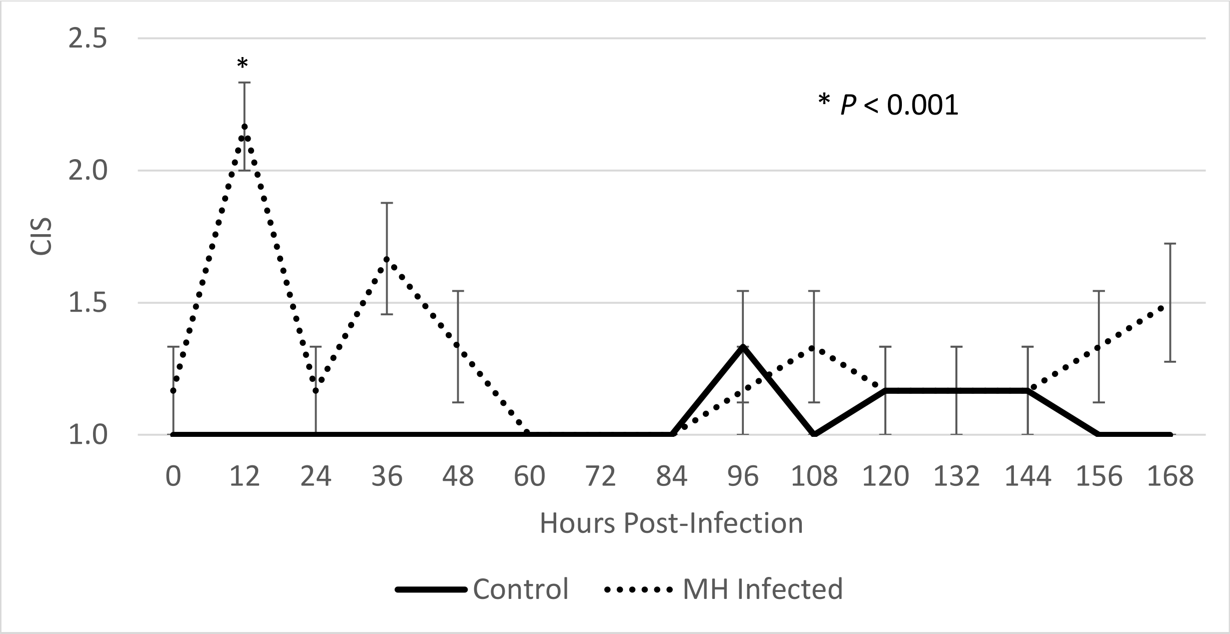

Figure 1.6. Mean clinical illness scores. Mean clinical illness scores by hours post inoculation and treatment groups. Hour 0 indicates time of inoculation. Calves infected with *M. haemolytica* had greater mean clinical illness score 12 hours post inoculation (P < 0.001). Clinical illness scoring was unblinded, due to the nature of the pen set-up, therefore caution should be taken when comparing scores between treatment groups.

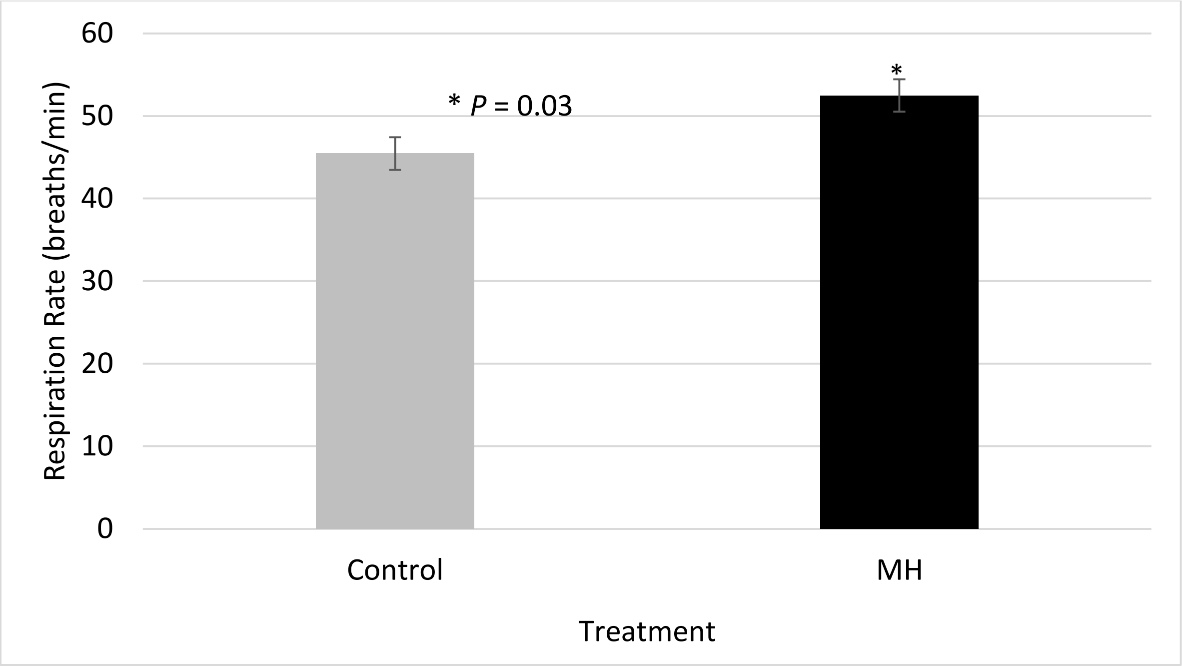

Figure 1.7. Mean respiration rates. Mean respiration rates by treatment groups. Infected calves had higher respiration rates than control calves over the course of the study (*P* = 0.03). There was no effect of time (*P* = 0.06) or treatment*time (*P* = 0.16) on respiration rates.

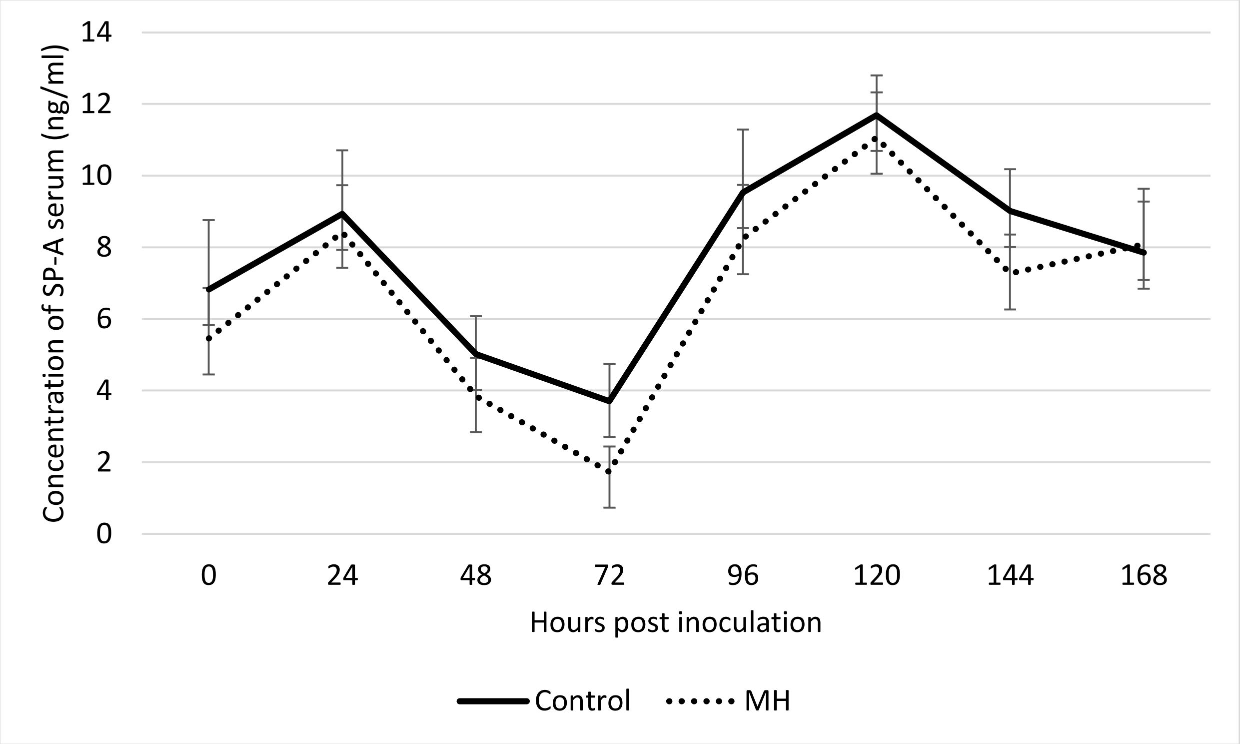

Figure 1.8. Mean Surfactant Protein-A serum concentrations. Mean (± SEM) of serum SP-A concentrations (ng/ml) across study period (hours) and treatment groups. Hour 0 indicates time of inoculation. No differences were found in serum SP-A concentrations between treatment groups (*P* = 0.40).

