EVALUATION OF IMMUNE RESPONSE AND PERFORMANCE IN STEERS OF KNOWN GENETIC BACKGROUND VACCINATED AND CHALLENGED WITH BOVINE VIRAL DIARRHEA VIRUS

A Thesis

by

CHASE ANTHONY RUNYAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Andy D. Herring
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ABSTRACT

Evaluation of Immune Response and Performance in Steers of Known Genetic Background Vaccinated and Challenged with Bovine Viral Diarrhea Virus.

(December 2010)

Chase Anthony Runyan, B.S., Oklahoma State University

Chair of Advisory Committee: Dr. Andy D. Herring

This research was directed at investigating the variation in immune response of cattle when administered a known challenge from Bovine Viral Diarrhea Virus (BVDV) following different Bovine Respiratory Disease (BRD) vaccine treatments. Cattle were assigned vaccine treatments with sire and cow family was stratified across treatments to assess the role genetic differences may impact immune function. The same BVDV strain and challenge technique were used in two trials (2008 and 2009) in Angus-sired yearling steers. Data from these two years were analyzed separately because the cattle were managed and fed differently.

Blood antibody Immunoglobulin-G (IgG) titers for IBR, BVD Type 1 and BVD Type 2 were higher for cattle in the Killed vaccine group than the MLV or NON vaccinated groups ($P < 0.05$) in both years. In the 2008 study, average daily gain (ADG) was higher for cattle from the Killed vaccine group ($P < 0.05$) for the 28 d following BVDV challenge, but no cattle were classified as morbid based on rectal temperature. In the 2009 study, differences in rectal temperatures were observed, and a total of 35 of 93

having over 40.0^{o}C (28 in the first 14 d following challenge). Cattle in the MLV vaccine group had lower overall mean temperatures, with no animals having rectal temperatures over 40^{o}C 14 d following viral challenges. Differences in rectal temperature were also observed due to sire. Differences in feed intake also occurred due to treatment, day, treatment × day interaction, and maternal-grandsire. The MLV vaccine group maintained more constant levels of intake as compared to Killed and NON vaccinated cattle at days 5 to 12. Although large differences in titers following BVDV challenge were observed, the relationships of this immune response with animal health and performance appears very complex.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

Bovine Respiratory Disease (BRD) is costly to U.S. beef production due to effects on weight gain, production efficiency, antibiotic therapy, and in some cases animal death. In spite of improved efforts for prevention and treatment, there is also evidence that morbidity manifests as sub-clinical illness and can result in irritations of the lungs and, in severe cases, pulmonary tissue damage in animals never identified for illness. Breed differences pertaining to BRD occurrences have been reported, but the genetic influences of BRD still remain largely unknown. There are also questions that remain about immune responses and protection offered by killed vs. modified-live BRD vaccines. Bovine Viral Diarrhea (BVD) is a disease that is a component of BRD and is a threat to cattle production systems not only from its threat of morbidity and mortality from transient infection, but also the incidence of cattle persistently infected with the virus. As a result, the objectives of this project were to: (1) evaluate genetic variation and vaccination effects on immune response and health measures in steers of known background that were challenged with Bovine Viral Diarrhea Virus (BVDV) following killed, modified-live or no BRD vaccination, (2) evaluate genetic and vaccination effects on weight gain and other traditional performance measures following vaccination and BVDV challenge, and (3) investigate potential genetic-vaccine treatment interactions for immune, health and performance measures.

This thesis follows the style of the Journal of Animal Science.

CHAPTER II

LITERATURE REVIEW

Animal health can be summarized as the overall physical condition and well being of animals. In beef cattle, morbidity (sickness) accounts for reductions in performance and creates additional costs associated with treatment mortality (death loss), which can result in huge economic losses. Morbidity and mortality in growing cattle on pasture and in feedlots can be the result of numerous disorders, most commonly respiratory and digestive associated diseases (Smith, 1998).

It is well understood that Bovine Respiratory Disease (BRD) is an enormous burden in beef cattle operations. In terms of financial strain on the U.S. cattle industry, Griffin (1997) summarized an estimated annual loss of $750 million due to BRD incidence. Chirase and Greene (2001) reported a similar range of estimated loss of $800 to $900 million annually from death loss occurrence, reduced feed intake resulting in reduced gain performance, and treatment associated costs. McNeill et al. (1996) summarized Texas A&M Ranch to Rail data and concluded that a difference of $93 added expense per animal can be attributed to cattle treated for BRD versus cattle that have not been treated.

These financial strains reflect the concern over the incidence of morbidity due to BRD. Galyean et al. (1999) calculated that 52% of U.S. feedlot morbidity was a result of BRD. Snowder et al. (2006) reported incidence of BRD as high as 43.8% in 18,112 cattle over a 15-year period from herds at the U.S. Meat Animal Research Center at Clay Center, NE. Edwards (1996) reported morbidity of up to 82% as the result of BRD and

summarized that morbidity and mortality from BRD was most common in the first 45 days after arrival at the feedlot. Cattle are at a higher risk early in the feeding period when viral and bacterial exposure is compounded with high stress during arrival periods. Pre-exposed cattle from various geographic locations, breed types, immune status, and management systems are comingled in the feed yard shortly before arrival through regional auctions (Edwards, 2010). Efforts to reduce the amount of stress incurred during the receiving period is important especially for those cattle who are deemed as higher risk than others. Metaphylactic programs and prevention methods deserve attention when dealing with more susceptible, high risk cattle (Duff and Galyean, 2007).

