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Predicting Immunization Status at Arrival in Tennessee Stocker Calves

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To the Graduate Council:

I am submitting herewith a thesis written by Claire E. Hunkler entitled "Predicting Immunization Status at Arrival in Tennessee Stocker 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.

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Predicting Immunization Status at Arrival in Tennessee Stocker Calves

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ABSTRACT

Bovine respiratory disease is a multifaceted disease with agent, host, and environmental factors. Stocker calves are at high risk of contracting the disease through many stressors like weaning, transportation, commingling, etc. The purpose of this project was to test if externally observed physical characteristics of calves on arrival at a stocker facility can be used to predict calfhood vaccination status determined based on antibody titer levels. Knowledge of highly correlated characteristics could allow stocker operators to reduce the occurrence of BRD through targeted management strategies, thus lowering morbidity, mortality, and treatment costs. Ear notches, blood, and visual characteristics were collected for 408 stocker calves at four farms in Tennessee. Each animal was tested for Bovine Viral Diarrhea Virus-Persistently Infected status and titer levels for three known viral agents. Multiple visual characteristics were predictive of immunization status, including the presence of a prior ear tag (1.6 times), heifers (0.82 times), steers (1.26 times), polled cattle (4.8 times), body condition score increasing by one (1.46 times), and possessing health records (2.2 times). When analyzed together, the calf factors of sex, tag, and possessing health records remained the most universally informative. When we evaluated the predictive nature of multi-variable models, predictability was generally low. Despite low model accuracy, this initial work creates a good foundation for further research focused on more robust data collection to build more robust models.

Additionally, we followed sixty calves through the stocker phase to ascertain the downstream impacts on health and production of preconditioning. We found that preconditioned calves had lower BRD incidence, had a higher presence of detectible titers, and gained more weight over 60 days than naïve cattle. However, many calves marketed as preconditioned did not have detectible viral titers on arrival. This work identifies practical solutions for stocker operations to make more informed purchasing and management decisions. It also lays the groundwork for future work identifying ways to deliver precision management to stocker cattle.

Keywords: Bovine respiratory disease, Stocker cattle, Tennessee, Precondition calves

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LIST OF ABBREVIATIONS

ADG, Average daily gain

BCS, body condition score

BHV-1, bovine herpes virus one.

BRD, bovine respiratory disease

BRSV, bovine respiratory syncytial virus

BVDV, bovine viral diarrhea virus

BVDV-PI, bovine viral diarrhea virus persistently infected

ELISA enzyme-linked immunosorbent assay

IBR, infectious bovine rhinotracheitis

MLV, modified live virus

SN, Serum neutralizing

VNT, virus neutralizing tests

CHAPTER ONE

INTRODUCTION AND GENERAL INFORMATION

Introduction

Bovine respiratory disease (BRD) is the primary cause of mortality and morbidity in the cattle industry (Griffin 1997, Smith 1998, USDA 2015) Southeastern stocker calves are considered high risk for BRD due to inconsistent weaning methodologies, extensive commingling at sale barns, transportation stress, and low levels of vaccination driving risk (Parish, Rhinehart et al. 2021). Over 50% of stocker cattle are marketed through an auction facility, so commingling is a big concern in relation to BRD (Schmitz, Moss et al. 2003). BRD is a multifaceted disease with numerous agent, host, and environmental factors. Vaccination has been shown to successfully reduce BRD prevalence, but factors like poor vaccine timing and improper administration can inhibit the efficacy of the vaccine. Vaccinating calves alone cannot completely control BRD (Parish, Rhinehart et al. 2021). Common viral agents associated with BRD include Bovine viral diarrhea virus (BVDV), Bovine respiratory syncytial virus (BRSV), Infectious bovine rhinotracheitis (IBR) also known as Bovine herpesvirus (BHV-1), Parainfluenza type 3 virus (PI3), and Bovine respiratory coronavirus (BRCV) (Fulton, Confer et al. 1995).

Our work focused on three crucial BRD viruses (BVDV, BRSV, and IBR) because these antigens are commonly present in vaccines commercially available in the United States (Chamorro and Palomares 2020). This study aimed to correlate visual indications of prior handling or processing to immunization status for BRD in calves at arrival at a stocker facility. Better predicting an animal’s immunization status could help producers make more informed purchasing and precise management decisions. Using these immunization calf indicators, we develop proof-of-concept predictive models for immunization status. Our ultimate goal is to help producers manage calves more precisely on-arrival, lowering the morbidity, mortality, and associated costs of BRD.

CHAPTER TWO

LITERATURE REVIEW

Introduction of the beef cattle industry

The beef cattle industry is unlike other meat production industries (i.e., poultry and swine) which are vertically integrated. One thing that is common among all livestock industries is that producers want to create high-quality, wholesome products for consumers. Cow-calf production represents the first segment of the beef value chain, where forage-based cows raise calves that will go on to feedlots and eventually slaughter. Nearly a third of cow-calf operations are located in the Southeastern states (McBride and Mathews 2011). The average size of a cow-calf operation in Tennessee is approximately 30 head, and the state has 1.81 million head of the total cattle inventory (USDA-NASS 2012) (USDA-NASS 2020). These inventories are consistent with other states in the Southeast where 35% of cow-calf operations in the Southeast have 20-49 head of cows; while in contrast, 35% of operations in the West have 500 head or more (McBride and Mathews 2011). Smaller operation sizes in the Southeast means that cattle tend to be marketed as smaller groups. This means that feeder calf buyers must purchase multiple groups of calves to make a “pot load” or “lot”. This leads to increased commingling of calves from numerous backgrounds, creating more opportunity for diseases transmission and stress in the calves, especially regarding recurrence rate of BRD (Wiegand, Cooke et al. 2020).

The Stocker industry is the intermediate phase between the cow calf and feedlot sector. The goals for the stocker industry are to add weight, uniformity, and money to calves before they are sent off to the feedlot. A stocker calf is an animal that is freshly weaned, six to nine months of age, and 400-700 pounds. The stocker segment adds these desirable characteristics to calves based on a forage-based diet for around 3-4 months. The Southeastern regional characteristics of abundant rainfall, high-quality forages, and higher stocking rates than other regions in the United States make it the perfect environment for the Stocker industry (McBride and Mathews 2011).

While the cow-calf and feedlot sectors have been extensively researched with regards to cattle health and wellbeing, the stocker sector remains largely unstudied. Health information and protocols from cow-calf production and the feedlot are not directly portable to stocker cattle which present a unique set of challenges, with less than a quarter of cattle being indicated as vaccinated when coming through the sale barn (Miller 2010). It is common for stocker producers to purchase calves without knowledge or records of previous health management. As such, many of these calves would be considered at high risk for developing BRD post-arrival. These calves are usually unthrifty, lighter-weight, and exposed to elevated levels of disease and stressors (Parish, Rhinehart et al. 2021). High-risk calves need to be treated and managed differently than calves that may be considered minimal risk. It is paramount to isolate these high-risk calves and calves that are visually ill away from the low-risk for the animals to limit further transmission (Parish, Rhinehart et al. 2021).

Some stocker calves will go through a preconditioning program while at the cow-calf farm before entering the stocker segment. In conventional preconditioning programs calves will be weaned at least three weeks prior to shipment or selling (Cole 1985). They will also have received vaccinations for BRD and clostridial diseases. They will be processed and be dehorned, castrated, and treated for internal and external parasites. This preconditioning program allows cattle to become more acclimated to the new environment of the stockering segment in front of them. This preconditioning period is able to largely ease the stressors associated with weaning, significantly lowering the risk of these animals (Cole 1985). Preconditioned cattle are considered low-risk animals as compared with traditional non preconditioned calves, and need to be managed differently on and post arrival (Parish, Rhinehart et al. 2021).

Bovine Respiratory Disease in the U.S.

The varying complexity, severity, and identification of BRD can be difficult to treat compared with other illnesses. Some animals can present with subclinical cases of BRD and will not show signs and symptoms of the disease until the disease has progressed substantially. BRD's clinical signs are also very subjective to the human observer (Blakebrough-Hall, Dona et al. 2020). Some of the typical clinical signs of BRD include, but are not limited to, cattle that are off feed, labored breathing, increased respiration rate, fever, depression, nasal discharge, coughing, weight loss, and a rough coat (Ward and J 2021). Visual signs can be a clear indicator of the health and quality of management of an animal, but the diagnosis of BRD can be complicated.

To combat some of this disease stocker cattle will be processed and evaluated after arrival to the stocker farm. Standard stocker arrival management practices include producers administering de-wormers, vaccinating for diseases like BRD and clostridial diseases, administration of antibiotics, castrating the intact bulls, etc. (Parish, Rhinehart et al. 2021). Because the calves are acquired in as a group, they are managed as a group rather than on a per animal basis. This can be problematic when animals that are not sick are administered mass metaphylaxis (antibiotics on arrival) when it is not needed. Overuse of metaphylaxis can create animal stewardship and antibiotic effectiveness issues, especially regarding antibiotic resistance pathogens (Patel, Wellington et al. 2020). González-Martín and colleagues observed that selective metaphylaxis reduced antibiotic use lowered drug-related production costs compared with mass metaphylaxis (González-Martín, Elvira et al. 2011).

Similar to stocker operations, BRD is the most common disease in the feedlot sector, where 16.2 % of cattle contract it during their stay (USDA-NAHMS 2013). To combat this, 59.3% of large capacity feedlots (>1,000 head), use metaphylaxis on almost a quarter (21.3%) of the incoming cattle that are placed on feed (USDA-NAHMS 2013). Metaphylaxis or treatment for the control of BRD consists of using antibiotics on arrival for a whole group of cattle with only a portion of the group showing clinical signs of disease. This is a preventive measure and control treatment used by the industry to limit future infections that may be incubating in apparently healthy animals. This frequent use of antibiotics poses a stewardship issue that can be mitigated through more appropriate management.

The overuse of antibiotics can create an efficacy issue with the creation of drug resistance pathogens in these cattle. Klima and others examined at the antimicrobial resistance of BRD bacterial pathogens and saw when performing a susceptibility test 50% of *P. multocida* isolates, and 72% of *M. haemolytica* isolates had multidrug resistance and resistant to least three or more antimicrobials (Klima, Zaheer et al. 2014). Specifically, *M. haemolytica* it possessed resistant phenotypes to all antimicrobials commonly employed, except Ceftiofur to treat BRD. They also saw that a third of the *M. haemolytica* samples and 12.5% of *P. multocidaisolates* were resistant to seven classes of antimicrobials.

However, several studies have demonstrated that when used appropriately, metaphylaxis can be cost effective and can reduce morbidity and mortality associated with BRD (Word, Wickersham et al. 2020). Word and others observed 25.2% lower morbidity rates for calves receiving metaphylaxis and a significant increase in AGD. Dennis and others concluded the appropriate use of metaphylaxis helped mitigate decreased performance in high risk stocker calves; thus seeing the aid of metaphylaxis strategies in the high risk animals (Dennis, Schroeder et al. 2020)

Not only is BRD a risk to the health and wellbeing of cattle across all sectors, but it also hinders every sector economically. The estimated annual direct cost of this disease is $54.12 million, while the indirect costs like labor and decreased performance can add up to over $5 billion (Johnson and Pendell 2017). The related treatment costs per head, per treatment have been shown to be $12.39- $23.60 (plus the cost of labor), with an average treatment rate of 1.7 times (Faber, Hartwig et al. 1999, USDA 2013). Calves who were treated three or more times for BRD earned $174 less net profit than calves who were never treated (Faber, Hartwig et al. 1999).