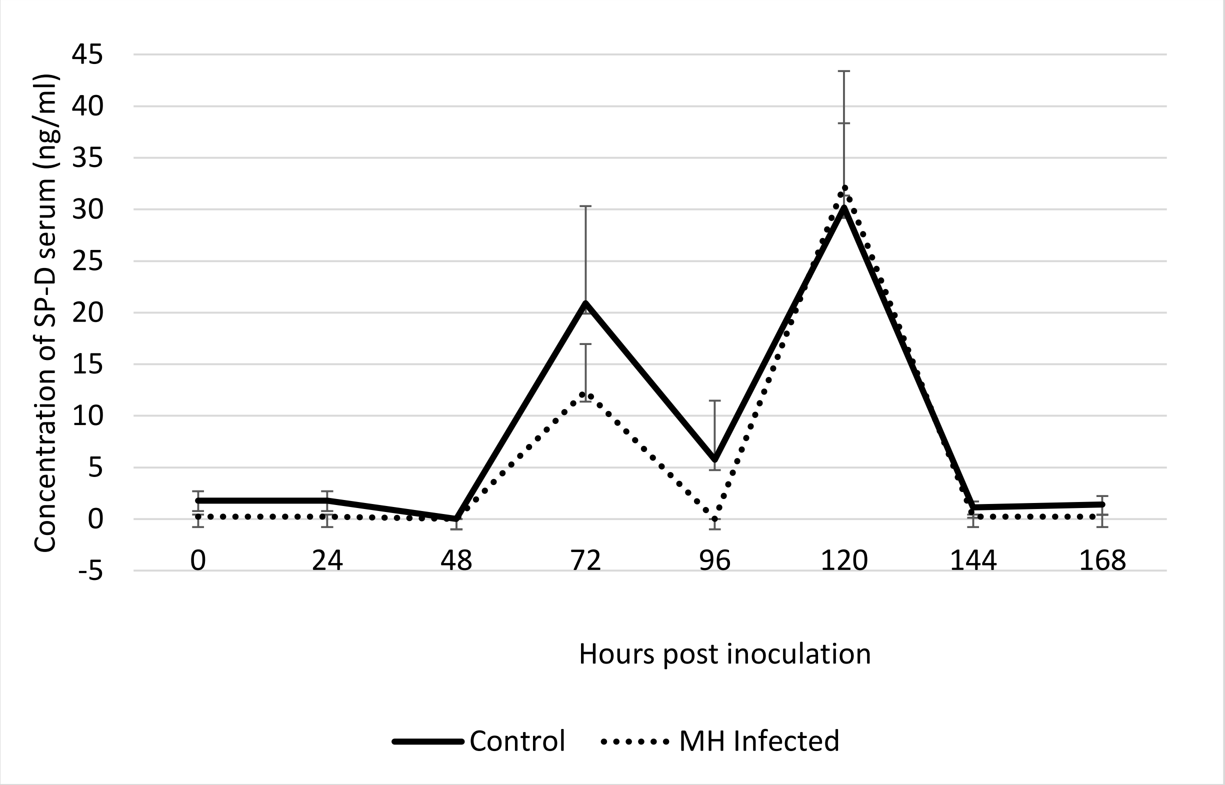

Figure 1.9. Mean Surfactant Protein-D serum concentrations. Mean (± SEM) of serum SP-D concentrations (ng/ml) across study period (hours) and treatment groups. Hour 0 indicates time of inoculation. No differences were found in serum SP-D concentrations between treatment groups (*P* = 0.41).

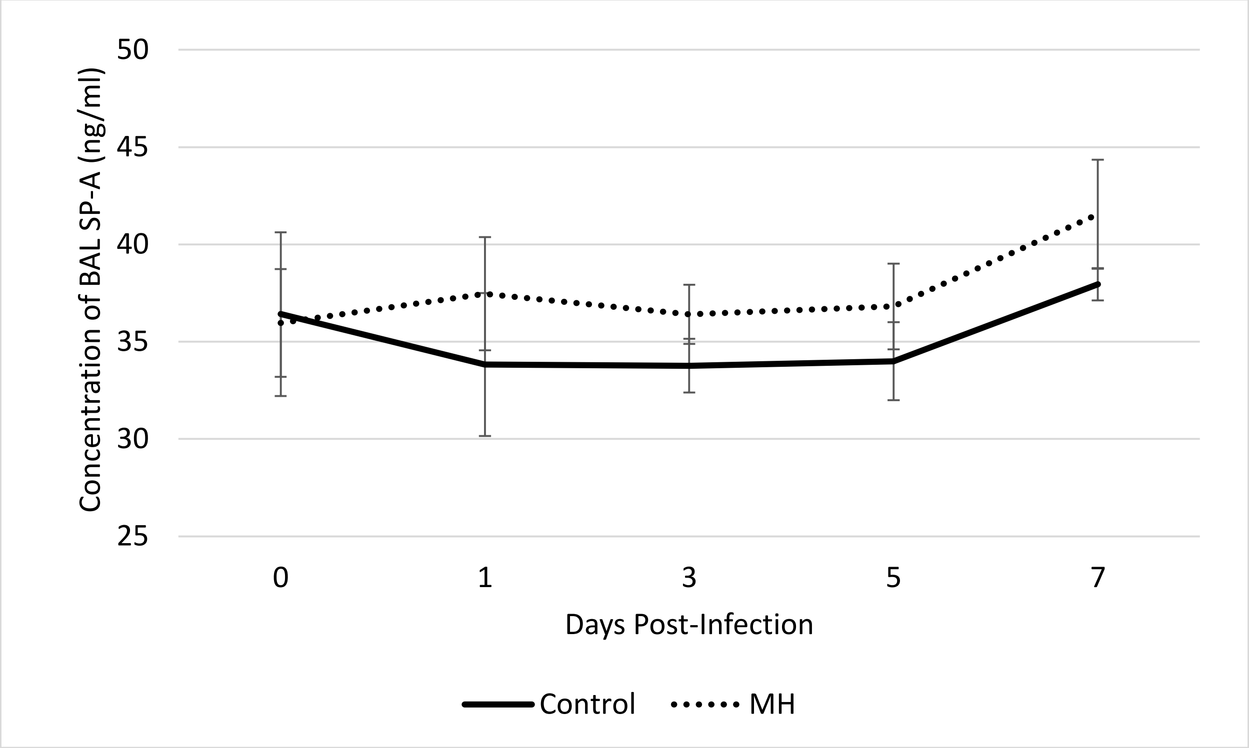

Figure 1.10. Mean Surfactant Protein-A lung fluid concentrations. Mean (± SEM) of bronchoalveolar lavage SP-A concentrations (ng/ml) across study period (hours) and treatment groups. Day 0 indicates day of inoculation. No differences were found in bronchoalveolar lavage SP-A concentrations between treatment groups (*P* = 0.38).