Occurrences of BRD are the result of pathogenic bacteria and the presence of one or more viral infections (Daniels et al., 2000). The viral agents that contribute to BRD are *Bovine Herpes Virus-1* (BHV-1), *Parainfluenza-3 virus* (PI-3), *Bovine Respiratory Syncytial Virus* (BRSV) and *Bovine Viral Diarrhea Virus* (BVDV). These viruses create suppressed immune response activity which facilitates bacterial pneumonia caused by *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma spp.* (Fulton et al. 2000; Fulton et al. 2002; Fulton et al. 2006).

Incidence and morbidity of BRD has been recorded between breeds, although it has not been heavily researched. Muggli-Cockett et al. (1992) reported Pinzqauer offspring had a higher incidence frequency for BRD postweaning at 24.6% as compared to the lowest incidence frequency of 11.8% for the Angus in herds from the Germ Plasm Utilization project where 10,142 cattle were evaluated from 1983 to 1988. Snowder et al. (2006) also evaluated MARC herd BRD data from 1987 to 2001, and observed

incidence frequency for BRD was highest for Pinzqauer, Braunvieh, Simmental, and Limousin at 35%, 34%, 33%, and 32%, respectively. The Angus breed was the lowest at 10.2%, and the overall mean was 12.8% in the 12 breeds represented. Snowder et al. (2005) also reported MARC III composite (¼ Angus, ¼ Hereford, ¼ Pinzguaer, ¼ Red Poll) as not only having a lower incidence frequency of BRD at 9.7%, but also one of the highest mortality rates at 17.2%. They concluded that some breeds or breed combinations are inherently more sensitive to BRD than others.

Signs of clinical illness from BRD often include fever, depression and reduced appetite. Buhman et al. (2000) recorded lower frequency and duration of feed intake behavior in newly arrived feedlot cattle that were identified as sick vs. non-sick. Sick steers were identified using a scoring system that included a rectal temperature of greater the 40.0°C, visual signs of changes in attitude, cough, nasal secretion, ocular secretion, and hematologic examinations. A reduction in weight gain was reported by Gardner et al. (1999) as they observed 1.47 kg/d for treated cattle and 1.53kg/day for untreated cattle using visual symptoms and rectal temperature greater than 40°C. Roeber et al. (2001) also observed similar response in cattle treated more than once having .25 kg/d reduction in average daily gain (ADG) as compared to cattle not being treated based on visual diagnosis of morbidity. Schneider et al. (2009) reported cattle that were treated for BRD or had presence of lung lesions had a 0.07 kg/d reduction in ADG as compared to non treated cattle with no lung lesions.

There appears to be disconnecting evidence in the literature for ADG response and BRD incidence, however. Wittum et al. (1996) found no difference in ADG

between treated and untreated cattle, but did conclude that 68% of untreated steers displayed pulmonary lesions at slaughter. Further investigation by Wittum et al. (1996) in the same study concluded that steers exhibiting lung lesions, regardless of treatment status, did have a 0.076 kg/d reduction in ADG as compared to cattle exhibiting no signs of lung lesions upon harvest.

Traditionally, illness in cattle has been identified by changes in behavior that differ from healthy cattle. Trained animal handling personnel, experienced in pointing out these changes, use signs of depression, increased rectal temperature, lethargy, nasal and ocular secretion, gauntness, or coughing as signs of clinical illness; followed by therapy or treatments that are applied accordingly. Even still, a gap exists between observations and evidence of infection. Wittum et al. (1996) observed that 78% of cattle treated for BRD had pulmonary lesions evident at slaughter, whereas 68% of untreated steers also displayed pulmonary lesions at slaughter as compared to cattle with no pulmonary lesions. Gardner et al. (1999) also observed the presence of lung lesion in 37% of non-treated cattle. Similar observations were made by Schneider et al. (2009) where 60.6% of cattle never treated had lung abrasions present, and 74% of cattle that were treated also displayed lung tissue damage. These observations reflect the ability of infection to arise unnoticed by accepted clinical symptoms, however these subclinical infections are also harmful in terms of additional health stress which results in lost performance.

The focus of this thesis is the impact of Bovine Viral Diarrhea Virus (BVDV) on immune response and performance, so it is discussed in more detail than the other viral

components and pathogens of BRD. The greatest threat of BVDV comes primarily from persistently infected (PI) cattle. PI calves result from vertical transmission of BVDV from the infected dam's bloodstream to her fetus during pregnancy (Larson et al., 2002). BVDV hinders beef cattle production on a substantial economic scale as the estimated cost of a single occurrence of PI in a beef herd has been estimated to range from $14.85 to $24.84 per cow annually (Larson et al., 2002). It is important to note that the occurrence of PI calves in-utero happens between day 45 and day 175 of pregnancy (Grooms, 2004) during the time when the calf immune system is developing. Persistently infected calves fail to create an immune response to the BVD virus because they do not distinguish the virus from itself, and serve as reservoirs of infection for other cattle (Chase et al., 2008). Because a failure to respond to the infection has occurred, PI cattle continuously shed BVD virus through horizontal transmission from one animal to the other through mucosal secretions (Larson et al., 2002).