BRD is the leading cause of morbidity and mortality in calves that were three weeks of age and older in the cow-calf sector, and it is the leading cause of morbidity and mortality for the beef cattle industry as a whole (USDA 2010, USDA 2015). Morbidity or when an animal is suffering from a disease and is sick, and mortality or when an animal dies from the disease, go hand in hand when it comes to extreme cases of bovine respiratory disease. It is not uncommon to see morbidity rates in the 60% range for the cattle industry (Faber, Hartwig et al. 1999, Thompson, Stone et al. 2006).

Since BRD is a multifaceted disease with no single cause for infection, it is hard to pinpoint the single viral or bacterial pathogen that is the culprit behind the illness in a particular animal. Instead, there are multiple agent, host, and environmental factors involved in an infection of BRD. It is common for cattle to have simultaneous infections of different pathogens that facilitate secondary infections.

Klima and colleagues dissected the pathogen load of a group (n=68) of animals that died due to BRD. The most common pathogen among animals in their study was *M. haemolytica* (91% infected) and BVDV was the second highest (69%). Sixty-three percent tested positive for both *M. haemolytica* and BVDV (Klima, Zaheer et al. 2014). Furthermore, they found that 97% of the cattle had been infected with multiple other pathogens. This is what makes treating BRD difficult, often leading to recurring treatments for this disease. These samples were collected via nasopharyngeal swabs, and observable pathogens were confirmed to be in the lung, nasopharynx, or both. Interestingly, 100% of the Texas in this study cattle had *Histophilus somni* and 100% of the Nebraska cattle had *Mannheimia haemolytica*, underlining the role that the source of animals is a major driver of the pathogens type and abundance.

Many studies have observed changes in BRD prevalence throughout different years and particular farms. A 15-year study by Snowder and colleagues showed that the instance of BRD varied from 10-43% in low instance years (Snowder, Van Vleck et al. 2006). Pre-weaned calves had lower rates of BRD of 3.3% to 23.6% compared to non-weaned calves (Snowder, Van Vleck et al. 2005). No single environmental factors in this study could be attributed to the years of high (1987, 1988, 2000, and 2001) and low incidence (1984, 1985, 1996, 1998, and 1999). This study shows the vast variability in BRD incidence year-to-year.

There also can be occasions where BRD prevalence increases seasonally, for example in the spring and fall months there are greater occurrences of the disease than in the winter or summer. Spring born calves coming into the feedlot in the fall, specifically in November had 2-8 times higher rate of fatal BRD infections than that of calves entering in September or December witnessed in one study (Ribble, Meek et al. 1995). This trend was consistent throughout all four years this study with the maximum risk of BRD occurring 2-4 weeks after the peak of calf sales at auction facilities. They found factors that attributed to this could include changes in population dynamics and density, labor efficiency and transportation availability, and weather. Another aspect of seasonal BRD that is not related to weather, but still largely an environmental occurrence is the influx of calves being marketed in the fall season. Producers tend to market cattle at sale facilities more in the fall for various reasons like more spring calving herds, location, tradition etc. (Taylor, Fulton et al. 2010). The incidence of BRD increases during this season with higher rates of commingling and stress. Some human aspects contribute to the higher rates during this season where facilities have higher workloads resulting in slower loading and unloading rates which can lead to more unintentional stress on the animals. (Taylor, Fulton et al. 2010).

It takes around 14 days for animals to show symptoms of BRD, and most of these cases occur in the first 75 days post-arrival (Thomson, Moore et al. 2012). Therefore, we chose to evaluate the first 60 days after arrival in our study two. By this later stage of an animal's time in the feedlot the animals have had time to commingle and spread disease throughout the population. Thompson and colleagues observed that by day 35, 87% of all treatments had been administered for BRD (Thompson, Stone et al. 2006). Fulton and others had a fatal disease onset mean (i.e. Only animals that died from BRD) of 32.65 days (Fulton, Blood et al. 2009). All these studies show BRD has a fast onset after arrival as animals are dealing with the greatest levels of stress and must be addressed accordingly to minimize morbidity and mortality levels.

Faber and colleagues identified that within the first 14 days of cattle arriving to the feedlot, 40% of the first treatments for BRD took place (Faber, Hartwig et al. 1999). They also saw morbidity rates between groups range from 0-59% with an average of 20.6% (Faber, Hartwig et al. 1999). Other studies observed much higher morbidity rates: 66%, 66.5%, and 64% in Thompson, Wilson, and Caucci respectively. (Thomson, Moore et al. 2012, Wilson, Step et al. 2017, Caucci, Di Martino et al. 2018)

Of the 66% morbidity in Thompson (2012), 15% of the calves were treated for BRD twice, while the remaining 51% were treated only once for BRD. Another study by the same group experienced treatment rates of 22.6% of the population (Thompson, Stone et al. 2006). Of those treated animals, 17.5% were treated for BRD two times or more

(Thompson, Stone et al. 2006). Between all the studies, there were similar time frames when cattle showed visual indicators of BRD and were treated, as well as similar morbidity rates. These studies demonstrate that cattle start to show clinical signs for BRD within the first few weeks after the arrival at feedlots, and that enormous variation can exist in BRD incidence rates.

The study by Faber and colleagues consisted of younger animals and animals received from multiple sources. 38% of the cattle were less than 200 days of age when received at the feedlot (Faber, Hartwig et al. 1999). At this younger age, it is possible that the calves had not been properly weaned prior to delivery. The average age of calves received at the feedlots in this study was 195 days at weights of 572 pounds (Faber, Hartwig et al. 1999). The average age for weaning beef calves in the U.S. was 221 days in 1997 and has steadily decreased to 195.8 days in 2017 (USDA:APHIS:VS 1997) (USDA-NAHMS 2009, NAHMS 2020). The Faber et al. (1999) study observed that calves that were weaned more than 30 days prior to receiving had significantly lower incidences of BRD compared with calves weaned less than 30 days or non-weaned at all (Faber, Hartwig et al. 1999). Further, calves who went into the feedlot at less than four months of age had a BRD morbidity rate of 50% (Faber, Hartwig et al. 1999). As calves got older, their BRD morbidity rate decreased such that calves that were seven months of age or older upon intake had a morbidity rate of only 12.2% (Faber, Hartwig et al. 1999). This data supports the need for proper calf weaning procedures and placing calves into the feedlots or stocker operations at a proper age to mitigate some BRD morbidity.

Proper weaning allows calves to be better acclimated to their new settings and prepared for the next segment of the industry.

Faber and others also found that BRD relapse rates were the highest in calves that were weaned less than 30 days (57%) (Faber, Hartwig et al. 1999). For non-weaned calves the relapse rate was at 38%; calves that were weaned more than 30 days had a relapse rate of only 31% (Faber, Hartwig et al. 1999). These rates coincide with other studies showing that weaning calves for an extended period prior to the feedlot provides numerous health benefits across the board. Non-weaned calves were treated at the highest rate (30%), followed by calves weaned less than 30 days (28%). Calves weaned more than 30 days had BRD incidences of only 13%. The percentage of calves treated three times or more in these two groups (the non-weaned and weaned 30 days plus) had an equal percentage of 6%, while only 1% of the calves that were weaned more than 30 days were treated that many times (Faber, Hartwig et al. 1999).

Another major calfhood health challenge in cattle industry is Bovine Viral Diarrhea Virus (BVDV). BVDV type 1 and type 2 are the two major causes of BRD infections in cattle. Bovine Viral Diarrhea and BHV-1 are primary surface infections (i.e. They infect the animal first, creating favorable environment for opportunistic commensal bacterial organisms enter and cause deadly cases of bacterial pneumonia) (Fulton 2009). They infect the epithelial cells that inhabit the nasopharyngeal mucosa and the lungs of the respiratory track (Fulton 2009). Bovine Viral Diarrhea virus can attack calves in-utero (45 to 175 days of gestation) and create a persistently infected (PI) calf (Chase, Hurley et al. 2008). Once born, this animal will shed BVDV its whole life, consequently infecting other animals. The threat of BVDV can come in many forms. A meta-analysis was conducted on a global scale over a 55-year period using 325 distinct studies showed that in the United States the pooled PI prevalence is around 0.41% of the cattle population (Scharnböck, Roch et al. 2018).

It has become more of a common management procedure to PI test incoming calves to a stocker or feedlot facility, as the presence of PI calves can create major issues by infecting other calves. There are rapid tests on the market that allow producers to obtain results in as little as 20 minutes compared to sending an ear notch test off to a lab (IDEXX and Laboratories 2011). With the advent of rapid tests, producers can isolate positive animals quicker and thus reduce potential infection rates. The 2008 National Stocker Survey showed that 16.5% of the surveyed 339 producers market and separate their cattle without testing or identifying whether a calf is PI-positive (Roe 2010). Only 18.9% of producers who BVDV-PI test inform buyers of positive cattle when marketing at a sale facility(Roe 2010). 42.7% of stocker producers test their incoming calves for BVDV-PI within the first two days after arrival to the facility, while 35.0% test the incoming cattle before arrival to the farm (Roe 2010). Once the cattle are identified as PI positive 46.6% of producers will separate and feed out PI animals in separate pens or areas of the farm, while 13% choose to euthanize (Roe 2010). These statistics show that majority of stocker producers want to test incoming cattle for BVDV-PI and when those cattle are positive, producers undertake management strategies that lower the risk of the animal further spreading BVD in their herd. As such, they separate PI animals and either try to salvage production loss from these animals or sell them.

The proper use of vaccinations for BVDV can help combat the disease in a herd. One study by Fairbanks and others showed that when giving a dam a commercial modified live vaccine (MLV) for BVDV that contained both BVDV type 1 and 2 prior to breeding, 100% of the fetuses were protected against BVDV type 1, and 95% protected against BVDV type 2 and therefore did not become PI animals in utero (Fairbanks, Rinehart et al. 2004). While today most MLV and killed virus vaccines in the United States contain both BVDV types 1 and 2. It is important that vaccines contain both strains, rather than a single strain where the protection can be lower (Fulton, Confer AW et al. 1995). The duration of immunity was found to be at 370 days after vaccinating with a MLV vaccine in heifers, where all calves were negative for BVDV throughout this time frame (Ficken, Ellsworth et al. 2006). The lasting immunity and high efficacy rates in this vaccine protocol demonstrate the utility of production management tools when attempting to limit the spread of this disease in a producer's herd.