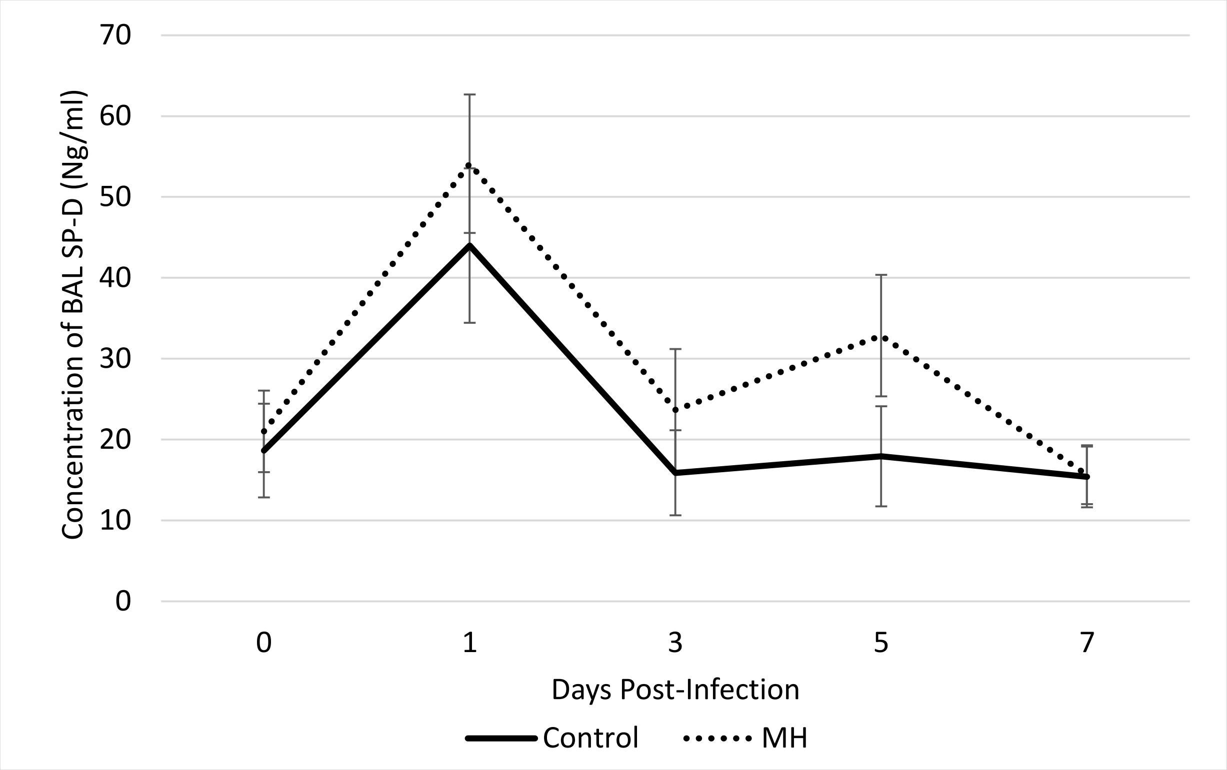

Figure 1.11. Mean Surfactant Protein-D lung fluid concentrations. Mean (± SEM) of bronchoalveolar lavage SP-D concentrations (ng/ml) across study period (hours) and treatment groups. Day 0 indicates day of inoculation. No differences were found in bronchoalveolar lavage SP-D concentrations between treatment groups (*P* = 0.13).

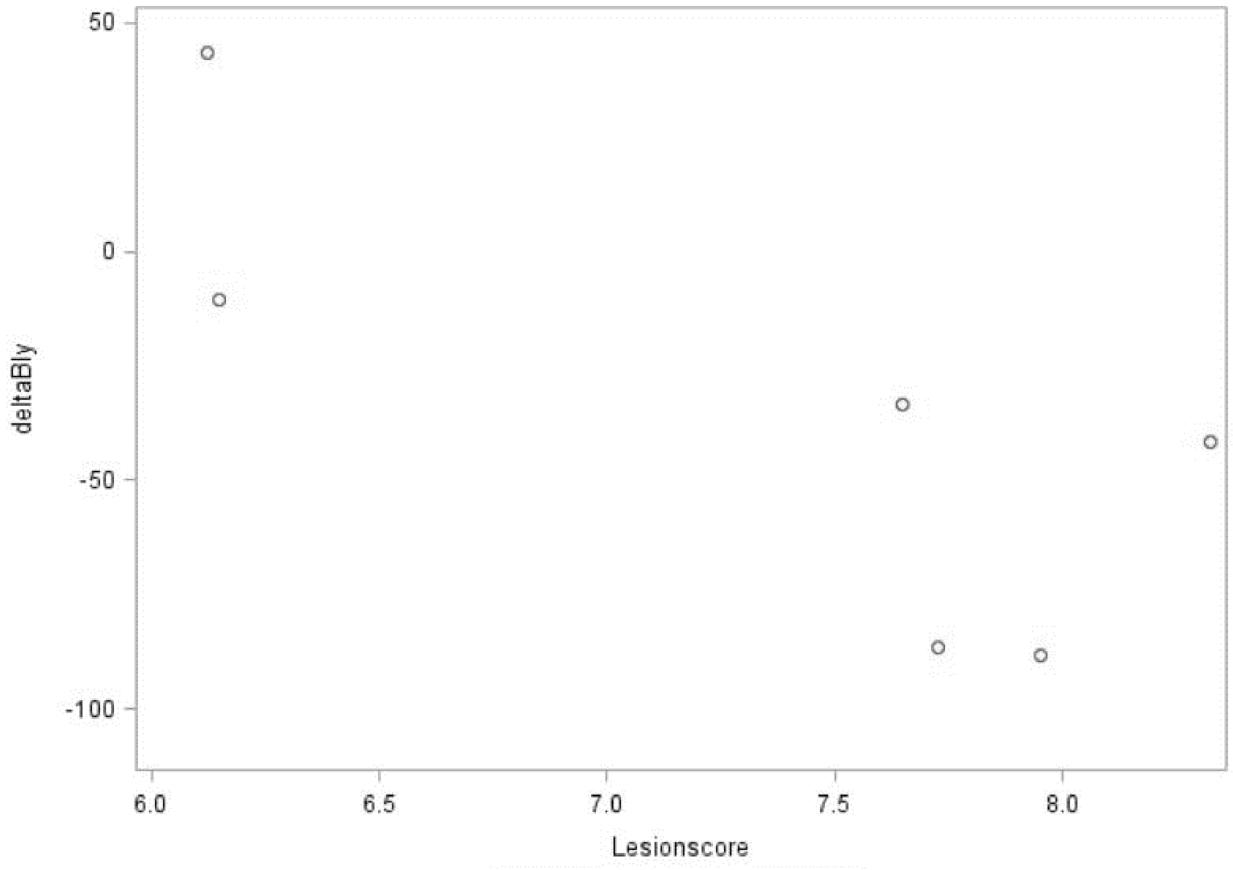

Figure 1.12. Change in lying time by lesion scores. Each marker indicates an individual calf. Baseline behavior was determined by the mean overall lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the overall lying time on day 0 (inoculation day). Lung lesion scores were determined following necropsy in infected calves.