Bovine Viral Diarrhea virus can be classified into 2 genotypes (BVD type 1 and BVD type 2). Beyond these distinctions, each type has several subtypes such as BVD1a, BVD1b, BVD1c, etc; BVD1b is the most prevailing subtype in U.S. feedlots, whereas BVD1d is the most common in Australia (Fulton et al., 2003b; Fulton et al., 2009). Beyond these genotype separations, there is also a difference in biotypes for BVD. The biotype is defined as cytopathic or non-cytopathic (Ahn et al., 2005; Peterhans et al., 2003). These biotypes are classified based on the presence or absence of visible cytopathic, degenerative or degrading, effects in infected BVDV cell cultures. Ahn et al. (2005) recorded differences in occurrence of biotypes for BVDV as 30% of samples

were defined as cytopathic (CP) and 70% were non-cytopathic (NCP). Fulton et al. (2000) reported similar figures in vaccinated and non vaccinated steers with 20.8% of collected samples were CP, and 79.2% of samples were NCP.

Clinical symptoms of BVDV as reviewed by Peterhans et al. (2003) and Fulton et al. (2003a) include those similar to other respiratory infections. Further, the virus can affect the digestive tract, fetal development, and the immune system in addition to causing mucosal disease. BVDV has a dual nature for infection; BVDV itself serves as an infectious agent, but it also serves as an immunosuppressor of health defenses (Baker, 1995). With defense mechanisms suppressed, secondary bacterial colonization can occur which commonly leads to subsidiary infections (Edwards et al., 1986).

In terms of handling the occurrences of BVD, an important aspect is accurate screening and diagnosis of Persistently Infected (PI) calves. Sandvik (2005) described in detail numerous methods of laboratory testing for BVD virus itself through virus isolations, or immune function response to BVD, or via virus neutralizing antibodies. Cornish et al. (2005) summarized important characteristics of laboratory testing for detecting PI calves to have large capacity, be economical, accurate, and timely in order to be suitable for practical application.

Immunity is the ability to resist disease or infection from foreign substance that threatens homeostasis and wellbeing, and the complexity of the immune system is very extent. Two major components of immune function exist. The innate system, which is a non-specific response mechanism, and the adaptive immune system, which is antigen specific and leads to immunological memory (Abbas and Lichtman, 2007). The

principal components of innate immunity are (1) physical and chemical barriers, such as epithelia and antimicrobial substances produced at epithelial surfaces; (2) phagocytic cells (neutrophils, macrophages) and natural killer (NK) cells; (3) blood proteins, including members of the complement system and other mediators of inflammation; and (4) cytokine proteins that regulate and coordinate many of the activities of the cells of innate immunity.

The mechanisms of innate immunity are specific for structures that are common to groups of related microbes and may not distinguish fine differences between foreign substances. Innate immunity provides the early lines of defense against microbes (Abbas and Lichtman, 2007). Conversely, the adaptive immune system has an extraordinary capacity to distinguish among different, even closely related microbes and molecules, and for this reason is also called specific immunity (Abbas and Lichtman, 2007).

There are two types of adaptive immune responses; humoral immunity and cell-mediated immunity. Cell-mediated is derived by different components of the immune system and function to eliminate different types of microbes. Humoral immunity is mediated by antibodies in the blood and mucosal secretions and produced by B lymphocytes (also known as B cells). Cell-mediated immunity, also called cellular immunity, is mediated by T lymphocytes (also known as T cells). Intracellular microbes, such as viruses and some bacteria, survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Defense against such infections is a function of cell-mediated immunity, which promotes the

destruction of microbes residing in phagocytes or the killing of infected cells to eliminate reservoirs of infection (Abbas and Lichtman, 2007).

Protective immunity against a microbe may be induced by the host's response to the microbe or by the transfer of antibodies or lymphocytes specific for the microbe. The form of immunity that is induced by exposure to a foreign antigen is called active immunity because the immunized individual plays an active role in responding to the antigen (Abbas and Lichtman, 2007).

Immunity can also be conferred on an individual by transferring serum or lymphocytes from a specifically immunized individual. The recipient of such a transfer becomes immune to the particular antigen without ever having been exposed to or having responded to that antigen. Therefore, this form of immunity is called passive immunity (Abbas and Lichtman, 2007), and is the primary mechanism by which cows provide immunity to their calves through colostrums.

Vaccine products generally contain modified-live (MLV) or killed viruses and bacteria toxins known to cause diseases. These products are administered to initiate the body's immune system into creating antibodies and develop immunological memory (Faries, 1999). Adjuvants are also included in available vaccines to slow the release of the antigen into the system to extend the immune response. Historically, most killed vaccine adjuvents are comprised of aluminum hydroxide or oil and water combinations, (Roth and Hednerson, 2001). Vaccines can contain an assortment biological agents, inactive toxins known as toxoids, killed bacteria known as bacterins and combinations of adjuvents which elevate the level of effectiveness of the antigens (Faries, 1999).

Faries (1999) also summarized that vaccines are identified as either infectious or noninfectious vaccines. Infectious vaccines contain an organism that is modified or altered to reduce its virulence so that it will not cause disease, but will still be infectious enough to provide immunity. MLV vaccines are infectious vaccines that achieve a desired level of infection. Immunity of the animal prevents the establishment of disease and provides immunity. Noninfectious vaccines are unable to infect or replicate infectious agents. Generally, noninfectious vaccines are weaker in their ability to illicit an immune response, so a second dose, or booster, is required 2-4 weeks later. The initial dose of vaccine is a priming, sensitizing dose that provides little to no protection and the booster provides protection for 6 to 12 months (Faries, 1999).

