INTRANASAL AND PARENTERAL RESPIRATORY VACCINATION IN HIGH-RISK, NEWLY RECEIVED BEEF CALVES

by

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ABSTRACT

Following discovery of acute bovine respiratory syncytial virus (BRSV) infection enhancing *Histophilus somni* (*H. somni*) clinical disease in calves, further understanding of the safety and efficiency of live-attenuated BRSV vaccines is important. Our objective was to determine the safety, efficiency and immunomodulation of an intranasal (IN), trivalent (infectious bovine rhinotracheitis virus [IBRV], parainfluenza-3 virus [PI3V], and BRSV) respiratory vaccine with parenteral, bivalent bovine viral diarrhea virus (BVDV) and a parenteral, pentavalent (BVDV type I and II, IBRV, BRSV, and PI3V) respiratory vaccine. High-risk beef calves (*n*=525) were received in 5 truckload blocks and stratified by body weight (213 ± 18.4 kg), sex, and presence of a pre-existing ranch ear-tag. Pens were spatially arranged in sets of 3 and randomly assigned to treatment with an empty pen between treatment groups. Treatments included: 1) no viral respiratory vaccination (**CON**), 2) cattle intranasally administered a trivalent, modified-live virus (MLV) respiratory vaccine with parenteral BVDV type I and II vaccine (**INT**), and 3) cattle administered a pentavalent, MLV respiratory vaccine (**INJ**). Pen was the experimental unit, with a total of 15 pens per treatment and 11 or 12 calves per pen in this 70-d receiving study. Performance, morbidity, mortality, BRSV, *H. somni, Mycoplasma bovis* (*M. bovis*)*, Mannheimia haemolytica* (*M. haemolytica*)*,* and *Pasteurella multocida* (*P. multocida*) prevalence and cycle time in nasal swabs via rtPCR on d 0, 7, 14, and 28

was determined, and BRSV-specific antibody titer and serum IFN-γ concentration via ELISA were evaluated on d 0, 14, 28, 42, 56, and 70. Morbidity ($P = 0.83$), mortality ($P = 0.68$) and average daily gain ($P \geq 0.82$) did not differ; however, feed efficiency from d 0 to 56 was improved ($P = 0.05$) for CON. Serum antibody against BRSV increased with time ($P < 0.01$), and was numerically greatest for INT. There was a treatment × time interaction ($P < 0.01$) for *H. somni* present in nasal swabs; on d 14 and 28, INT (21.1 and 57.1%) more frequently ($P < 0.01$) became *H. somni* positive than CON (3.6 and 25.3%) or INJ (3.4 and 8.4%). Also, INT had reduced ($P = 0.03$) cycle time of *H. somni* positive samples on d 28. There was a tendancy ($P = 0.06$) for a treatment effect for BRSV cycle time; CON had a reduced mean (30.80) than INT (33.53) and (32.93). No treatment × day interaction ($P \geq 0.17$) existed for *M. bovis, M. haemolytica,* or *P. multocida* prevalence and cycle time. No treatment differences ($P = 0.55$) were detected for serum IFN-γ concentration. There was a treatment effect ($P < 0.01$) for the rate of *M. haemolytica* positive culture from lung tissue specimens; INT had less (0.0%) *M. haemolytica* positive lung tissue cultures than INJ (45.5%) or CON (74.0%). These data indicate MLV vaccination of high-risk calves, either parenterally or intranasally, did not clearly impact health or growth during the feedlot receiving period. However, INT caused increased prevalence of *H. somni* in the naris and resulted in less *M. haemolytica* cultured from lung tissue samples upon necropsy. The intranasal administration of MLV vaccines may alter the microbial community in the upper respiratory tract of cattle; specifically, we observed that MLV IN increased the prevalence of *H. somni* in high-risk feedlot calves.

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CHAPTER I

REVIEW OF LITERATURE

1.1 Bovine Respiratory Disease Overview

Bovine respiratory disease (BRD) is the most important disease affecting feedlot cattle; it is the leading cause of morbidity and mortality in the feedlot (Woolums, et al., 2005) and poses animal welfare concerns. As arguably the most complex mammalian disease, its economic impact is often underestimated; however, it has been stated that BRD costs the industry over 1 billion dollars annually (Griffin, 1997). In a Kansas feedlot survey published in 2006, an increase in respiratory mortality during the previous decade was reported (Babcock, et al., 2006). The expense of medicine and labor to treat BRD affected cattle is perhaps the primary cost to the industry. However, the associated losses in performance and carcass composition has continued to negatively affect beef producers (Duff et al., 2007). The segmented beef production system induces physiological stress as cattle change ownership and location via routine marketing channels. Vaccination is a cost-effective method intended to prevent future respiratory virus infection (Van Orishcnot, 1999). However, only 26% of cow calf operations with less than 50 head, and 63% of cow calf operations with less than 100 head vaccinated their calves against respiratory disease before marketing (USDA-APHIS, 2011). Preconditioning programs that include preweaning viral vaccination in addition to

castration, weaning and other husbandry procedures reduce BRD impact at the feedlot (Holland, 2010). Metaphylaxis on arrival at the feedlot continues to be effective; however, growing concerns of antimicrobial resistance (AMR) warrants addition research into alternative management strategies.