Other than viral or bacterial agents that effect the likelihood of calves being ill with BRD there are multiple host effects related to an individual animal's risk level. Some of the host factors that play a role in BRD are the differing antibody titer levels in their blood per animal to combat the disease. Other host factors include breed, sex, and castration methods. A 15-year study by Snowder showed that castrated male calves were more likely to become sick with BRD compared with heifers (Snowder, Van Vleck et al. 2006). Switching from surgical castration used for three years (1987-1989) to a banding method (1990-2001) also coincided with a decreased BRD instance in the cattle. This study also identified breed differences in BRD incidence, with Herefords being more susceptible compared to composite animals, indicating a possible role of heterosis. While the study resulted in similar infection rates of purebred cattle and composite breed, the mortality was the highest in red poll calves at 8.9% compared to an overall average of 4%.

In another study, the same group evaluated the different influences of breed, heterozygosity, and disease instance of BRD in pre-weaned beef calves over a 20-year period (1983 to 2002). They saw that the highest instance of BRD in these pre-weaned calves was in the Braunvieh breed with 18.8% across 12 other different breeds ($P \leq 0.05$) (Snowder, Van Vleck et al. 2005). They did note that the Braunvieh breed's instance rate being higher than the rest could be compounded by the higher incidence of calving difficulty in the breed and in this study. The added stress on the calf and dam with difficult births can lead to reduced performance, or even death of the calf, dam, or both. They observed a negative genetic correlation between maternal and direct genetic effects for BRD incidence. This suggested that genetically superior dams that were better at resisting BRD produced and raised calves that were actually more predisposed to BRD (Snowder, Van Vleck et al. 2005). Research from this study now suggests that the dams supplied superior passive immunity to their calves, and this may have caused a delay the development of the calves' direct immune system. (Snowder, Van Vleck et al. 2005). Since immune system development was delayed, the calves were more prone to developing BRD in the pre-weaning stage of their life.

Bovine Respiratory Disease impacts on animal performance.

Beyond loses to mortality, BRD can cause substantial decreases in performance. BRD accounts for the loss of Average daily gain (ADG) in the cow-calf, stocker, and feedlot sectors. The 15-year study by Snowder showed that healthy animals gained 0.08 lb. more per day than animals that had a case of BRD (Snowder, Van Vleck et al. 2006). This correlates to a 17.637-pound difference that can be expected between healthy and BRD instance-stricken cattle (Snowder, Van Vleck et al. 2006). For each additional BRD treatment, a stocker calf lost on average 17.6 pounds of total gain (Griffin CM, Scott JA et al. 2018 Spring). Thomson, Moore et al. also observed that within the first 75 days of arrival, the calves that were infected with BRD had lowered ADG and lower overall gain in the finishing phase (Thomson, Moore et al. 2012). Schneider and others observed a decrease in ADG of the acclimation period. They saw 0.81 ± 0.06 lb. decreasing in the acclimation period and the overall test period decrease of 0.15 ± 0.02 lb. (Schneider, Tait et al. 2009). In this study they also looked at the impacts on hot carcass weight (HCW) and marbling. They saw a decrease in HCW of 17.9 ± 3.04 lb. and a reduction of a marbling score of 0.13 ± 0.04 in cattle that were treated for BRD (Schneider, Tait et al. 2009). This reduction in yield and quality of an animal carcass resulted in a reduction in carcass value of $23.23, $30.15, and $54.01 in the carcass value for cattle that were treated once, twice, and three times respectively for BRD (Schneider, Tait et al. 2009).

Overall, in the study by Thompson (2012), they noted that if a calf is treated for BRD, they would be able to reach the same yield and quality grade as a non-treated calf if the feeding period is prolonged. This supports the idea from many studies that when a calf is sick, they go off feed, and have reduced ADG compared to a healthy animal. A prolonged feeding period gives the sick animals a chance to catch up with healthy animals, but this does not go unnoticed. There are associated costs of prolonging the feeding period. Thompson and others also saw a 0.05 lb. reduction in ADG caused by the effects of BRD (P= 0.02) (Thompson, Stone et al. 2006). This correlated to an increase of 5.1 days on feed to make up for the reduction. As a producer, evaluating the cost to feed that animal five more days to obtain similar results to a non-treated animal can become costly in the long run with large operations.

When comparing the number of treatments a calf receives to performance measures, it is conclusive that the more the animal is ill and must be treated, the less profitable and lower quality product that animal creates. Wilson and others examined that non-treated calves weighed 714.3 lb. while calves treated three or four times weighed only 573.2lb at the start of finishing period (Wilson, Step et al. 2017). Calves that were treated once for BRD and the calves that were treated twice, had a 68 lb. difference in body weight, creating a major weight gain deficit that they would need to make up (Wilson, Step et al. 2017). Their data agreed with the Thomson study in that a calf that has been treated for BRD can reach the same yield grade as healthy animals if they are fed for an extended period. They did note the calves treated for BRD multiple times may eventually reach the same yield grade as healthy calves, but the likelihood is low. That said effective treatment can prevent animals from experiencing major reductions in AGD (Faber, Hartwig et al. 1999).

The timing of BRD vaccinations is another concern for stocker and feedlot operations. Rogers and others compared on-arrival vaccines and delayed delivery on day

30 in feedlot heifers. No difference was found in final grade performance, yield and carcass quality, feed conversion, or dry matter intake in any of the treatment groups (Rogers, Miles et al. 2016). A study by Richeson and others showed that ADG was greater for calves that received delayed MLV vaccines (by 14 days), than that of on-arrival vaccines for the study period of 0-42 days (Richeson, Beck et al. 2009). For the delayed group of calves they saw an increase of body weight by day compared to the on-arrival group of calves on days 0-42: (1.65 vs. 1.4 ± 0.19 lb./d) (Richeson, Beck et al. 2009). This is likely due to allowing animals to cope and respond to intake stress prior to subjecting the immune system to pathogens via vaccination.

Titers to Bovine Respiratory Disease.

A simple blood or tissue test for BRD does not exist like for BVDV-PI. The best option for diagnosing whether an animal has been sick is to assess its serum antibody levels. In evaluating the antibody levels, we can determine whether the calf has received a vaccine, has been exposed, or is naïve to a given pathogen. In addition, there exists much literature regarding titer levels of animals that have contracted BRD, and then the animals' titer levels after vaccinations for BRD. The use of a virus neutralizing test (VNT) can be used to measure the presence and magnitude of the viral load in an individual. The VNT is an enzyme-linked immunosorbent assay (ELISA) serial dilutions of serum are added to a plate. In each well there is an increasing dilution of serum up to the point all of the viral particles are bound. So, the first well will have one part serum two parts diluent, subsequent wells receive double the dilution (i.e., 1:4, 1:8, and so on). The dilution in which the viral content is fully neutralized is the value that the sample receives with regards to viral titer load. When evaluating antibody titers, a fourfold increase in the titer levels can indicate an active infection (Fulton 2009). BHV-1, PI-3V, BRSV, and BVDV antibody tests use virus neutralizing tests in cell culture routinely (Fulton 2009).

We would expect animals with elevated levels of multiple pathogens to have received a vaccination (Grooms and Coe 2002). A vaccination like the five-way or seven-way vaccines that fight against multiple viral pathogens would be more likely to result in moderately high viral titers for multiple pathogens, rather than a single heightened titer occurring from a viral infection. One study suggested that an animal vaccinated with MLV Bovi-Shield would have a titer level of ≥ 1:10 to BHV-1, and ≥ 1:4 for BVDV (Fulton, Confer AW et al. 1995). This level of antibodies will change over time and can differ in each animal. All five animals in this vaccination group in this study had similar titers at day 14, but by day 126, only one out of the five still had an antibody titer of ≥ 1:10 for BHV-1. By day 154 all calves in all vaccination groups had titer levels of ≥ 1:4. Similar results were had for BVDV; day 28- 126 all calves in the group had elevated titers of ≥ 1:4 BVDV.

A study by Ross and others looked at a 150 head commercial cow-calf herd that had reports of cows having abortions that were suspected to be related to BVDV infections. Out of the sample of 15 breeding females, all animals were seropositive, meaning they had an elevated titer levels concurrent with a BVDV infection (Ross 2003). Eleven of those females also had extremely high titers for BVDV (1: 972 to 1: 8748) (Ross 2003). It is important to note that the sampled cattle were less than two years of age, and the researchers avoided using mature cattle that might have had high titers due to previous exposure from multiple vaccinations. It is also important to note that older animals that receive the recommended vaccinations will have higher titers than animals that are young have not received a vaccine. In cases where the titer levels were this high, it was likely that this herd was likely naturally exposed to persistently infected BVDV cattle. If a herd has a high level of seropositive animals in it (>90%) it is likely caused by a persistently infected animal in the herd (Ross 2003). Additionally, a titer log level of zero or the complete absence of titer is also important to note. When looking at vaccination history, if an animal has no titer to different viral pathogens, then that animal has not been exposed to BRD naturally or through vaccinations. This animal will need to be vaccinated to prevent future infections. Houe and colleagues evaluated titer levels for BVDV and detection of BVDV-PI cattle in a sample population. They determined that a titer level of <1:16 would indicate the animal as naïve to the BVDV (Houe, Baker et al. 1995). They also found that animals that are vaccinated for the virus could have titer levels between 1:32 and 1:1,024 for BVDV (Houe, Baker et al. 1995).

Kirkpatrick and others in 2001 studied the passive transferred immunity in newborn calves and the rate of antibody decay and its effect on subsequent vaccination with an MLV. The 30 dairy calves in their study had estimated seronegative statuses of the following: 65.1 days for IBR, 117.7 days for BVDV 1, 93.9 days for BVDV 2, 183.8 days for PI-3V, and 200.2 days for BRSV (Kirkpatrick, Fulton et al. 2001). These time frames represent the persistence of antibodies that animals receive from colostrum to the time that they reach zero. Following the depletion of this maternally acquired immunity, calves become at increased risk of becoming sick. Menanteau-Horta and others observed that unvaccinated calves' maternal antibodies for BVDV decreased to a titer level of zero by day 200, and 170 days for IBR (Menanteau-Horta, Ames et al. 1985). This demonstrates that depending on the virus, time frames vary in which animals return to a seronegative status. This depends largely on the quantity and quality of antibodies in the colostrum. In this study they also observed that starting titers for IBR (when the animals were two days old) were substantially lower (1:32) than for BVDV (1:692) (Menanteau-Horta, Ames et al. 1985). With this lower starting titer, their results concluded that the detectible IBR titers were lost 30 days sooner than BVDV titers (Menanteau-Horta, Ames et al. 1985). Brar and others, observed results for average half-life of 21 days for both viruses, and Menanteau-Horta and others saw results of 19 days for IBR and 20 days for BVDV (Brar, Johnson et al. 1978) (Menanteau-Horta, Ames et al. 1985). Work by Kirkpatrick and others collected blood at day 32 ± 4 after vaccination and found that at vaccination, all 27 calves were seronegative to IBR, and of those calves 18 of them seroconverted (0 to >1:20) to IBR. Six other calves stayed seronegative and the remaining three had their titers increase 1:20 (Kirkpatrick, Fulton et al. 2001). This also supports the ideas that every animal reacts differently to vaccines and that titer levels can fluctuate in each animal, but there also can be consistent ranges of detectible titer levels that represent likely vaccination.