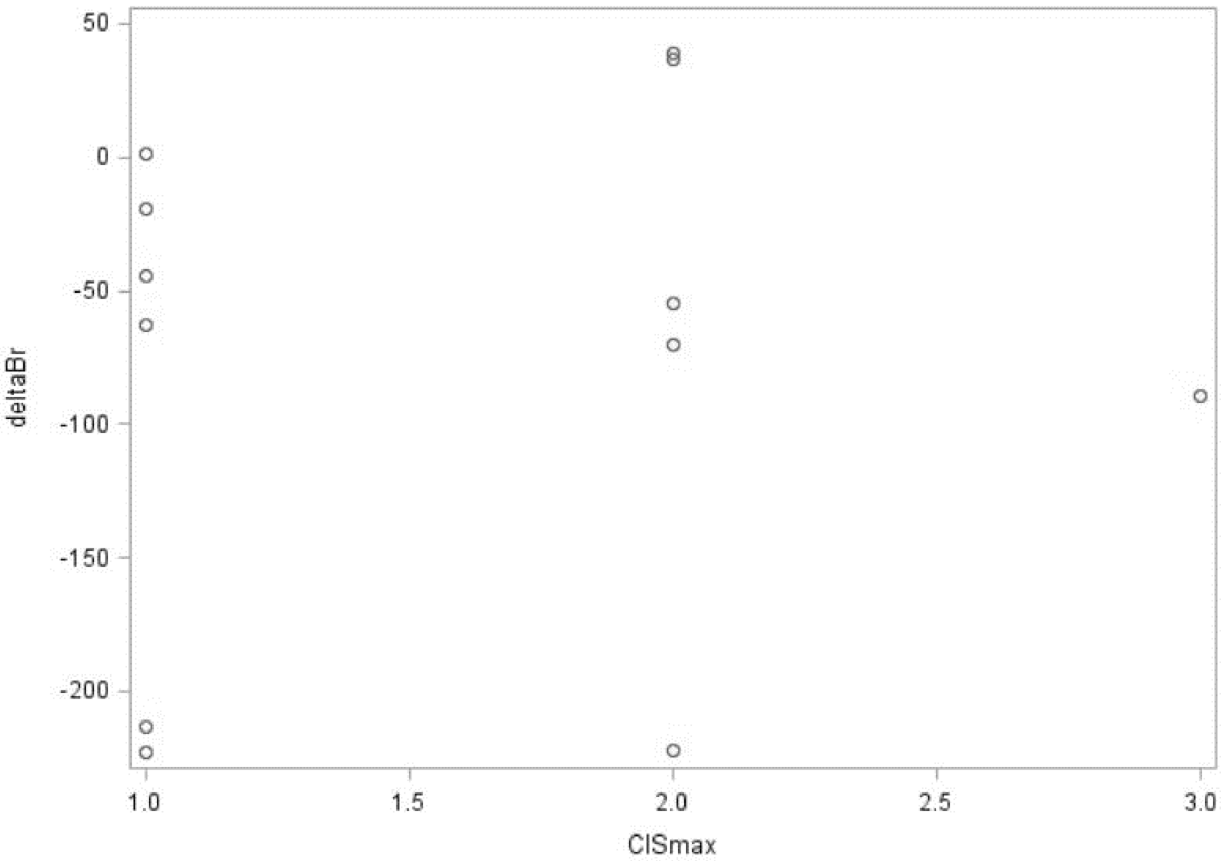

Figure 1.13. Change in right side lying time by maximum clinical illness score. Each marker indicates an individual calf. Baseline behavior was determined by the mean right side lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the right side lying time on day 0 (inoculation day). Maximum clinical illness score was the greatest score given to each calf in the 24 hours following inoculation.

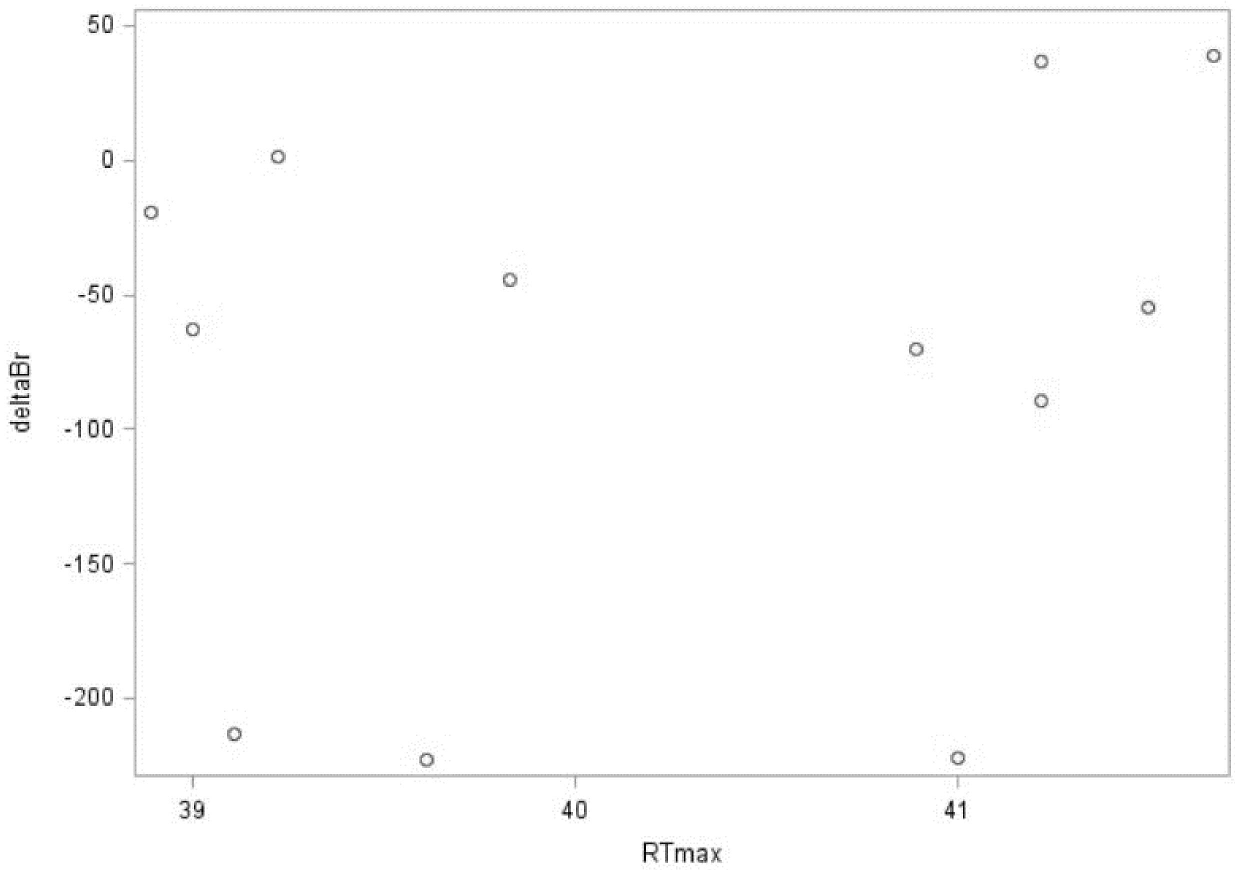

Figure 1.14. Change in right side lying time by maximum rectal temperature. Each marker indicates an individual calf. Baseline behavior was determined by the mean right side lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the right side lying time on day 0 (inoculation day). Maximum rectal temperature was the greatest rectal temperature recorded from each calf in the 24 hours following inoculation.

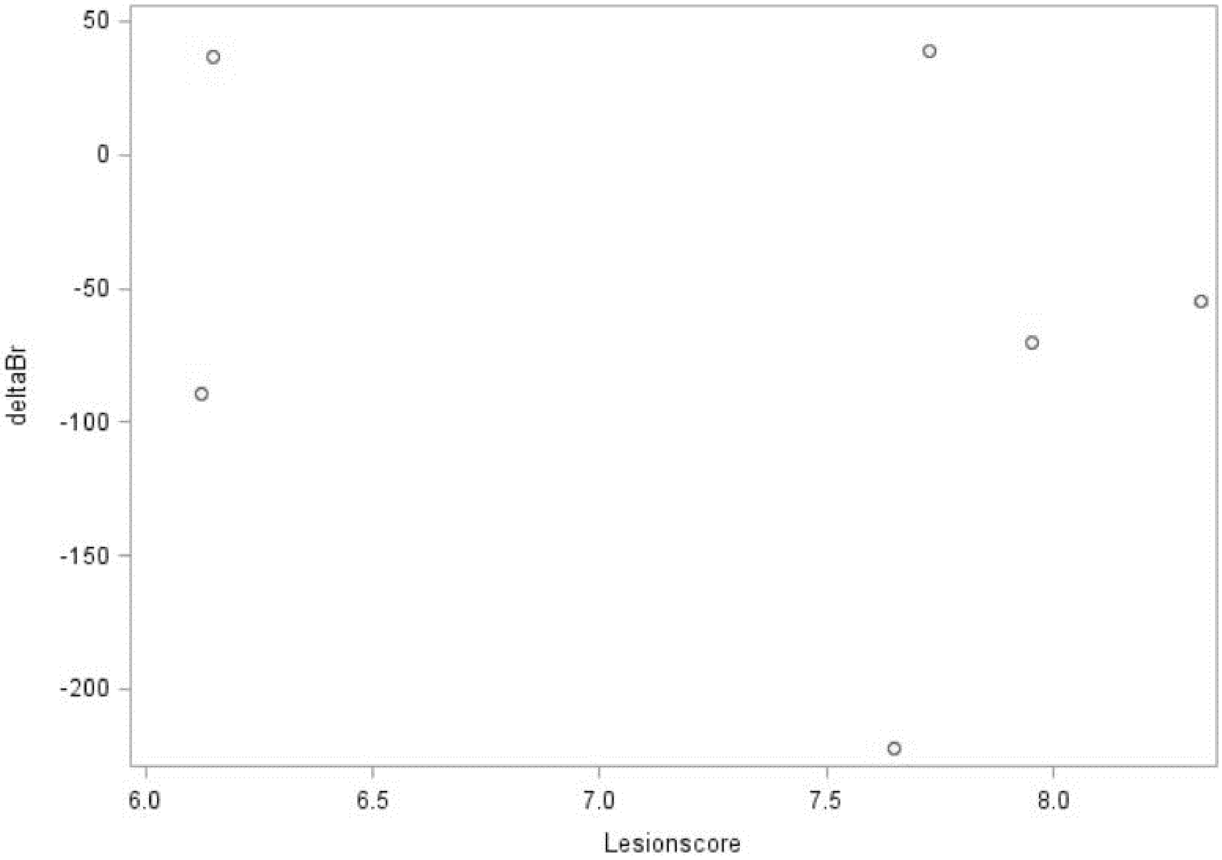

Figure 1.15. Change in right side lying time by lung lesion score. Each marker indicates an individual calf. Baseline behavior was determined by the mean right side lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the right side lying time on day 0 (inoculation day). Lung lesion scores were determined following necropsy in infected calves.