There appears to be some variance among reported studies pertaining to the duration of protection that vaccines provide. Cortese et al. (1998) concluded that vaccination with a MLV BVD Type 1 triggered antibodies to numerous strains of both, BVD type 1 and BVD type 2 that were detectable 18 months post vaccination. Reports from Fulton et al. (1995) indicate a decline in BVD antibody titers by day 140 following vaccination.

Some vaccines, which target specific antigens, such as toxoid vaccines, are meant to completely prevent disease from infection, whereas vaccines which are formulated for more complex agents of disease that tend to have more numerous antigenic strains are less likely to achieve a complete prevention level of protection (Faries, 1999). Callan and Garry (2002) summarized respiratory vaccines as disease modifiers rather than absolute preventative agents.

Additional benefits of vaccinatation exist which not only protects vaccinated individuals, but also reduces the ability to shed infections to pen mates or newly arrived comingled calves (Frank et al., 2003). Vaccinations are an important feature in reducing the spread of diseases, but practices that prevent pathogen introduction, limit exposure, and reduce transmission are all important steps upon entry into the feedlot (Snowder et al., 2006).

Viruses use two different strategies to infect hosts. Peterhans (2003) reviewed that a virus may either cause a persistent infection in individual animals to infect new hosts, or, viruses can also use the 'hit-and-run" strategy which is a short duration followed by a rapid transfer to a different host and continuing.

Virulence of BVDV has been characterized as having a broad range of effects based upon the amount of infection or severity of infection induced (Ridpath and Neill, 2007). Liebler-Tenorio et al. (2003) observed differences in clinical observations and rectal temperature changes in colostrum deprived, non-vaccinated steers less than 4 months of age when challenged with 2 different BVDV type 2 strains. Observations were made following an intranasal challenge of a naturally occurring low virulent strain, or a highly virulent strain isolated from a severe field outbreak. Calves receiving the low virulent strain expressed a mildly elevated body temperature at day 7 following challenge with no changes in feed intake or observable behavior differences. Conversely, calves inoculated with the highly virulent strain exhibited elevated body temperature (>40^{o}C) shortly following challenge which persisted for several days. Calves in the high virulence group also became lethargic and apathetic in behavior.

Similar findings were made by Kelling et al. (2002) where 5 different isolates of BVDV type II (2 highly virulent strains and 2 lowly virulent strains) were experimentally induced into 6 to 9 month old crossbred calves. Kelling et al. (2002) observed that calves inoculated with high-virulence strains developed more signs of respiratory tract disease, displayed elevated rectal temperature 6 days following inoculation, and exhibited more lethargic demeanor as compared to calves challenged with low-virulent strains.

The variation in virulence exposes challenges from a research standpoint. Efficacy of vaccine and vaccine use protocol studies in the field have been limited by the lack of repeatability and under-developed accepted criteria for appropriate research models. Traditionally, results from studies using BVDV type 2 have been readily available; however results from BVDV type 1 challenges are less common. One such BVDV1 strain that has been used in vaccines as well as a popular challenge strain is BVDV NY-1. Isolated from a field case in New York, calves infected with BVDV NY-1 were reported to respond with symptoms of pyrexia, reduction in white blood cells, diarrhea, reduced appetite, depression, and reddening of the gums (Baker et al., 1954). Ridpath and Neill (2007) summarized two problems in using BVDV NY-1 when conducting viral challenges; one, was that clinical presentation of infection was reported as mild in comparison to BVDV strains R5013, R2360, and CA0401186A, and two, was the conflicting results in vaccine efficacy in studies using BVDV NY-1 in the treatment vaccine as well as the challenge virus simultaneously.

One of the aforementioned BVDV strains used by Ridpath and Neill (2007) that compared BVDV NY-1 to other field strains was used in this research as well. Bovine Viral Diarrhea Virus CA0401186A was discovered from a PI calf in California that was one of 24 calves with brain and skeletal deformities born to heifers in a single herd. Tissues containing CA0401186a were submitted to the USDA-ARS National Animal Disease Center in Ames, IA from the Tulare Laboratory of the California Animal Health and Food Safety Laboratory. It was characterized as a noncytopathic biotype and found to be of the BVDV1b subgenotype (Ridpath and Neill, 2007).

Wilson (1989) defined the efficacy of a vaccine as its ability to reduce the overall level of respiratory disease. With this broad definition, a limited amount of literature exists pertaining to positive results for the efficacy of vaccination strategies in field trials. Reasons for this limitation were summarized by Wilson (1989) in that the methods of identifying respiratory disease are often based on clinical observation only. Variables including severity of illness and length of illness are un-detected. Finally, differences of repeatability issues which are unavailable in field studies as compared to laboratory evaluations (Wilson, 1989).