Menanteau-Horta and others observed that by six months of age calves' maternal antibodies for BVDV and IBR had decayed to nearly zero (Menanteau-Horta, Ames et al. 1985). This was similar to what the results that Woods and others saw in unvaccinated control animals that had no detectible antibodies to IBR or BVDV (Woods, Mansfield et al. 1973). Numerous other studies focused on quantifying titer levels in cattle in stocker phase suggested that if they were not vaccinated against BRD that they would not have detectible antibodies for BVDV or IBR. This also supports the idea that calves who are not vaccinated for respiratory disease will have titer levels close to zero by the age they enter the stocker phase (Woods, Mansfield et al. 1973).

The half-life of antibodies, as well as the time to estimated seronegative status for 30 dairy calves in a study, was as followed for IBR it was (65.1 days), BVDV 1 (117. 7 days), and BRSV (200.2 days) (J. Kirkpatrick 2001). Menanteau-Horta and others saw that unvaccinated calves' maternal antibodies to BVDV decreased to a titer level of zero by day 200, and for IBR by day 170. By six months of age calves' maternal antibodies for BVDV and IBR decayed to nearly zero (Menanteau-Horta, Ames et al. 1985).

Woods, Mansfield et al. evaluated at the timing of immunity responses to vaccinations and saw that after a modified live vaccine containing IBR, BVDV, and PI-3, that titer levels were highest against BVDV, then PI3. Only two steers developed detectable titer levels (1:2) to IBR. The serological titers (all in geometric mean titers) for IBR were at zero at pre-vaccination samplings for the vaccinated group, and after first vaccination it increased to 1.1 but decreased back to zero after that. For the control group they all had titers of zero for the whole extent of the trial period. For the vaccination group they started out with BVDV titers of zero and spiked to an 11.6 after first vaccination then steadily decreased to a 9 after revaccination. For the control group they stayed at a constant zero until the last collection point where they increased to a 1.3 (Woods, Mansfield et al. 1973).

Grooms and Coe observed that all the vaccinated cattle in their study had higher titers to BRSV, PI-3, and BVDV but did not have higher titers to BRSV This study used serological data at a 21-day interval, and the type and timing of the vaccine given for BRD (killed, MLV and timing of 0, 21, or 42 days). The day of weaning was day 21. They concluded that for BVDV, by day 63, all groups of vaccinated calves had higher titers than nonvaccinated animals. The geographic means on day 63 for the vaccinated groups ranged from 12.7 to 2463.8, with two doses of killed virus vaccine being the lowest, while the controlled group had a mean titer of 1.1. For BHV-1, by day 63 all vaccinated groups had mean titers between 3.3 to 19.2, whereas the unvaccinated group had a mean of 1.0. For this vaccination study killed virus generated the lowest titer levels. For BRSV by day 63 the mean titers were not significantly different in the vaccinated group compared with the unvaccinated group. The calves that were vaccinated with MLV on days 0 and 21 though, had significantly higher titers than all other groups on day 42 ($P \leq 0.05$). Lastly for PI-3, all calves, regardless of vaccination status, showed increased titers to PI-3 over time, indicating a natural infection within the herd. Although the exposure, by day 63 all the vaccinated calves still had significantly higher titers than that of the control group ($P \leq 0.05$). The vaccinated groups had titer levels ranging from 595.9 to 977.8, compared with the control group of 388.0 (Grooms and Coe 2002).

A similar study by Kramer and colleagues evaluated decay rates of BRD vaccinations. They did see that across multiple viruses (BVDV1, BVDV2, BRSV, and

BHV1), cattle showed an increase in titers from initial vaccination (D0), booster (3 weeks), and 6 weeks after initial vaccination (Kramer, Mayes et al. 2017). All these studies above indicate that it takes around two weeks after vaccination to exhibit elevated titer levels.

The prevention and vaccination of Bovine Respiratory Disease

Many vaccines are available to producers to combat multiple viral pathogens that cause BRD. A single BRD vaccine can range from $2.25-$6.25 per animal (Wang M, Schneider LG et al. 2018). In response to vaccination, the antibody levels in an animal will be raised for numerous pathogens, compared to natural exposure to a single pathogen.

Since the timing of immunity in an animal is so important, the timing of vaccines is also crucial to stimulating an animal's overall immune function. Vaccination is suggested before calves are six months old, followed by revaccination to avoid possible maternal antibody interference (Wenzel, Mathis et al. 2015). Annual revaccination with a single dose is recommended for sustained immunity against BRD. Although there is evidence that passive immunity could inhibit IBR vaccinations, once the subsequent immunization is given, the first will act as a primer for the secondary response. With revaccination at the time when maternal antibodies have disappeared, the calf will have a proper immune response to an IBR vaccination (Brar, Johnson et al. 1978). If vaccines are given at a time frame that is too young or too old, the drug efficacy may be negatively impacted.

Numerous studies have evaluated the impacts of vaccination timing for stocker cattle, and results are largely inconclusive. Many factors can affect the efficacy of vaccines based on timing. A meta-analysis and systematic review by Snyder and others integrated eight studies that compared feedlot cattle vaccination timing. They performed and calculated Mantel-Haenzsel risk ratios for each outcome and observed no difference in BRD mortality, morbidity, or retreatment risk for calves vaccinated at arrival versus those where vaccinations were delayed. This meta-analysis also mentioned that when evaluating the effects of vaccination timing, outside producer factors like the use of Mannheimia leukotoxin vaccines, Metaphylaxis, or other treatments can make isolating the timing variable difficult (Snyder 2019).

Delays in vaccination can be anywhere from a few days to a few weeks. The idea of postponing vaccinations for stocker and feedlot calves a few days is to allow the newly transported cattle settle into their new environment before being vaccinated (Griffin CM, Scott JA et al. 2018 Spring). The stress associated with travel and relocation can inhibit the effectiveness of the vaccines (Richeson, Beck et al. 2009). While some studies observe no notable impacts of delaying or not delaying vaccinations, other studies show that vaccinating calves on arrival can have detrimental effects on animal health, growth, and performance. Griffin and colleagues compared vaccinating on arrival to a group of non-vaccinated calves to see the impacts on each group. They saw that BRD incidences and mortality rates were significantly higher for calves vaccinated on day zero vs. nonvaccinated calves. They also concluded that stocker calves vaccinated on arrival to the facility had an overall loss of 10.3-pounds in weight gain versus calves that were not vaccinated (Griffin CM 2018 Spring).

Rogers and others evlauated the act of delaying vaccines and adding an immunostimulant and its effect on feeder heifers' performance, health, and carcass merits. Using 5,179 high-risk heifers in their study, quantified the potential benefits of delaying on-arrival respiratory vaccines by 30 days. They used an MLV vaccine with and without an additional immunostimulant. They had groups consisting of an on-arrival vaccine, a delayed vaccine of 30 days, an on-arrival vaccine with an immunostimulant, and a delayed vaccine of 30 days with an immunostimulant. The immunostimulant helped reduce BRD mortality and the overall morbidity of the heifers at 60 and 116 days on feed (DOF) ($P \leq 0.05$). They also found that delaying the vaccine lowered the percentage of calves that were treated twice for BRD ($P \leq 0.05$) (Rogers 2016). They conclude that with the addition of the immunostimulant and the delaying of on-arrival viral respiratory vaccine, there was an overall improvement in health outcomes related to BRD (Rogers, Miles et al. 2016).

Another study observed that delaying vaccines to day 14 can improve acquired immune response in calves (Richeson, Beck et al. 2009). The rate of BRD morbidity for the on-arrival MLV group was 71.5%, and for the delayed MLV group was 63.5%. These differences in BRD morbidity were not significantly different from one another ($P = 0.12$). The 2008 National Stocker Survey found that 27.15% of stocker operations process their calves the day after the calves arrive at the operation, while 22.22% of operations process calves on the day of arrival to the farm (Roe 2010). In contrast, only

1.91% of operations process their calves after 14 days of arrival on the farm. While possessing the calves does not necessarily mean that the calves will receive vaccinations for BRD, this does mean that the cattle would have been worked and possibly evaluated for disease at this critical point and hopefully there are health management actions taken accordingly. The type of vaccine used is an important contributor to efficacy. There are two types of BRD vaccines. They include killed virus vaccines and modified live vaccines. The 15-year study by Snowder saw higher instances of BRD in previous years (1987 to 1992) than in later years (1993 to 2001). In the last years, this lower instance can be attributed to the switch from the less effective killed virus vaccine to the new modified live vaccine (Snowder, Van Vleck et al. 2006).

Faber and colleagues observed comparable results when looking at killed versus modified live vaccines given to feedlot cattle. Killed virus vaccines had higher instances (10%) of calves receiving three or more treatments for BRD than in groups vaccinated with modified live vaccines (3%). They also saw that calves that received killed vaccines for BRD were 2.2 times more likely to experience BRD than the MLV calves, independent of other factors. Calves who received a killed virus vaccine had almost two times the percentage of treated animals for BRD (32%) versus the group who received an MLV (18%) (Faber, Hartwig et al. 1999). Fulton and others showed that a MLV BHV-1 vaccine had a longer antibody titer duration than the killed (inactivated) vaccine (Fulton 1995). This again supports the idea that MLV vaccines can be more effective and provide extended immunological benefits.

A systematic review and meta-analysis by Theurer and colleagues examined 31 studies encompassing 88 trials evaluating the effectiveness of vaccinating cattle with commercially available viral antigen vaccines to combat BRD. As expected, vaccinated calves had significantly lower BRD morbidity risks than nonvaccinated controls in natural exposure trials. When evaluating BHV-1 and MLV BVDV vaccines, the vaccinated calves had lower BRD morbidity risk than control calves in experimental challenge models. In contrast, in experimental challenge trials, the MLV BRSV and PI3 vaccines had no significant difference in morbidity and mortality risk between vaccinated and unvaccinated calves (Theurer, Larson et al. 2015).

The titer means on day 63 for the vaccinated groups consisted of 12.7 to 2463.8, with two doses of killed virus vaccine being the lowest, while the control group had a mean titer of 1.1 (Grooms and Coe 2002). For BHV-1 by day 63 all vaccinated groups consisted of mean titers of 3.3 to 19.2 compared to the unvaccinated of 1.0. For BRSV, by day 63 the mean titers were not significantly different in the vaccinated group than the unvaccinated group. In the study by Houe they found that animals that are vaccinated for BVDV would have titer levels of 1:32 and 1:1,024. They determined that a titer level of <1:16 would indicate the animal as naïve to the BVDV (Houe, Baker et al. 1995).

Vaccine type and the timing of antibody detection can result in differences in detectability via VNT. With a BRD vaccination, elevated titers can be observed by day 14 following a vaccine. Fulton and others evaluated antibody responses to four commercial viral vaccines containing BHV-I, BVDV, PI-3V, and BRSV immunogens. Overall, all four vaccines induced an increase of BHV-1, by day 14, with the chemically altered and MLV vaccines having higher responses compared with the inactivated vaccine. BVDV titers took longer to develop on average but exhibited higher titers through 140 days. The BRSV titers developed more rapidly. For the MLV it had elevated BRSV titers by day 7 and was faster than the inactivated vaccine. Each virus responds and presents differently by titer levels. Different vaccines can induce more or less rapid responses, but animals who are fully immunized will have greater titers than calves who are not.