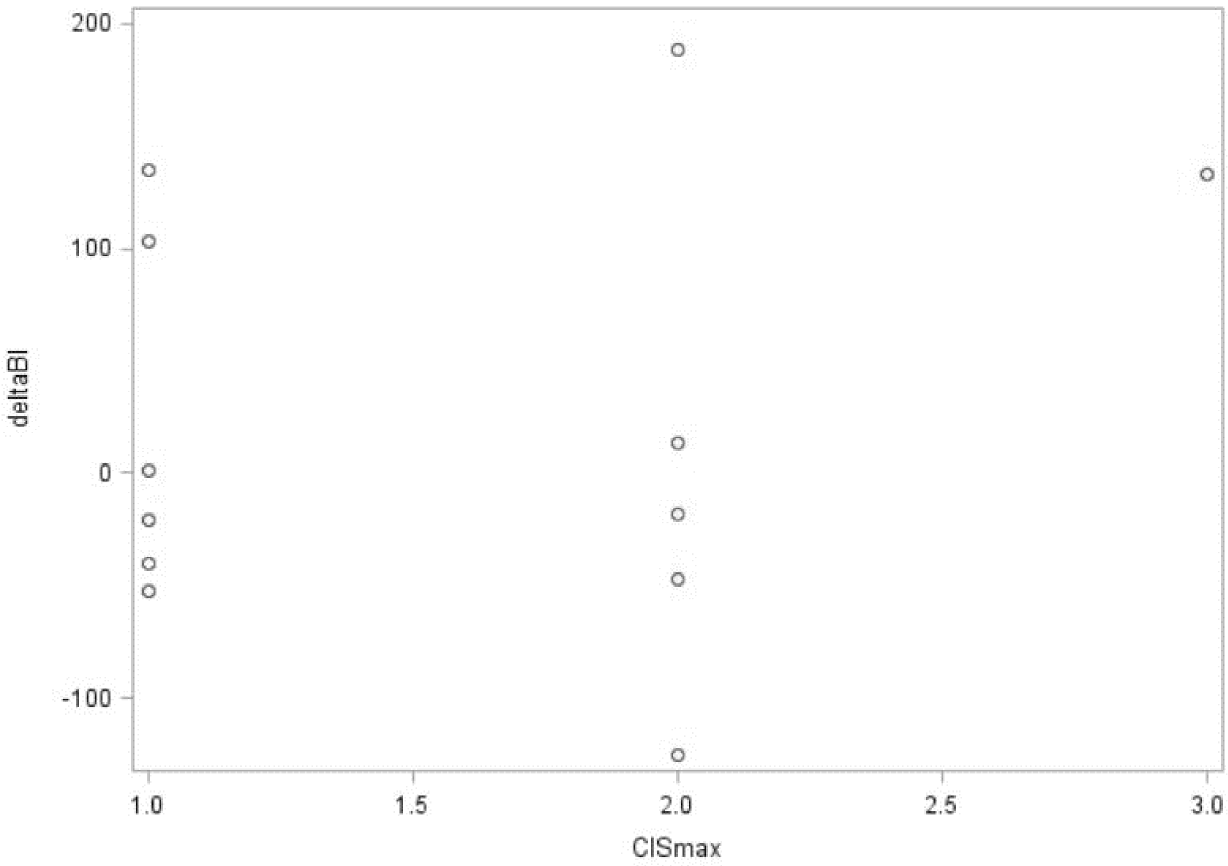

Figure 1.16. Changes in left side lying time by maximum clinical illness score. Each marker indicates an individual calf. Baseline behavior was determined by the mean left side lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the left side lying time on day 0 (inoculation day). Maximum clinical illness score was the greatest score given to each calf in the 24 hours following inoculation.

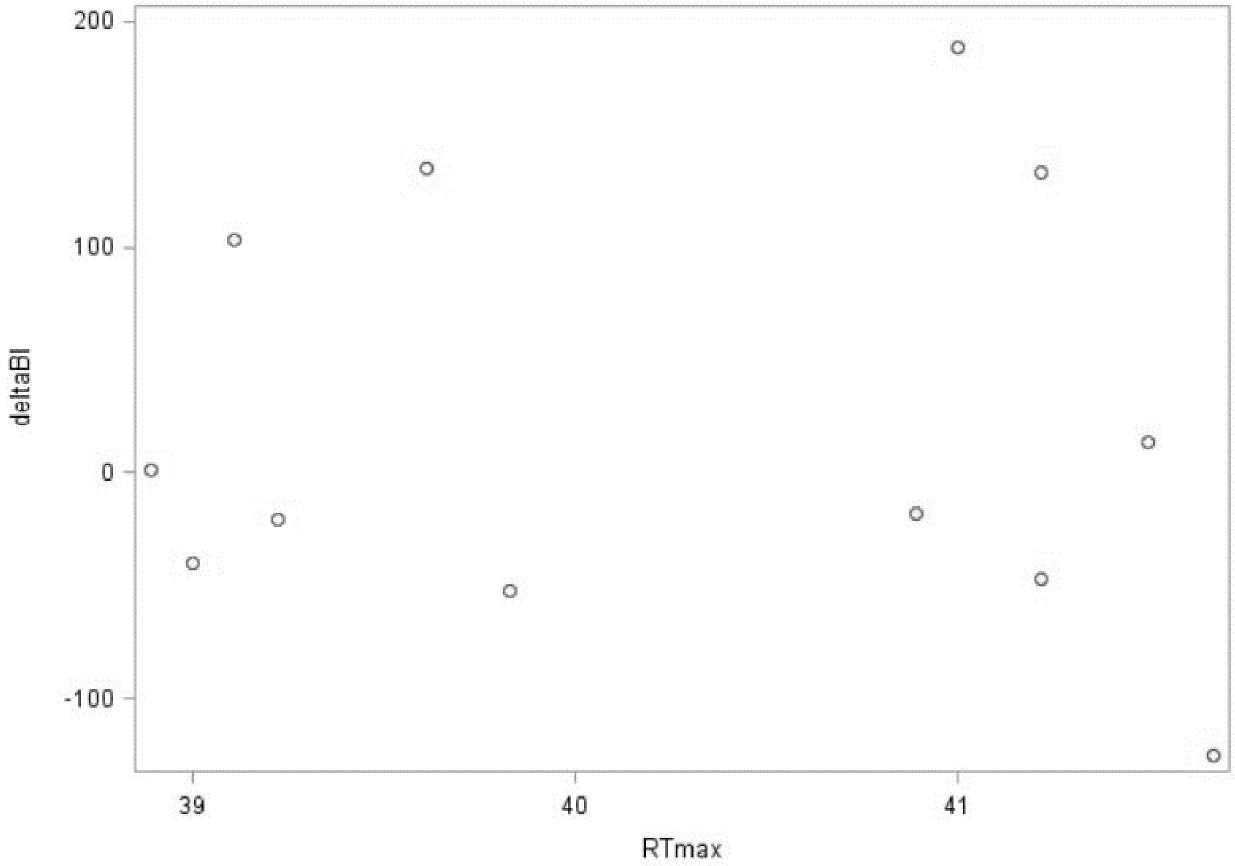

Figure 1.17. Change in left side lying time by maximum rectal temperature. Each marker indicates an individual calf. Baseline behavior was determined by the mean left side lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the left side lying time on day 0 (inoculation day). Maximum rectal temperature was the greatest rectal temperature recorded from each calf in the 24 hours following inoculation.