Considering the complex characteristics of BVDV, there is also complexity in BVDV vaccination strategies. Fulton et al. (2002) observed that BVDV was an important contributing factor to BRD in 2 studies, with BVDV subtype 1b being the most predominant. Fulton et al. (2002) and Fulton et al. (2005) also briefly reviewed popular BRD vaccines containing BVDV, and summarized that only a small number of commercially available vaccines actually contained BVDV 1b antigenic material. The

problem here is that even though cattle are vaccinated with BVDV 1a or 2, they may still be susceptible to the most common BVDV subtype, 1b. Fulton et al. (2005) concluded that vaccination with a MLV vaccine containing BVDV subtypes 1a and 2 did not prevent infection in vaccinated calves when exposed to PI calves with BVDV1b. Fulton et al. (2003a) also reported that vaccines containing BVDV1a induce lower antibodies to BVDV1b than to BVDV1a in 3 separate USDA licensed vaccines.

Summary

Bovine Respiratory Disease Complex is costly and remains widespread in beef cattle production systems. Morbidity and mortality result from exposure to viral and bacterial infections that work in concert to activate immune responses. Breed types and combinations of breeds have also been linked to a lower incidence of BRD. One major component of BRD is Bovine Viral Diarrhea Virus. One of the reasons BVDV draws much attention is due to Persistently Infected (PI) calves that go unidentified as a major source of spreading infection. Even in events of immunization, exposure to varying subtypes to BVDV can still result in infection leaving questionable results of vaccine efficacy. Thus, research is needed that addresses the genetic influences related to health, immune responses and animal performance in cattle exposed to BVDV.

CHAPTER III

EVALUATION OF WEIGHT GAIN, RECTAL TEMPERATURE AND IMMUNE RESPONSE OF ANGUS-SIRED STEERS CHALLENGED WITH BOVINE VIRAL DIARRHEA VIRUS

Introduction

Growing cattle on pasture and in feedlots can experience limited productivity from the presence of viral and bacterial agents that make-up a complicated disease system known as Bovine Respiratory Disease (BRD) complex (Gardner et al., 1999; Roeber et al., 2001). During the growing and feeding phases in the beef cattle production, a variety of stress elements work in concert with BRD pathogens to reduce immune function and ultimately allow for infection and morbidity (Fulton et al., 2000). Genetic variation in BRD occurrence is another subject with very little reported findings. In those studies however, differences between breed types in susceptibility have been identified (Muggli-Cockett et al., 1992; Snowder et al., 2006).

One disease that is an important component in the BRD complex is Bovine Viral Diarrhea (BVD). As a serious threat to worldwide production (Peterhans et al., 2003), BVD infections suppress immune function and initiate morbidity. Furthermore, BVD is important from a cow-calf production standpoint because, BVD can cause abortions, and, cow herds are the point of origin for persistently infected (PI) cattle. Calves born PI are the result of their dams becoming infected with BVD in mid gestation before the calf's immune system has fully developed. As a result, PI calves are born

immunotolerant to BVD infection and serve as harbors of the virus and facilitate constant exposure to non-PI cattle (Grooms, 2004).

Vaccines for BVD are commercially available as a means to manage and hopefully minimize occurrences of BVD infection or offset effects of viral infection. Efficacy of BVD vaccines have been questioned based on the genotypes and biotypes of virus included (Fulton et al., 2003a); for instance, BDV type 1a is typically in vaccines, but BDV type 1b has shown to be the most prevalent strain in the USA (Fulton et al., 2003a; Fulton et al., 2009). Additionally, virulence of BVDV seems to be subject of much variation as well in terms of symptoms associated with infection (Ridpath and Neill, 2007).

With such complicated factors that go into BRD complex from BVDV infection, the objective of this research was to investigate the health measures and performance traits of growing cattle of known genetic background that were vaccinated for BRD and then challenged with BVDV.

Materials and Methods

Animal methods were approved by the Texas A&M University Institutional Animal Care and Use Committee (AUP 2007-130).

Animals. Seventy-three Angus-sired steers of known genetic background were used in this trial. Steers were born in spring of 2007 and out of half *Bos indicus* cows from the Texas A&M University McGregor Genomics project. No prior exposure to viral vaccinations occurred before the trial began, and all steers were confirmed to be seronegative to BVD and IBR prior to trial initiation. Cattle were confirmed to be PI-

free through evaluation of ear notch sample by antigen capture ELISA at the Texas Veterinary medical Diagnostic Laboratory (TVMDL, Amarillo, TX).

Vaccine treatments. Steers were stratified by sire and genomics cow family across the vaccine treatments of Killed group (Killed, n = 33), modified-live group (MLV, n = 32), and non-vaccinated group (NON, n = 8).

Steers in the MLV treatment group were administered Arsenal 4.1® (Novartis Animal Health US, Inc.) on day -21 as per label instructions. Steers in the Killed treatment group were administered Vira-shield® (Novartis Animal Health US, Inc.) on day -42 and a booster at day -21 as per label instructions. Eight steers (4 from killed group and 4 from MLV group) were not vaccinated. Steers in the MLV group were kept separate from killed and NON steers for 5 days following vaccination.

Viral challenge. At day 0, steers were challenged with a type 1b non-cytopathic BVDV strain (CA0401186a) obtained from the USDA-ARS National Animal Disease Center (NADC) (Ridpath and Neill, 2007). This strain was isolated from a persistently infected calf and submitted to the NADC from Tulare laboratory of the California Animal Health and Food Safety Laboratory. Each steer was administered 5 mL of inoculum (1×10^5 TCID/mL) by placing 2.5 mL in each nasal passage; the animal's nose was elevated until it was visually observed that the steer had swallowed to confirm challenge virus was ingested. This particular strain of BVDV was chosen for this study because it had previously been reported to cause recognized immunological and clinical signs of morbidity, but without risk of extreme illness or death (Ridpath and Neill, 2007).