Hypothesis:

I. Immunized calves will tend to possess certain characteristics that distinguish them from their non immunized contemporaries.

II. Preconditioned calves will differ from naïve calves regarding disease instances and weight gain.

Objective:

I. Develop a model using easy-to-measure characteristics that can predict on-arrival immunization status.

II. To address if preconditioned calves differ from naïve calves regarding immune health, disease incidence, and performance.

CHAPTER THREE
MATERIALS AND METHODS

Study procedures

Animal Care and Use Committee approval was granted for this study. Private producers that were not associated with the university and agreed to client consent forms. For both studies, whole blood samples and ear notches were collected, and visual attributes were recorded from weaned calves on arrival at four Tennessee stocker operations from sale barns (n=408). There were 7 different sampling dates over 8 months (September to April). The number of animals sampled by season is as follows: Fall n=51, Winter n=167, Spring n=191. Thirteen visual attributes were collected on each animal during arrival processing: Sex (heifer, steer, or bull), castration status (freshly cut, cut and fully healed, stag, or intact), approximate frame score (small, medium, large) (AMS-USDA 2000), coat score 1 to 5 (1 = slick to 5 = full winter coat), body conditioning score 1 to 9 (National Academies of Sciences and Medicine 2016, BIF and Parish. 2018), a prior ear tag being present, approximate body weight estimated to the nearest 25 lb., horn status (horned, dehorned, polled), dominant breed influence, coat color, *Bos indicus* influence (yes, no), and signs of illness on arrival. Another parameter collected was if producers stated that calves received vaccines prior to shipment and receiving, based on sale information or buyer confirmation. After data collection, calves were handled and managed by the producer’s normal health management practices. To ensure there was no discrepancy in visual data collection one individual collected all visual data throughout the study.

Animal information

The four farms in our study varied in buying practices regarding weights, sex, and preconditioning status. Calves ranged from low risk to high-risk, sourced from multiple different sale facilities for each farm. At all farms, each sampling group was made up of calves originating from multiple sources. Two of the four farms used prophylaxis on arrival in high-risk calves. Excluding calves utilized in our second study focused on preconditioning, all other farms processed one to two days after arrival. Calves were given at least 24 hours to rest following arrival before processing occurred. At all farms, in-tact bulls were castrated, horned cattle were dehorned or tipped, and calves were tagged. Some farms provided multi-min, implanted calves, and gave BRD vaccines on arrival with boosters two weeks later.

BVDV-PI Testing

Ear notches (1 cm x 1 cm) were collected from each calf during processing. Refrigerated ear notches were shipped to the Tennessee Department of Agriculture's Kord Animal Health Diagnostic Lab in Nashville Tennessee for Bovine Viral Diarrhea Virus (BVDV-PI) testing within one day of collection. Ear notches underwent an ELISA (enzyme-linked immunosorbent assay) test and were analyzed in batches as directed by demand and were reported positive or negative on an individual animal basis.

Blood collection and titer evaluation

Approximately 10 ml of whole blood was collected from all calves via the jugular vein prior to calves receiving any vaccines or metaphylaxis. Whole blood was centrifuged at 2,000 × g for 10 minutes at 4°C. After, serum was aliquoted into 2 mL microcentrifuge tubes and stored at 4°C until shipped. Refrigerated serum was sent to the Iowa State Veterinary Diagnostic lab (ISU VDL; Ames, IA) for virus neutralizing tests (VNT). The titer detection limits were <1:4 for BRSV and <1:2 for BVDV and IBR. The antibody agent A51908 was used for the ELISA for all viruses.

We classified animals into two groups based on titer levels. Group one had no or low viral titers (never exposed). We set naïve viral titer thresholds at <1:32 for BVDV, <1:4 for BRSV, and <1:2 for IBR based on known titer decline levels (Menanteau-Horta, Ames et al. 1985, Kirkpatrick, Fulton et al. 2001). Group two consisted of animals likely vaccinated or exposed to a single virus. Those calves had to have titer values above the set naïve thresholds (Fulton, Confer AW et al. 1995, Houe, Baker et al. 1995, Grooms and Coe 2002). Finally, we identified animals with measurable titers to at least two of the three viruses in our study as likely immunized for BRD, as at least two of these pathogens are present in nearly all BRD vaccines on the market. Exposure to multiple of these pathogens, resulting in detectible titers is unlikely in the absence of vaccination.

Statistical analysis

Our first study was purely observational, where calves served as experimental units, and we collected data from them prior to any treatment or processing. In all statistical models, we treated the stocker operation of origin as a random variable to account for distinct buying practices that could potentially confound the interpretation of results. We performed data cleaning, reformatting, and summary statistic calculations in R using various packages from the tidyverse (Wickham, Averick et al. 2019, R-Core-Team 2020). We modeled likely immunized (i.e., possessing two detectible titers) as a binary dependent variable in univariate linear mixed models that included stocker operation as random to assess individual associations between visual calf factors and likely immunization status. Using the individually significant calf variables ($p < 0.05$), we developed multiple-variable logistic regression models to test combinations of factors associated with the probability of likely immunization. Similarly, these models used likely immunized as a binary dependent variable and stocker operation as random. We assessed these models' fit to the dataset using Akaike Information Criterion (AIC). Using the best-fitting models, we performed 10-fold cross-validations to evaluate the predictive ability of each combination of calf factors. We used true and predictive values to calculate correlation coefficients (R^2), Root Mean Squared Errors (RMSE), and accuracy for each model that we tested. Lastly, we evaluated whether model predictive abilities were significantly different with paired t-tests.

Study two comparing immune health, disease incidence, and performance in preconditioned to naive calves:

A subset of 60 animals (30 naïve and 30 marketed as preconditioned) were purchased and housed at the University of Tennessee Middle Tennessee Research and Education Center (MTREC, Spring Hill, TN). Cattle were sourced from 19 different farms and purchased at two sale facilities in Tennessee on 11/29/2021 (n=17) and 12/6/2021 (n=43). Mixed lots of preconditioned and naïve calves were bought at each sale and transported approximately 40 minutes to the research farm. The 30 calves marketed as preconditioned had reported average weaning dates of 50.5 days prior to shipment. Sale health records indicated that some preconditioned calves were vaccinated with Covexin 8 w/Tetanus and Triangle 10 and given Cydectin Dewormer. Metaphylaxis on arrival was not utilized by the farm where this trial took place. Calves received the following vaccines and medications on arrival after sampling: Express 5 HS, Alpha 7 MB-1, Presponse HM, Autogenous Bacterin (Pinkeye Vaccine) includes Moraxella bovis & bovoculi AND Mycoplasma bovis & bovoculi, and lastly Eprinex Pour on Dewormer. After processing, all calves were comingled and managed as one unit. Calves were placed on a fescue-based forage diet with a period spent on a ryegrass field. Calves had access to free choice hay and were given supplemental feed through a total mixed ration (TMR) from an automated smart feeder (C-Lock Super SmartFeed Pro). Calves were fed 40 pounds of corn silage with a 5-pound 30% concentrate mixed in the TMR and smart feeder. They were kept for four months from processing date of December 8th, 2021, to sale date of April 4th, 2022. All 60 head were sold together with an average weight of 834 lbs.

Health observations were recorded and treatment was administered based on the evaluation detailed in Step et al. (Step, Krehbiel et al. 2008). BRD treatment occurred if a calf had a clinical severity score of 1 or 2 and with a rectal temperature of greater than 104˚F or if they had scores of 3 or 4 regardless of rectal temperature. If a calf had a rectal temperature greater than 104˚F, regardless of clinical severity score they were given banamine. For the first treatment for BRD calves receded Nuflor, for their second treatment they received Draxxin, and for their third treatment they received Excede. Additionally, weights were collected post-processing on days 14, 35, and 56 am before animals were fed in the morning. The 60 calves in this study are also included in the 408 animals used to develop predictive immunization models.

In analyzing our subset of calves to evaluate how preconditioning affected calf weight gain and disease instance, we used SAS 9.4 (SAS Inst., Inc., Cary, NC) to calculate summary statistics between the two groups of cattle (preconditioned and naïve). We used Chi-squared statistics to evaluate if preconditioned animals were 1) less likely to be treated at least once for BRD and 2) if they performed better (based on ADG) compared with their naïve contemporaries. We also performed a Chi-squared analysis for BRD incidence for animals that possessed detectible titers for BVDV and/or IBR. We considered group differences significant when $p<0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

Out of the sampled population, 37% (151/408) of calves were considered immunized for BRD on arrival at the stocker facilities. Only one calf was BVDV-PI positive (0.0025%). Thirty-five percent (143/408) of calves had no titer for any of the three viruses tested. We observed that 28% of animals had detectible tiers for a single virus: 5% for BVDV, 15% for BRSV, and 8% for IBR (Figure 1). For calves to be considered vaccinated by sale, these animals were sold at sales that stated they had received vaccines for BRD. Only 53% (36/68) of those animals were considered immunized for BRD based on VNT measurements. Calves without health records were considered as naïve at sale. Of those, only 34% (115/340) were considered immunized for BRD by VNT. Out of 37% of the total population considered immunized 75% of those animals had two detectible titers and only 25% had three. Almost half of the animals considered immunized had elevated titers to BRSV. There were no animals that had detectible titers for BRSV until the spring months (Figure 2). Animals collected in the spring tended to have higher titer levels than those sampled in other months. This could be indicative of calves having seasonal exposure to BRSV, as we saw with almost all calves in the spring month sampling time frame had elevated titers to BRSV. These cattle in the sampled spring months born in the fall, could come from cow calf farms with higher levels of management (i.e., controlled breeding seasons, health management protocols in place, etc.).

Descriptive statistics for visual characteristics for sampled cattle are presented in Table 1. Severn percent of calves showed signs of visual illness on arrival. The top three health issues were eye lesions (4.4%), warts or some skin issues (2.0%), and nasal discharge (1.5%). Other illnesses included bloat, and visual dehydration. There was an even split regarding sex, and almost all the steers in the study were cut and healed. Calves that were freshly cut were calves that were mainly recently banded. Over 60% of the calves were black-hided and likely of Angus descent. Cattle ranged in weight from 200 to 900 pounds, but majority of the animals weighed between 400 and 500 pounds. Most calves did also not have any health or vaccination records on arrival.

Correlations between calf factors, and detectible titers are shown in Figure 3. We observed correlations between immunization and calf factors prior tag (0.14), BCS (0.13), and having health records (0.09). Calves who had to be treated for BRD in the stockering phase (-0.24), calves visually ill on arrival (-0.10), had a smaller frame score (-0.21), and lighter visual body weight (-0.20) all had a negative correlation with immunization status.