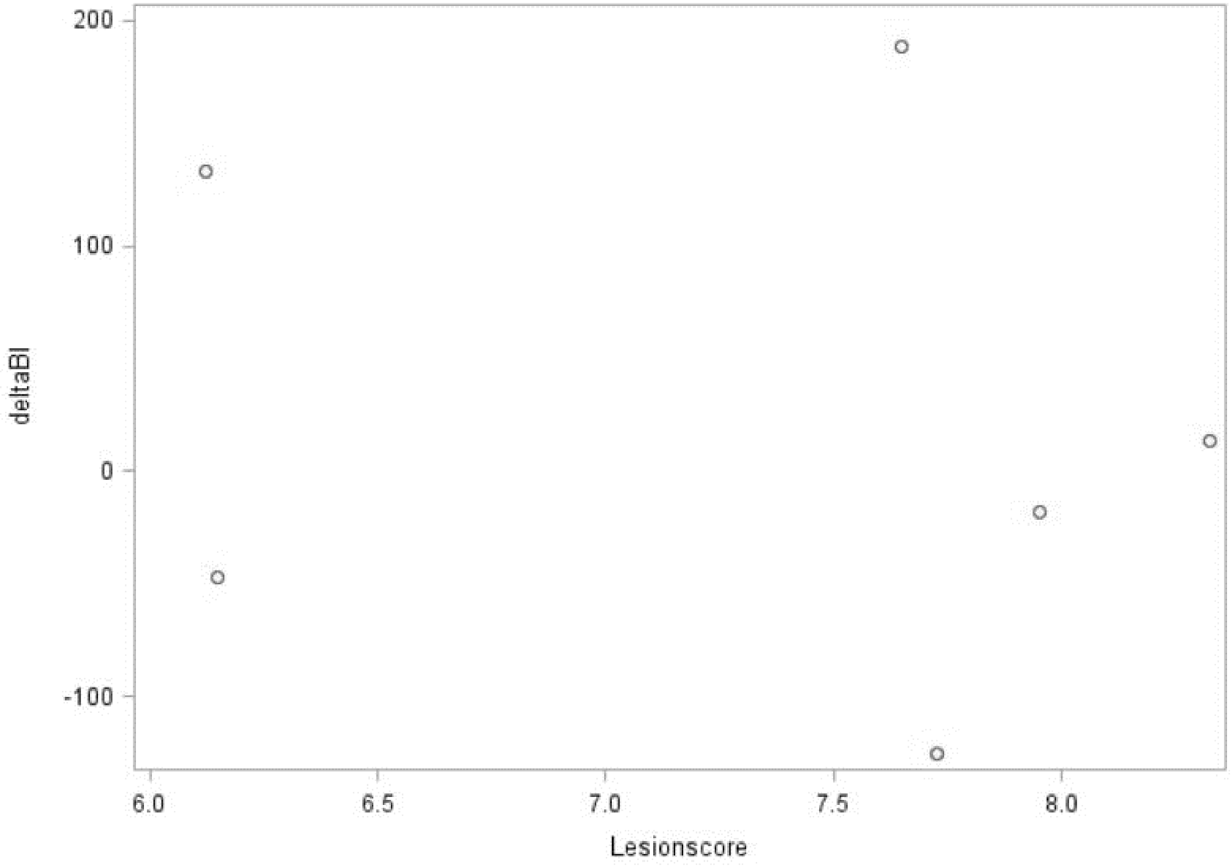

Figure 1.18. Change in left side lying time by lung lesion score. Each marker indicates an individual calf. Baseline behavior was determined by the mean left side lying time of the two 24 hour periods prior to inoculation. Change in behavior was determined by subtracting the baseline from the left side lying time on day 0 (inoculation day). Lung lesion scores were determined following necropsy in infected calves.

CHAPTER TWO

The Effect of Hock Injuries and Lameness on Lying Laterality in Croatian Dairy Cows

ABSTRACT

Lactating dairy cattle spend approximately the same amount of time lying on their left side as their right side. However, incidences of discomfort such as pregnancy and cannulation can cause a cow to shift lying side preference to the left side. Hock injuries and lameness have been associated with changes in lying behavior and overall cow discomfort. Lying behavior, cow records, and health scores were collected from 188 cows across four commercial farms in eastern Croatia. Lying behaviors were recorded with data loggers attached to the metatarsus of cattle for a minimum of 3 d. Cow records were provided by farms. Health scores including hock injuries (scores from 0 to 3, both hocks) and locomotion (scores from 1 to 3) were collected once per cow. Lying side preference was analyzed using a mixed model to determine effect of hock injuries, hock injury laterality, lameness, and parity, plus split-plot effects of lying side and their interactions. Cows with unilateral hock injuries spent more time lying on their left side than their right compared to cows with bilateral injuries and uninjured cows. The change in detectable lying behaviors associated with these injuries suggest the potential for improved recognition of environmentally induced discomfort.

Introduction

The high prevalence of hock injuries observed worldwide indicates a larger issue within the dairy industry; previous research has reported injury rates of 57% in France (Veissier et al., 2004) and 50% in Germany and Austria (Brenninkmeyer et al., 2013). High prevalence of hock injuries also have been found in the northeastern United States (81%) (von Keyserlingk et al.,

2012) and southern British Columbia (73%) (Weary and Taszkun, 2000). High prevalence of hock injuries may indicate potential decreased welfare for cows experiencing these injuries.

Cows with hock injuries are more likely to become lame (Klaas et al., 2003); and both hock injuries (Brenninkmeyer et al., 2013) and lameness (Coulon et al., 1996, Bicalho et al., 2008) impact production and welfare. The relationship between hock injuries and lameness may be due to similar risk factors such as inadequate stall design (Brenninkmeyer et al., 2013). Lameness in itself is a common problem on farms; 23.9% of cows in the United States in 2007 were reported to have been lame at least once in a 12 month period (USDA, 2009). Furthermore, producers may overlook lame cows (Whay et al., 2003), resulting in uncomfortable cows going undetected.

Lactating dairy cattle spend approximately the same proportion of time lying on their left and right sides (Bao and Giller, 1991, Tucker et al., 2009). However, conditions of discomfort may drive cows to favor the left side (spending 61% of lying time on the left), including pregnancy (Bao and Giller, 1991, Forsberg et al., 2008). Cows may favor the right side (70% of time lying on the right side) following cannulation (Grant et al., 1990). Laterality variation may be more apparent in individual cows, particularly non-lactating pregnant cows (Tucker et al., 2009). Therefore, abnormal laterality could indicate cow discomfort.

When dairy cows experience discomfort such as lameness, normal lying behaviors can be altered (Ito et al., 2010). Overstocking and inadequate bedding can also alter normal lying behaviors by decreasing lying time in cows (Camiloti et al., 2012, Krawczel et al., 2012). This suggests that inadequate housing conditions could cause increase risk of lameness and hock injuries. While a shift in lying laterality can occur when cow discomfort occurs (Grant et al., 1990, Forsberg et al., 2008); the importance of hock injuries in the alteration of lying laterality have yet to be illustrated. Therefore, the objective of this study was to determine the impact of hock injuries and lameness on laterality of lying behavior.

Materials and Methods

Animals, housing, and management

This study utilized data collected previously (N. Eberhart et al., unpublished data).The previous work reports lying time, prevalence of lame cows and injured cows, and udder hygiene. This study incorporated the lying behavior data with the lameness and hock injury data and cow records to determine relationships between laterality and these conditions. Lying behaviors and health scores (locomotion and hock injury scores) from four farms across eastern Croatia were evaluated. Lying behavior data was collected from 278 cows: 81 cows from farm 1, 93 cows from farm 2, 42 cows from farm 3, and 62 cows from farm 4 (approximately 25 cows from each pen identified as "high" production by the farm were used). Health scores were collected from 792 cows across all farms: 381 cows from farm 1, 213 cows from farm 2, 82 cows from farm 3, and 116 cows from farm 4 (numbers represent 30% of cows housed in pens identified as "high" production). Cow records from all farms were combined with lying behavior results and sorted by injury status (injured = score of 1 or above; uninjured = score of 0), hock injury side (left, right, both), lameness status (lame or sound), and parity (primiparous, n = 80; and multiparous, n = 108). For data analysis, only cows with lying behavior, health score, and production were used (n = 188). Farm 1 had 39 cows meeting these criteria, 61 on farm 2, 39 on farm 3, and 49 on farm 4.

Cows were housed in either freestalls with straw (farms 1, 2, and 3) or deep bedded packs with straw (farm 4). Farm 1 used a 40 cow rotary parlor milking twice daily, farm 2 used a 24 double sided herringbone parlor milking three times daily or twice daily for late lactation cows, farm 3 used a robotic parlor system (2 robots per pen) with free choice milking, and farm 4 used a 20 double sided parallel parlor with cows being milked twice daily. All farms had less than 100% stocking density in housing (freestalls), except for farm 4 which had over 100% stocking density. Stocking density in the bedded pack was determined by allotting 9.3 m^2 per each cow. All heifers from all farms were raised at a common location with bedded packs and pastures. All farms fed cows twice daily and used Delaval milking equipment (Tumba, Sweden).