Sample collection. Rectal temperatures were measured via digital rectal thermometer on days 0, 1, 3, 9, 14, 28, and 42 following viral challenge. Body weights were also recorded, with those observations made at days -42, -21, 0, 14, 28, and 42. Average daily gain (ADG) was calculated for three phase increments of increment 1 (days 0 to 14), increment 2 (14 to 28), and increment 3 (28 to 42).

Serology. Collection of blood was conducted via jugular venipuncture into evacuated tubes on days -42 and -21 before the viral challenge, day 0, and days 14, 28, and 42 following viral challenge. Following collection, blood serum was harvested via centrifuge 2 to 4 hours following collection; centrifuge settings were to spin samples for 20 minutes at 4^{o}C at 4000 RPM. Serum samples were then stored at -20^{o}C until they were sent to Texas Veterinary Medical Diagnostic Laboratory (TVMDL) for quantification of serum neutraliazing (SN) antibody immunoglobulin G (IgG) titers for BVD Type 1, BVD Type 2, and IBR.

Clinical evaluation. Clinical observations were conducted twice daily for 14 days following challenge to assess apparent health with a score of 0 (no symptoms), or 1 to 5 (least severe to most severe) for commonly associated symptoms of BVD/BRD (cough, ocular secretion, nasal secretion, depression, diarrhea, and gauntness/shrink). From days 15 to 42 observations were only conducted once daily.

Diet. Steers were on wheat pasture from days -42 to day 0. From days 0 to 14, steers were fed in a set of holding pens. From days 14 to 42 steers were held in a small (1 hectare) pasture. During the period following BVDV challenge, the diet consisted of

average quality coastal bermudagrass hay fed, *ad libitum*, with no additional supplement following challenge.

Statistical analysis. Mixed model procedures of SAS (SAS Inst. Inc., Cary, NC) were used so that rectal temperature and antibody titers (transformed to a log base of 2) were analyzed as repeated measures with a model that contains vaccine treatment, day, sire, vaccine treatment × day, and vaccine treatment × sire, as fixed effects. An autoregressive covariance structure was utilized. Least squares means were separated with two-tailed t-tests following significant F-tests ($P < 0.05$) from the mixed model analyses. Average daily gain was analyzed with a similar model that included vaccine treatment, day, vaccine treatment × day, pen, maternal grandsire, and vaccine treatment × MGS interaction and day 0 weight as a covariate. Eight steers (n = 4 from killed vaccine group, n = 4 from MLV vaccine group) were removed from this analysis due to them not receiving viral challenge, and are not included in these results.

Results and Discussion

A general summary of the traits evaluated is presented in Table 3.1.

Table 3.1 Summary statistics for traits measured in 2008 trial

Variable	Mean	SD	Minimum	Maximum
IBR[1]	2.19	2.3	0	9.0
BVD Type 1[1]	3.65	3.8	0	13.0
BVD Type 2[1]	3.44	3.7	0	13.0
Rectal Temperature, ^{o}C	38.8	1.58	37.44	40
ADG, kg/day	.38	.26	-.87	.89

[1] Titer transformed to log base of 2

Results of the statistical analyses are presented in Table 3.2.

Table 3.2 Significance levels from mixed model analysis for health measures

Effect	IBR	BVD1	BVD2	Rectal temperature
Vaccine treatment	< 0.001	< 0.001	<0 .001	0.66
Day	<0 .001	< 0.001	< 0.001	0.12
Vaccine treatment × Day	<0 .001	< 0.001	< 0.001	0.94
Sire	0.33	0.17	0.12	0.80
Vaccine treatment × Sire	0.54	0.06	0.09	0.98

IBR titers. Titers for IBR were evaluated in an attempt to evaluate vaccine effectiveness and pathogen exposure. Steers in both vaccinated groups should have developed titers to IBR, whereas those not vaccinated should not have developed titers, and, this was observed. Differences in IgG titer levels for IBR were observed due to vaccine treatment, day, and vaccine treatment × day interaction ($P < 0.01$). Least squares means for the interaction are presented in Figure 3.1.