Using these variables, logistic regression models showed that steers were more likely to be immunized on arrival (P<0.02), calves possessing an ear tag (P=0.004), calves possessing health records (P<0.001), and small framed caves (P=0.003). Univariate logistic regression models identified three calf factors that were significantly associated with likely immunization. Polled cattle were 4.9 times more likely than horned cattle to be immunized (P= 0.01). Calves sold as preconditioned were 2.2 times more likely than cattle with no health history (P= 0.003) to be immunized. Finally, calves that had a prior ear tag present were 1.6 times more likely than calves with no tag on arrival (P= 0.03) (Table 2) to show VNT indicative of immunization. We also found that post-arrival, there was an association of immunization status and calves having to ever be treated for BRD (P=0.015). This explains that a calf who was considered immunized in our study was less likely to receive a treatment for BRD. Although this is limited by reporting from producers, as every producer used different health management protocols and treatment regimes. Weight was also a significant calf factor ($p<0.05$) (Figure 4). We saw a higher percent of animal in the lighter and heavier weight classes being considered immunized. The weight range with the lowest immunization rates were calves in percentage of animals that are considered immunized were calves in your true stockering weight range.

Fitting multiple calf factors simultaneously further demonstrated that sex, tag, and seller-reported health records were the most consequential (Table 3). We evaluated models using AIC to evaluate best fit to our data. Models 1 (sex and tag) and 4 (sex, tag, frame score, and health records) had the lowest AIC, making them the best fit to our data. Sex and tag were significant in model one, so adding other variables like horns did not affect model fit. Horns were never significant when added to multiple-variable analyses. Frame score was a significant predictor in model 3 and 4. When increasing the model complexity, we observed that when adding health records, tag was no longer statistically significant. Health records were likely confounded with tag variable, overpowering it in the multi-variable analysis.

Using these models, we assessed their predictive ability using a 10-fold cross validation. Increasing model complexities resulted in increases in the mean R^2 from 6% of the variation being explained by the data to 23.4% variation explained. RMSE and accuracy changes did not differ across cross-validated models. The relatively low predictive ability of our models is likely rooted in the low number of farms and number of animals sampled.

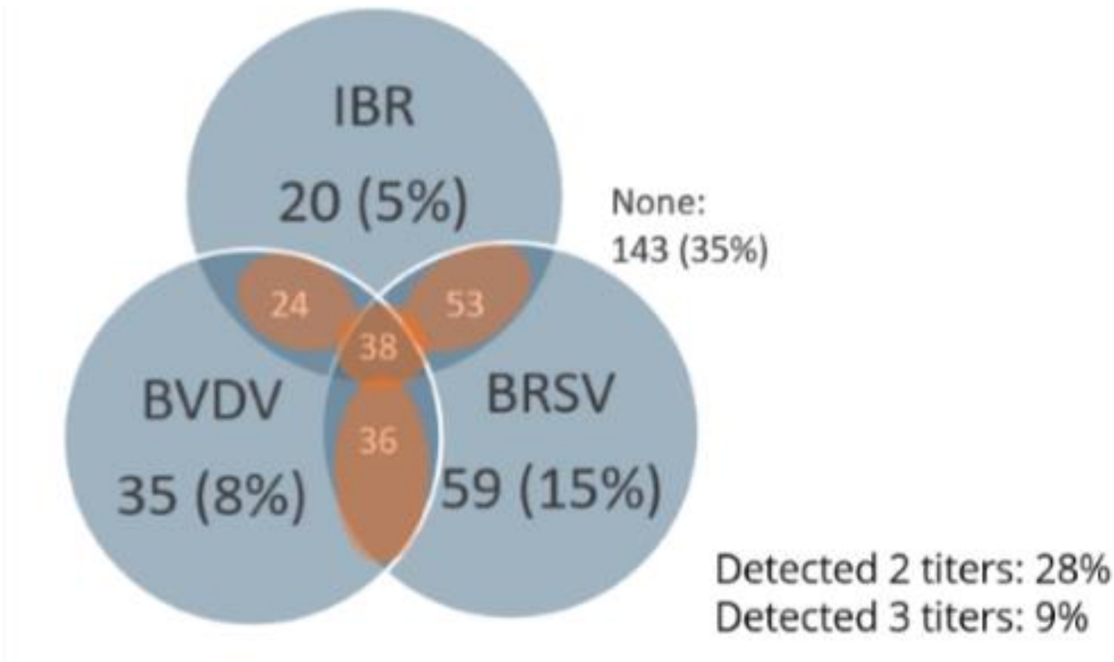

Figure 1. Titer Venn Diagram

Venn diagram of viral titer presence for three BRD pathogens (BVDV, BRSV, and IBR) in 408 weaned stocker calves in Tennessee. The overlaps for animals that were considered immunized are highlighted in orange.

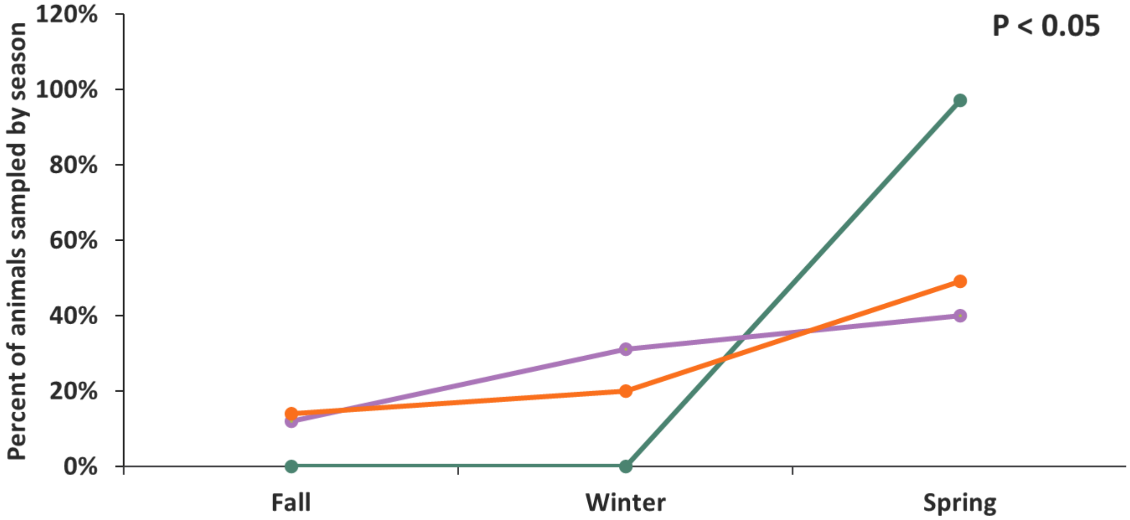

Figure 2. Titer presence by season sampled.

The percent of calves that were sampled in each season that had detectible titers on arrival. Based on 408 stocker calf observations. Statistically significant p-values were those <0.05.

Table 1.Descriptive stats on the cattle sampled.

Visual Characteristic	State	Number of animals	Percent of population
Sex	Heifer Bull Steer	145 138 125	35.5% 33.8% 30.6%
Tag present	No Yes	231 177	56.6% 43.4%
Visual approximate body weight (Lb.)	200 300 400 500 600 700 800 900 +	4 62 122 115 55 39 8 3	0.98% 15.2% 29.9% 28.2% 13.5% 9.6% 2% 0.74%
Body condition score[1]	4-borderline thin 5-moderate 6-slightly fleshy	52 319 37	12.8% 78.2% 9.0%
Docility score[2]	1-Docile 2-Restless 3-Nervous 4-Flighty	220 124 40 24	45.8% 30.4% 9.8% 5.8%
Castration	Cut and healed Freshly cut Stag Cryptorchidism	116 5 3 1	28.4% 0.98% 0.74% 0.25%
Frame score[3]	Small Medium Large	116 217 75	28.4% 53.2% 18.4%
Horn status	Polled Horned Dehorned	382 26 0	93.6% 6.4% 0%
***Bos indicus* influence**	No Yes	404 4	99% 0.98%
Visual apparent breed	Angus Hereford X Angus Charolais cross Simmental cross Angus X dairy Other	259 64 46 18 7 6	63.5% 15.7% 11.3% 4.4% 1.7% 1.5%

Table 1. Continued

Visual Characteristic	State	Number of animals	Percent of population
Coat color	Black Black Baldy Smoke Red White Red Baldy	271 63 27 20 20 7	66.4% 15.4% 6.6% 4.9% 4.9% 1.7%
Coat score	1- Slick 100% shed 2- Mostly 75% shed 3- halfway- 50% shed 4- initial - 25% shed 5- full coat- 0% shed	73 60 135 48 92	17.9% 14.7% 33.1% 11.8% 22.5%
Possessing health records	No Yes	340 68	83.1% 16.2%
Visually sick on arrival	Eye lesions Skin issues Nasal discharge	18 8 6	4.4% 2% 1.5%

Descriptive statistics for the 14 visual characteristics collected on 408 Tennessee stocker calves.

Definitions:
[1] 1 to 9 scale based on BIF guidelines (National Academies of Sciences and Medicine 2016, BIF and Parish. 2018)
[2] 1 to 9 scale based on BIF guidelines (BIF and Parish. 2018)
[3] USDA, US standards for grades of feeder cattle in 2000 (AMS-USDA 2000)

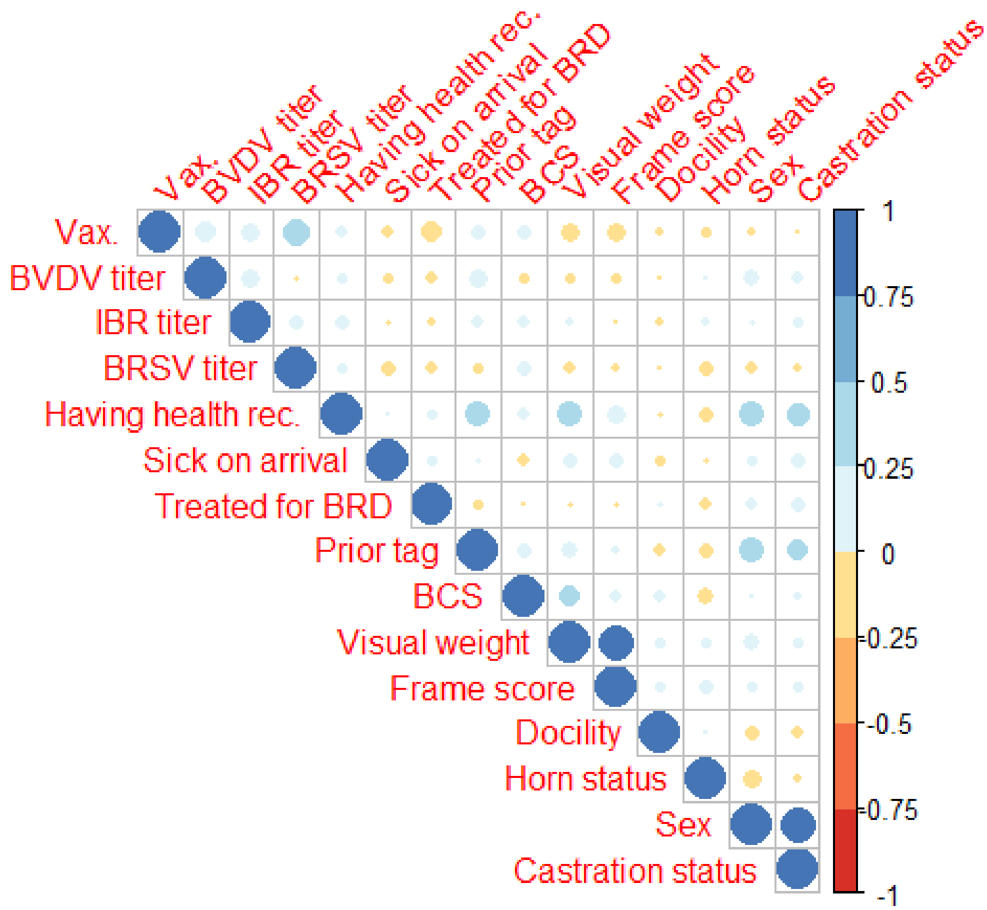

Figure 3. Correlation of calf factor to immunization status.