Data Collection

Lying behaviors were collected using Hobo Pendant G data loggers (Onset Computer Corp., Bourne, MA) as previously validated (Ledgerwood et al., 2010) for a minimum of 3 days and summarized with a SAS code (AWP, 2013). Locomotion was evaluated using the NAMHS scoring system (NAHMS, 2014) with a sound cow being represented by a score of 1, a moderately lame cow being represented by a score of 2, and a score of 3 representing a severely lame cow. Hocks were scored on a 0-3 scale where 0 indicated no visible injury on the hock, 1 indicated hair loss but no swelling, 2 indicated the presence of swelling, and a score of 3 indicated major swelling (Fulwider et al., 2007). Right and left hocks were scored separately. Locomotion and hock injury assessments were conducted

Data Analysis

Lying side preference was analyzed using a mixed model to determine effect of hock injuries, hock injury laterality, lameness, and parity, plus split-plot effects of lying side and their interactions. The experimental unit was cow and the random variable was farm. Hock injury laterality was categorized into three categories: unilateral (cow has only one hock that is scored at 1 or above), bilateral (cow has both hocks scored at 1 or above), and neither (both hocks were scored as 0).

Results

Cows with unilateral hock injuries spent more time lying on their left side than cows with bilateral injuries or cows without hock injuries (Figure 2.1; $P = 0.02$). No differences in lying side preference were found in lameness status (Figure 2.2; $P = 0.45$) or parity (Figure 2.3; $P = 0.86$). A greater percentage of primiparous cows had hock injuries than multiparous cows (Table 2.1), however the percentage of multiparous lame cows was greater than primiparous cows (Table 2.2).

Discussion

Changes in lying laterality may occur when cows experience discomfort (Bao and Giller, 1991, Forsberg et al., 2008), including incidences where the condition is not bilateral such as cannulation (Grant et al., 1990). However, the full extent of uncomfortable conditions that may cause a preference in lying laterality is unknown. Cows in the present study with unilateral hock injuries spent more time lying on their left side compared to cows with bilateral injuries or uninjured cows, indicating potential discomfort.

Currently, to the authors' knowledge, no research has been published looking at the direct relationship between lying behavior laterality and hock injuries. In the present study, hocks were considered injured if scored as a 1 or greater. Although research has yet to quantify the pain experienced by cows with hock injuries, hock injury scoring is included in welfare assessments (Burow et al., 2013), indicating that the presence of injured hocks decreases cow welfare. Therefore, the shift in lying laterality in unilaterally injured cows may indicate decreased cow welfare.

Severe hock injuries are observed more commonly unilaterally than bilaterally (Potterton et al., 2011), however, all severe hock injuries in the present study were found on cows injured bilaterally. Because hocks were scored once during the study period, determining the potential development of the severe injuries in bilaterally injured cattle is not possible. However, heifers housed on freestalls with mats may have increased hock injury scores over time (Livesey et al., 2002). Therefore, it is possible that severe hock injuries developed from mild injuries in bilaterally injured cows.

In the present study lameness was not associated with a change in lying preference. Although lameness has been associated with hock injuries (Klaas et al., 2003, Brenninkmeyer et al., 2013) the timeline from hock injury to lameness or vice versa has yet to be determined. Cows with severe hock injuries are more likely to become lame (Klaas et al., 2003); however, minor hock injuries such as hair loss have been found to occur after a bout of lameness has occurred (Lim et al., 2013). It is possible that cows in the present study with mild hock injuries experienced a bout of lameness previous to hock injury scoring. However, because hock injury and lameness scores were taken at the same time and not evaluated over the course of a period of time, the likelihood of one condition leading to the other cannot be postulated.

Parity was not associated with a change in lying laterality. Although age has previously been associated with hock injuries (Weary and Taszkun, 2000, Rutherford et al., 2008), in the present study, primiparous cows had a greater percentage of hock injuries than multiparous cows. Research suggests a positive correlation between age and dominance and dominant cows have priority choice in freestalls (Friend and Polan, 1974). Therefore, it is possible that less dominant, younger cows may be restricted to stalls that have the least amount of bedding (Brenninkmeyer et al., 2013), causing an increased risk for hock injuries. Multiparous cows had a greater percentage of hock injuries than primiparous cows, similar to previously reported findings (Sarjokari et al., 2013).

Conclusions

The shift in laterality observed in cows with unilateral injuries indicate an increase in discomfort and a decrease in cow welfare caused by hock injuries. The change in detectable lying behaviors associated with these injuries suggest the potential for improved recognition of environmentally induced discomfort.

Appendix

Table 2.1. Hock injuries. Number of cows with hock injuries categorized by injury laterality, lameness status, and parity. A greater number of cows that were injured had bilateral injuries and were sound and on their second or later lactation.

	Hock Injury Status	
	Uninjured	Injured
Total	29	159
Lateral hock injuries		
Unilateral	-	16
Bilateral	29	143
Lame (locomotion score of 2 or 3)	14	58
Sound (locomotion score of 1)	15	101
Parity		
Primiparous	5	75
Multiparous	24	84

Table 2.2. Lameness. Number of lame cows categorized by injury status and laterality and parity. A greater number of cows were sound than lame. A greater number of lame cows were injured bilaterally and in their second or later lactation.

	Lameness Status	
	Sound	Lame
Total	116	72
Injured (hock score of 1 or higher)	101	58
Uninjured (hock score of 0)	15	14
Lateral hock injuries		
Unilateral	11	5
Bilateral	90	53
Parity		
Primiparous	58	22
Multiparous	58	50

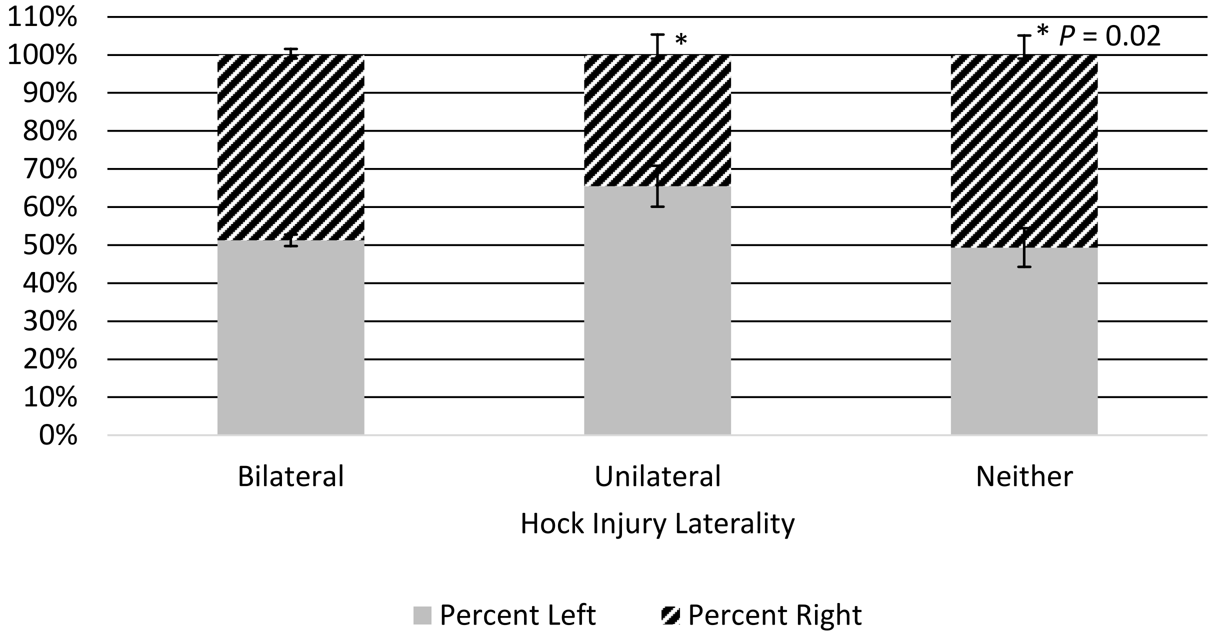

Figure 2.1. Hock injury status and lying laterality. Proportion of time cows spent lying on the left and right side categorized by hock injury status. Hocks were considered injured if they had a score of 1 or above. Hock injury scores of 0 designated cows with uninjured hocks. Cows with bilateral injuries had both hocks injured, cows with unilateral injuries had only one hock injured, and cows with no hock injuries were categorized as “neither.” Cows with unilateral injuries spent more time lying on their left side than cows with bilateral injuries or no injuries (*P* = 0.02).