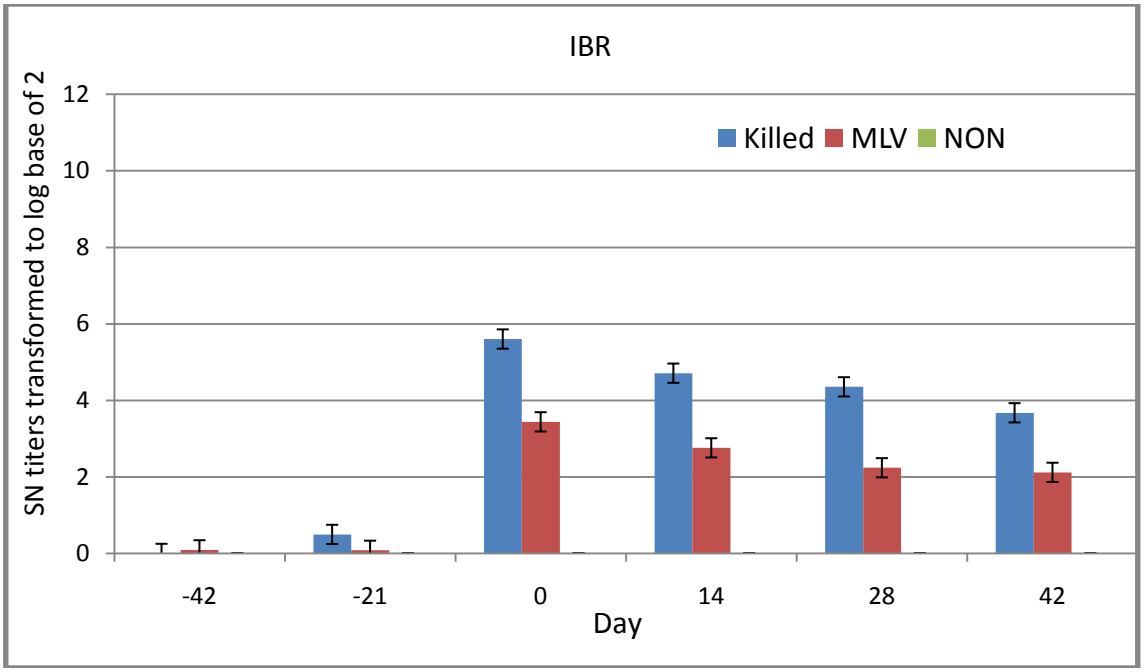

Figure 3.1. Least squares means for serum neutralizing antibody titer (log_2) for IBR in steers of killed vaccine (Killed), modified-live vaccine (MLV), or non-vaccinated control (NON) groups across time in 2008

Large differences between treatment groups were observed with steers from the Killed group being the highest at days 0, 14, 28, and 42 as compared to MLV and NON vaccinated cattle. Titer levels within the Killed group were highest at day 0 ($P < 0.01$), followed by day 14, then 28, and, these were higher than days -42, -21, and 42 for cattle within the Killed group. Titers for IBR within the Killed group at day 42 were higher than days -42 and -21.

Steers in the MLV group had higher IBR titers than steers from the NON group at all dates measured after the viral challenge including day 0. Differences within the MLV group across collection dates did vary however, with IBR titers for the MLV group being highest at day 0 and decreasing steadily through day 42 ($P < 0.01$). After the

challenge on day 0, serum neutralizing IBR titers for MLV steers were lowest at days 28, and 42 ($P < 0.01$).

BVD Type 1 titers. Differences in BVD Type 1 titers were observed due to vaccine treatment, day, and vaccine treatment × day interaction ($P < 0.01$). Figure 3.2 shows the least squares means from the interaction.