A correlation plot of the relationships of visual calf factors, titer abundances, and likely immunization status for 408 weaned Tennessee stocker calves. Point size is proportional to the absolute value of the magnitude of correlation. Positive and negative correlations are represented as a scale from blue (positive) to red (negative).

Table 2. Odds ratios for likelihood of a calf being immunized to BRD

Calf Variable	Odds Ratio	P- Value
Health records (Yes Vs. No)	2.20	0.003
Horns (Polled vs. Horned)	4.84	0.011
Sex to bull Steer Heifer	 1.26 0.82	 0.367 0.422
Coat score For every increase in coat score by 1	Calf odds increase by 0.80	0.004
Prior tag (Yes Vs. No)	1.56	0.030
Docility For every increase in docility score by 1	Calf odds increase by 0.86	0.224
Body condition score For every increase in condition score by 1	Calf odds increase by 1.46	0.071
Visual approximate body weight For every increase in weight class	Calf odds increase by 0.99	0.0002

Odds ratios for likelihood of a calf being immunized for eight visual calf observations. Based on 408 stocker calf observations. Statistically significant p-values were those < 0.05.

Table 3. Best fit and predictive model building and analysis.

Best fit Models analysis				Predictive Models Analysis		
Model	Variables	AIC	Statistically Significant variables	Mean[1] (R^2)	Mean[2] (RMSE)	Mean[3] (ACC)
1	Sex + Tag	536	Sex: P= 0.048 Tag: P= 0.031	0.062	0.484	0.630
2	Sex + Tag + Horn status	540	Sex: P= 0.048 Tag: P= 0.035	0.125	0.480	0.625
3	Sex + Tag + Frame	537	Sex: P= 0.017 Tag: P= 0.029 Frame: P= 0.003	0.176	0.478	0.615
4	Sex + Tag + Frame + Health records	514	Sex: P= 0.005 Frame: P= <0.001 Health rec: P=<0.001	0.234	0.474	0.607

Models are increasing in complexity. Best fit models on the left-hand side and the predictive analysis is on the right side of the table.

Definitions:
Mean (R2): measuring the proportion of the variance the independent variable (calf characteristics) explains the dependent variable (immunization status) in a regression model.
Mean (RMSE) Root mean square error: this is the prediction errors, standard deviation of the residuals
mean (ACC) accuracy: The accuracy of the times the predictive model correctly predicts immunization status based on calf characteristics.

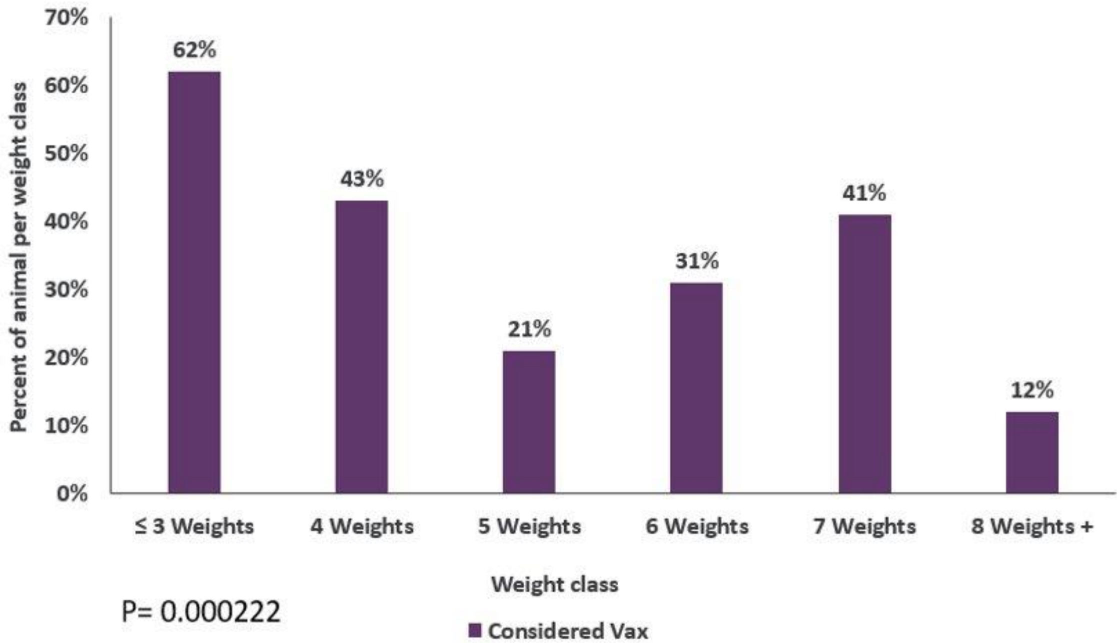

Figure 4. Percent considered immunized by weight class.
The precent of calves that had a likely immunization status by the weight group, for 408 weaned Tennessee stocker calves.

Study two

Comparing immune health, disease incidence, and performance in preconditioned to naive calves.

A subset of 60 male weaned calves (average body weight 581 lb. [SD = 15.8]) were followed through the stocker phase where we were able to collect sale information, health & treatment records, and scale weights on days 14, 35, and 56 post-processing on arrival. Thirty calves were bought at preconditioned sales with health records, and the remaining 30 calves were bought at a weekly non-preconditioned sale. We refer to these two groups throughout as preconditioned and naïve, respectively.

These animals underwent the same onboarding sample collections and evaluation described by the larger stocker calf survey. Of these calves, 28% (n=17) had a detectible titer to BVDV, and 23% (n=14) of the calves had detectible titers to IBR. None of the calves showed a detectible titer for BRSV. None of the naïve calves had detectable titers for IBR, and all but two lacked detectible titers to BVDV (Figure 5). Twenty percent (12/60) of the total population of calves were considered immunized for BRD based on possessing two detectible titers. Only 40% (12/30) of these preconditioned calves were considered immunized based on VNTs. None of the calves in the naïve group were considered immunized by VNT.

The incidence of BRD in the population was as follows: 52% (31/60) of calves were treated at least one time for BRD. Of the preconditioned calves 33% (10/30) were treated at least one time for BRD, whereas 70% (21/30) received at least a single treatment. Illness occurrence peaked two weeks after intake, consistent with previous observations (Thomson, Moore et al. 2012). In addition to becoming sick less frequently, the preconditioned group got sick over two times slower than the naïve group. We observed a significantly lower incidence of BRD in calves that had detectible BVDV titers vs. not (23.5% vs. 68.2%), IBR titers vs. not (21.4% vs. 60.9%), and considered immunized on arrival vs. not (16.6% vs. 60.4%). Calves without titers for BVDV were 2.7 (95% CI: 1.1, 6.6) times more likely to contract BRD as calves with titers, and calves without titers to IBR were at 5.7 (95% CI: 1.4, 24) times more likely than calves with titers. Calves not considered immunized were at 7.4 times more likely to be treated for clinical BRD than animals considered immunized (P=0.006). The presence of a single viral titer to BVDV or IBR appeared to lend helpful immunity that allowed animals to avoid clinical illness. In addition to avoiding disease, the preconditioned calves gained significantly more weight on average (22.7 lb.) than the naïve group over the first 60-days (P= 0.003). Average daily gain of individual animals in differing categories are represented in Figure 6. The animals that were treated for BRD are separated out within their precondition status in figure 7. Lastly in figure 8, we have the overall ADG over 60 days, for calves based on precondition status. We saw that animals in each group became more uniform regarding AGD as they spent more time in the stockering phase. This can be attributed to calves acclimating to new environments, learning how to eat out of a feed bunk and drink from a water trough, and calves recovering from early BRD and returning to normal feed intake.

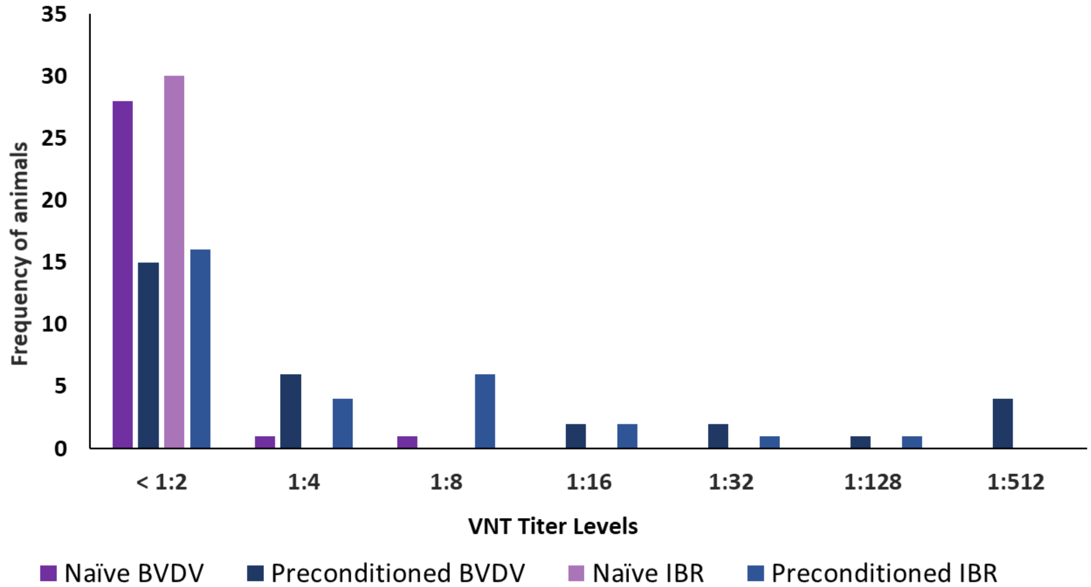

Figure 5. Detectible titers by precondition status.

Titer levels on arrival for preconditioned (n = 30) and naïve (n = 30) calves on arrival. Note that no animals showed detectable titers for BRSV.