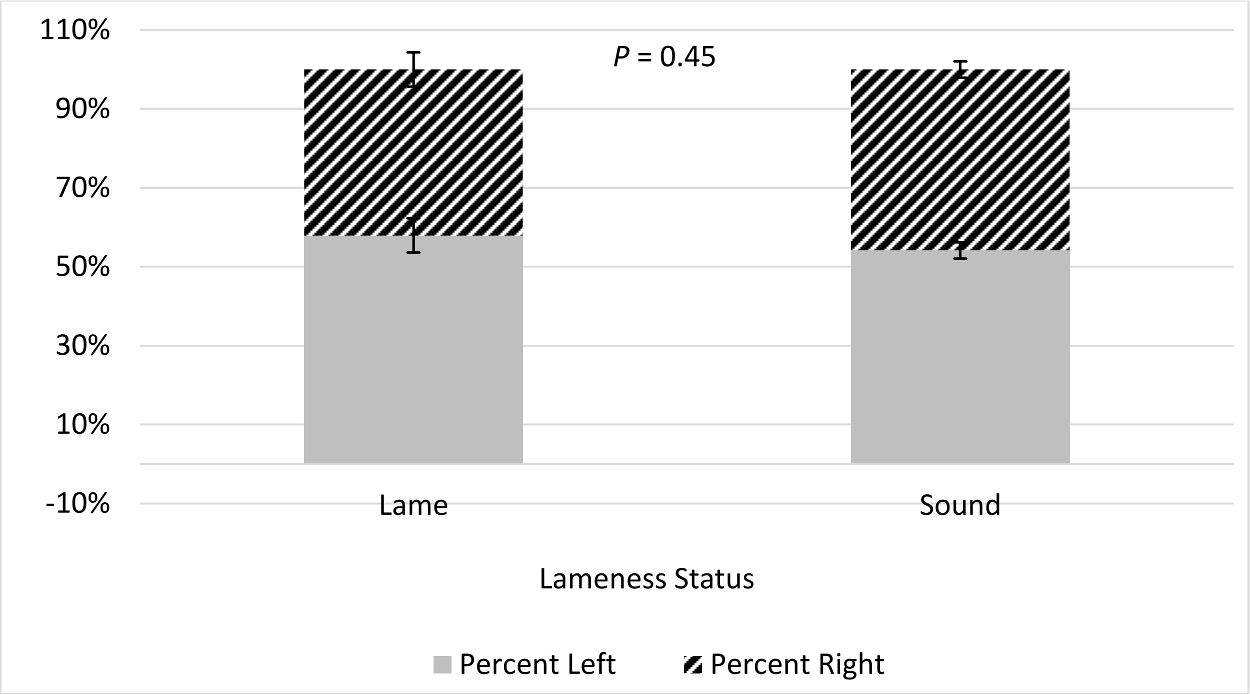

Figure 2.2. Lameness status and lying laterality. Proportion of time cows spent lying on the left and right side categorized by lameness status. Cows were considered lame if they had a locomotion score of 2 or 3. Cows with a locomotion score of 1 were considered sound. Both lame and sound cows did not shift their proportion of left or right side lying from 50%. (*P* = 0.45).

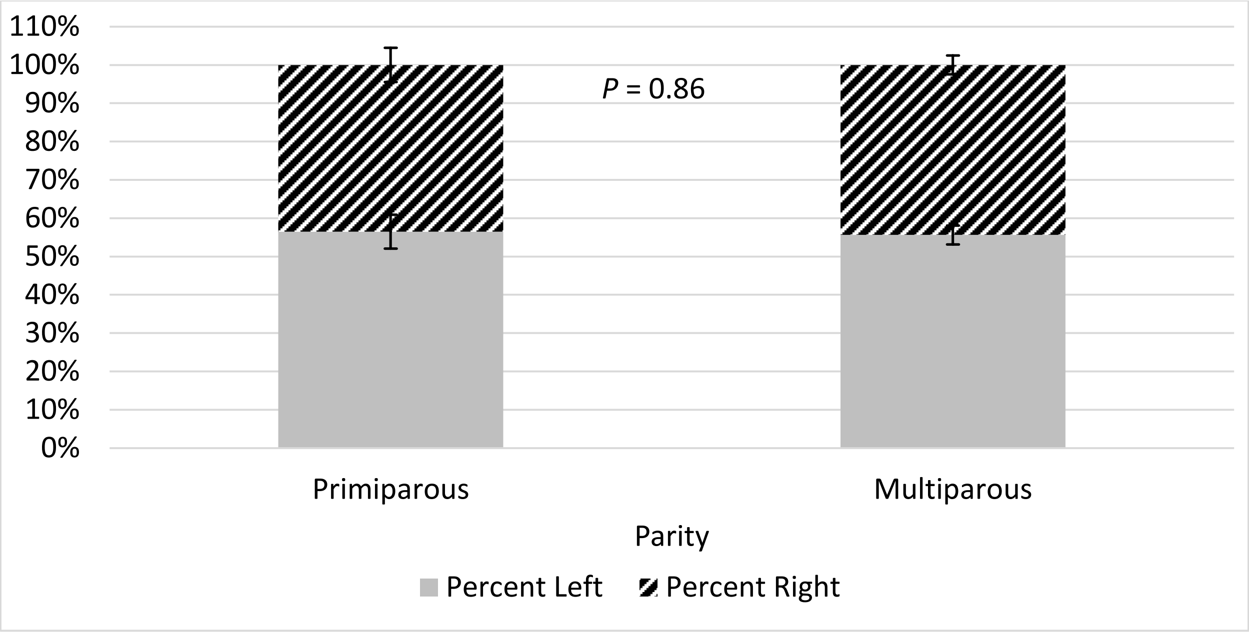

Figure 2.3. Parity and lying laterality. Proportion of time cows spent lying on the left and right side categorized by parity. Primiparous cows were in their first lactation. Multiparous cows were in their second or greater lactation. Both primiparous and multiparous cows did not shift their proportion of left or right side lying from 50%. (*P* = 0.86).

CONCLUSION

Disease and ill-health in dairy cattle impact the production, economy, and welfare of both the dairy and beef industries. These conditions cause disruption in the normal behaviors and physiology of cattle. However, these changes in behavior and physiology give researchers, producers, and veterinarians tools to detect and diagnose sick cattle. One such behavioral change is lying laterality, which, although has been tied to painful or uncomfortable situations such as cannulation or late pregnancy, has not been previously identified as a behavioral change associated with respiratory disease in calves or hock injuries in adult cows.

Calves with experimentally induced respiratory disease spent more time lying down on day of inoculation compared to healthy calves. Infected calves also spent more time lying on their right side over the course of the study compared to healthy calves, which showed no significant preference in lying side. In the second study, cows with unilateral hock injuries spent more time lying on their left side than their right side compared to cows with bilateral hock injuries or cows without any injuries. The shift in laterality observed in infected calves indicates discomfort, potentially from the infected lung; calves were inoculated with *M. haemolytica* in the right lung. The laterality observed in cows with unilateral hock injuries suggests that cows are motivated to change lying positions to alleviate discomfort associated with hock injuries. The discomfort illustrated in the laterality shift further emphasizes the importance in early detection and treatment of respiratory disease as well as the importance of recognizing and correcting potential environmental conditions causing hock injuries in order to improve animal welfare.

By using automatic behavioral monitoring systems, changes in lying laterality as well as other behavioral changes such as increased or decreased lying time can be objectively evaluated to determine animals with potential health conditions.

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VITA

Nicole Eberhart grew up in a small town in Kansas. She obtained her undergraduate degree in Animal Science from Kansas State University in Manhattan, Kansas. Upon graduation, she decided to take time and think about what she was doing with her life. In that year, she discovered her love of dairy cows and decided to pursue a Master's degree in Animal Behavior and Welfare with a focus in dairy cattle. Through a mix of fate and good luck, she made contact with her now graduate advisor, Dr. Peter Krawczel and he obliged to give her the opportunity to follow her dreams. Upon graduation with her Master's, Nicole will continue her journey of self-discovery with her cat and dog, Meridon and Lana, in tow.