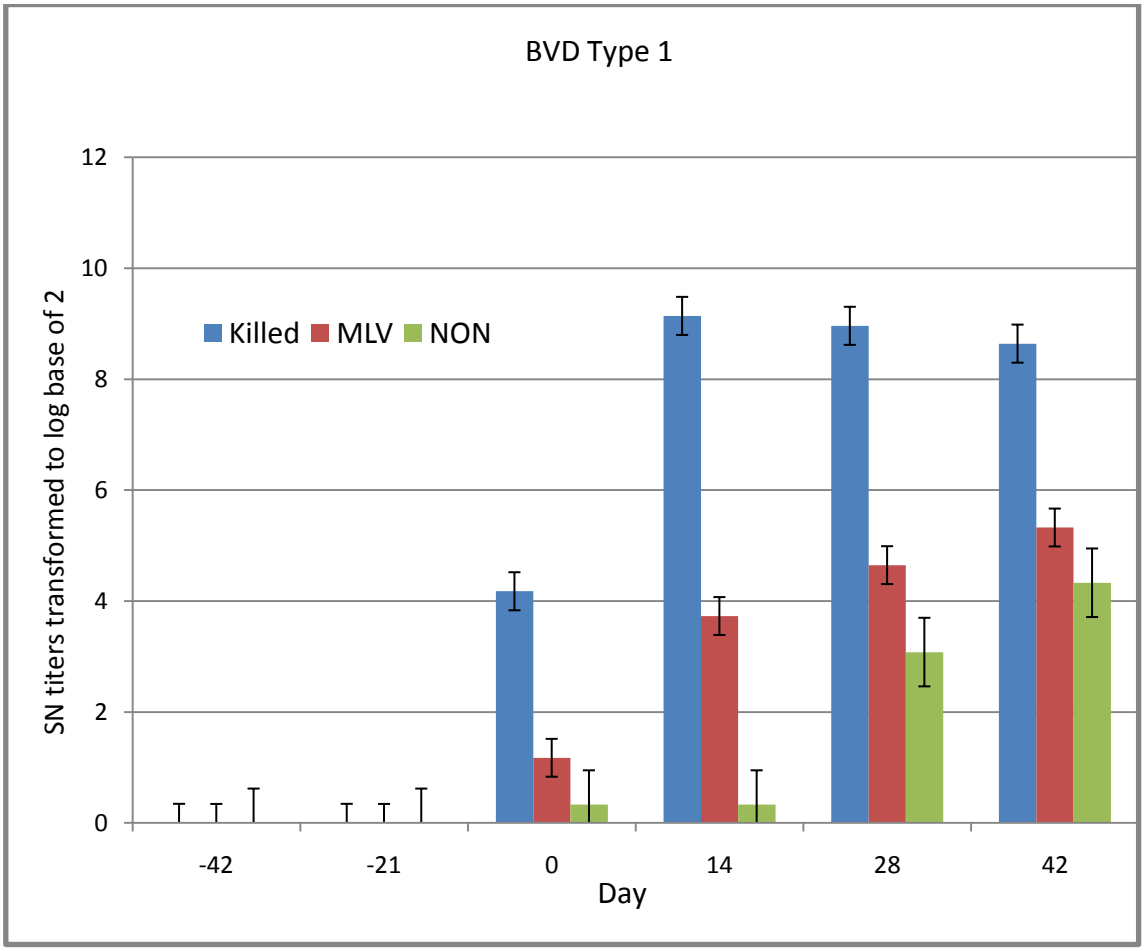

Figure 3.2 Least squares means for serum neutralizing antibody titer (log_2) for BVD Type 1 in steers of killed vaccine (Killed), modified-live vaccine (MLV), or non-vaccinated control (NON) groups across time in 2008

On days 0 through 42 it was observed that BVD Type 2 titers from the Killed group were higher than the MLV or NON vaccinated group at each day. A large difference in titers from day 0 to day 14 was observed ($P < 0.01$). Following this peak BVD Type 1 titers from the Killed group remained similar through day 42.

Calves in the MLV group displayed higher IgG titers for BVD type 1 than the NON group at day 14, 28, and 42. Titers from the MLV group steadily increased from day 0 through 42, which was the day with the highest level observed for cattle in this group ($P < 0.01$).

Titers for BVD Type 1 from calves within the NON group were observed to resemble titers from the MLV group. Titers from the NON group increased gradually until their highest point was observed at day 42.

BVD Type 2 titers. Differences in BVD Type 2 titers were observed due to vaccine treatment, day, and vaccine treatment × day ($P < 0.01$). Least squares means for the vaccine × day interaction are presented in Figure 3.3.

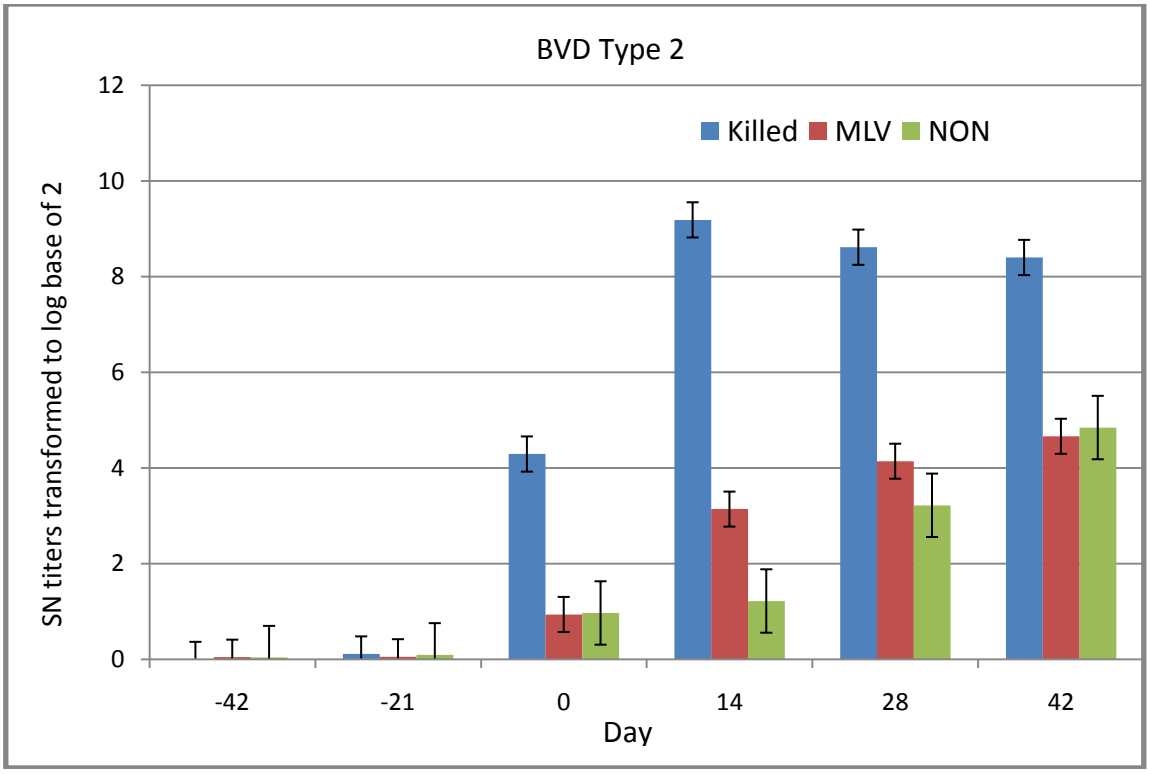

Figure 3.3. Least squares means for serum neutralizing antibody titer (log_2) for BVD Type 2 in steers of killed vaccine (Killed), modified-live vaccine (MLV), or non-vaccinated control (NON) groups across time in 2008

Serum neutralizing titers for BVD Type 2 from cattle in the Killed group were the highest at all days measured following the viral challenge including day 0 as compared to cattle in the MLV and NON groups ($P < 0.01$). A large increase in blood titers for BVD Type 2 from the Killed group was observed from day 0 to day 14. Titer levels for BVD Type 2 within the Killed group peaked at day 14 ($P < 0.05$). Following this peak a slight decrease in titers were observed and titer held at this level with no significant changes from day 28 through 42.