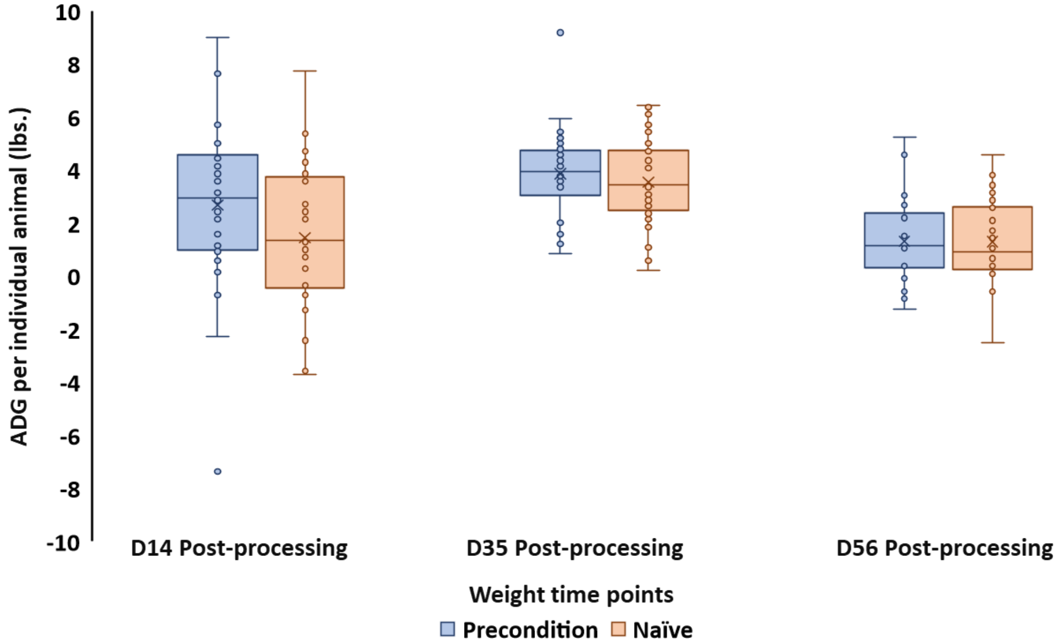

Figure 6. Average daily gain in preconditioned and naïve calves over the first 60 days post-processing.

Average daily gain of preconditioned (n = 30) and naïve (n = 30) calves post processing.

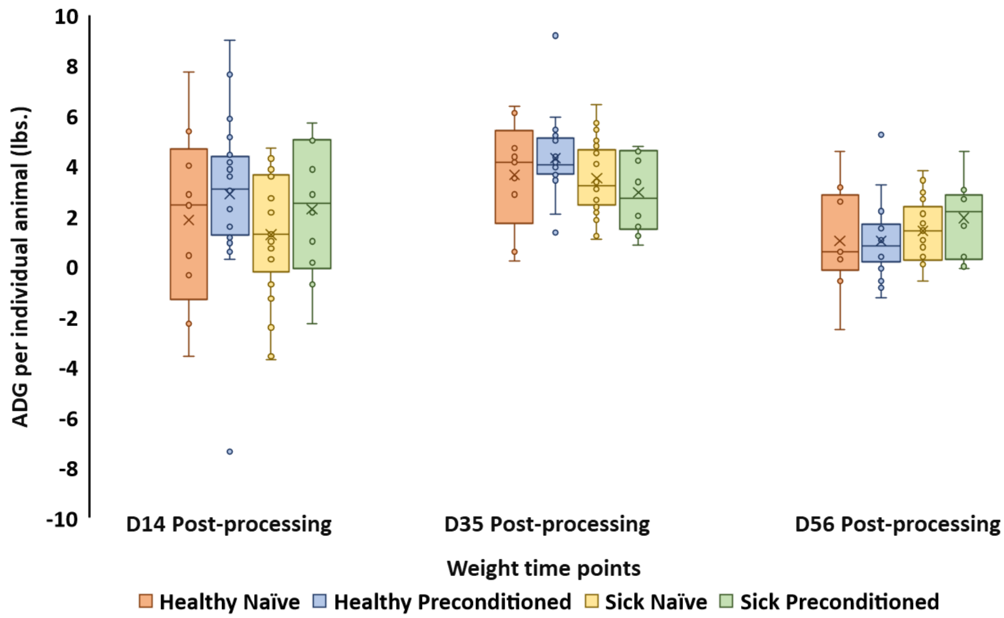

Figure 7 Average daily gain in healthy and sick preconditioned calves and healthy and sick naïve calves over the first 60 days post-processing.

Average daily gain of preconditioned (n = 30) and naïve (n = 30) calves post processing with healthy and sick animals separated out by preconditioned status.

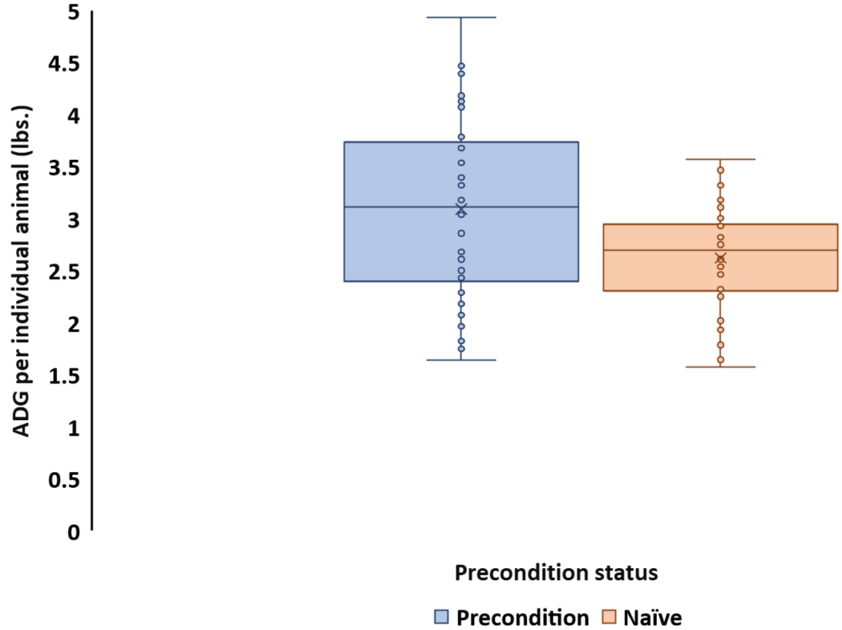

Figure 8 Overall average daily gain in preconditioned and naïve calves over the first 60 days post-processing.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Our study used data from four stocker operations across the state of Tennessee to evaluate whether on-arrival visual indicators were associated with calfhood vaccination for BRD. We found that animal sex, tag, and possessing health records on arrival were the factors most significantly associated with likely immunization (i.e., detectable titers for at least two of: BVDV, BRSV, IBR). Cattle with a tag present, were polled, had better body condition, had health records, and were more docile had better odds of being immunized then cattle that did not possess those individual attributes. This likely stems from calves having been processed and managed at a higher level. We would expect that if they have been managed and processed at some point in their life, they would be more likely to have received calfhood vaccines. Calves considered immunized exhibited lower BRD incidence than naïve animals. This was limited though by producer reporting of BRD illness in their calves and treatments of these animals.

We were able to develop a model to predict the immunization status of calves on arrival based on significant visual indicator traits. In addition to the indicators mentioned above, the predicative ability of our models was also improved by knowledge of a calf's sex and tag status. The predictive ability of our model was low, likely due to a limited sample population only from middle and east. Sampling calves across regions and from many more farms could better represent the variability in buying and management practices that are related to immunization. This additional data could drive further increases to the predictive abilities of models.

Despite having low predictive abilities in our models, knowledge of these visual indicators can serve as important tools for helping producers make more informed purchasing decisions and tailoring management strategies to the likely needs of individual animals. For example, producers attempting to buy lower-risk cattle might look for calves with a prior ear tag present that have been castrated. We did find when building the best fit models that adding health records makes the variable tag non-significant. These are cofounded variables as producers that give multiple vaccinations document health records would almost certainly have tagged their calves. With limited information available though (i.e., no health records), multivariate models with other easier-to collect factors are useful. When adding more information to a model or even animal management protocols, producers can evaluate animals more accurately and manage them according to likely immunization status.

Regarding the weights in Figure 4, we would expect the eight-weight class to possess increased immunization status compared with to the seven weights, but we assume that this small subset of calves were likely poorly managed if they were sent to the stocker this late on in life. The lighter-weight calves tended to appear more immunized, likely due to retaining elevated titer levels from calfhood vaccinations or maternal antibodies.

In following 60 preconditioned and naïve calves through a stocker phase, we observed that the preconditioned group had reduced BRD incidence, resulting in significantly more weight gain over 60 days compared with naïve cattle ($p=0.003$). Better management prior to sale, including a substantial weaning period, proper health protocols, and introduction to bunk feeding likely led to this improvement in performance for the preconditioned animals. Surprisingly, our results indicated that many calves marketed as preconditioned did not have titers indicative of immunization on arrival. This suggests that the premium that producers pay for preconditioned calves may not be accompanied by the enhanced immunity that they would expect from a preconditioned animal. (Cole 1985). While the calf performance data does suggest that preconditioned calves may have been weaned and trained to eat out of a bunk, their on-arrival titer levels suggest they may not be fully immunized for BRD. It is important to note that these cattle were not purchased from a verified preconditioned program. Rather, they were marketed as preconditioned. Regardless of immunization status, the interventions that preconditioned calves received did lead to better weight gain and reduced disease instance. We can recommend that if a producer is purchasing only preconditioned cattle from a single source that grouping those animals together could help mitigate some disease by not allowing a continuous source of infection from new cattle.

We did not observe titers for BRSV in any sampled calves until the spring months. Future research that samples over multiple years and across seasons would allow for a more in-depth look at the seasonality of immunization status. This could be a function of management differences for groups of available cattle over the course of a year, or due to seasonal changes in the viruses that we tested for (Ribble, Meek et al. 1995). Since the cattle we sampled in the last two trips had positive titers for BRSV and most also had a titer to IBR and/or BVDV, we expected that the elevated BRSV titers were concurrent with exposure to a vaccine, not necessarily from active infection (Fulton, Confer et al. 1995, Grooms and Coe 2002). The decreased BRD incidence in likely immunized cattle could help producers evaluate and separate calves on arrival and deliver more precise management practices. On-arrival titer status could assist producers regarding separating high-risk animals from low-risk to combat continued exposure of virus through the stockering phase (Parish, Rhinehart et al. 2021). Each farm will have different calf buying practices and management strategies in place, so it is important for a producer to always consult with their veterinarian on what health and arrival management practices they should consider are best for them.

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VITA

Claire Hunkler obtained her dual bachelor's Degrees in Agriculture and Agribusiness from Abraham Baldwin Agricultural College in Tifton, Georgia, in December 2020. She went on to pursue and graduate with a Master's in Animal Science from the University of Tennessee in Knoxville, Tennessee, in December 2022. Under the mentorship of Dr. Troy Rowan and Dr. Liesel Schneider, with committee members Dr. Lew Strickland, and Dr. Marc Caldwell. Her work focused on predicting calfhood immunization status for bovine respiratory disease in Tennessee stocker calves based upon on-arrival visual calf characteristics. In addition, she plans to work closely with producers in the Southeast to assist them in better managing their cattle through more precise livestock management systems.