Weaning and backgrounding practices allow cattle to more rapidly adapt to feed and watering systems in the feedlot; however, its contribution to future health status is perhaps the most rewarding. Step et al. (2008) compared three different management practices at the ranch prior to feedlot shipment. Freshly weaned claves were either shipped directly to the feedlot (WEAN), held at the ranch origin for 45 days (WEAN45), or held at the ranch origin for 45 days and administered a pentavalent modified-live virus respiratory vaccine (WEANVAC45). Additionally, a group of auction market cattle were purchased and evaluated simultaneously (MARKET). Both WEAN and MARKET calves had increased morbidity, which also revealed a difference in treatment costs at the feedlot for the same two treatments. Weaning at the ranch improved overall health status and reduced treatments costs. Prices at the marketplace support the added value of preconditioning. King et al. (2006) reviewed several years of video-auction purchase price data which revealed premiums up to $7.92/45.45 kg paid for calves that had been weaned for a minimum of 45 days. Preconditioned calves are clearly better prepared to transition from the ranch of origin to into the stocker or feedlot sector of the beef production system.

The perceived risk of BRD has a significant effect on the value of calves; however, the health outcome of cattle is difficult to predict. In a large Canadian study,

Ribble et al. (1995) traced several thousand steer calves in a large feedlot back to their ranch origins. It was discovered that individual producers supply an average of 2 calves per truckload. This suggests that calves experience extensive commingling at the auction facility to form homogenous truckloads. Small cow-calf operations that possess less than 100 cows account for approximately 90.4% of beef cattle operations, and 45.9% of the total beef cow population in the United States (USDA-NASS, 2007). The positive association with commingling and BRD cases is reported in Alexander et al. (1989) and Step et al. (2008). While producer numbers and total herd sizes have only slightly changed over the last twenty years, the challenges with commingling remains. Exposure to novel pathogens during a period of stress-induced immunosuppression results in increased morbidity at the feedlot, and commingling promotes stress and pathogen transmission.

Cattle feeders continue to purchase high-risk calves due to price discount incentives and the availability of antimicrobial metaphylaxis. Metaphylaxis is a management tool that allows for mass-medication of high-risk animals, using an FDA-approved antimicrobial, with the goal of controlling a likely respiratory disease outbreak. There are currently 8 injectable and 4 oral antimicrobial products available for metaphylactic use (Ives and Richeson, 2015). Injectable antimicrobials allow for more accurate dosing and delivery, but the added labor in the event of an unexpected BRD outbreak after initial processing is a disadvantage. Munoz (2020) reported calves that received antimicrobial metaphylaxis with tulathromycin upon arrival had a reduced morbidity rate, and the clear improvement in clinical health for metaphylaxis in that study

agrees with a multitude of other published studies evaluating metaphylactic treatment with various drugs (Kilgore et al., 2005; Wellman and O'Connor, 2007; Ives and Richeson, 2015; Baptiste and Kyvsgaared, 2017).

Despite significant research investment, and improved antimicrobials and vaccine technologies, BRD has remained the leading cause of mortality in the feedlot for several decades. The percentage of feeder cattle that died of BRD was the same in 2007 as it was in 1991 (Miles, 2009). Early diagnosis of BRD is imperative for treatment success; however, BRD is challenging to accurately diagnose because cattle possess a strong herd instinct and desire to mask sickness behavior. Behavior such as depression or isolation is traditionally assessed as a clinical sign of BRD along with nasal discharge, and anorexia. There is low sensitivity in these current methods of diagnosis (White and Renter, 2009). Improved diagnostics through technological innovation is theorized to be capable of earlier detection and improved accuracy. Continued research of pathogenesis, causative agents, and diagnostic tools will improve management and treatment of BRD.

1.1.2 Overview of Bovine Respiratory Disease Causative Agents

Respiratory Viruses

There are several known viral and bacterial pathogens involved in or with BRD, and numerous potential interactions between these agents. The viral agents include infectious bovine rhinotracheitis virus (IBRV), parainfluenza-3 virus (PI3V), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), and bovine enteric coronavirus. The primary bacterial agents are *Mannheimia heamolytica, Pasteurella*

multocida, Mycoplasma bovis and *Histophilus somni (*formerly *Haemophilus somnus)* (Plummer et al., 2004).

Infectious bovine rhinotracheitis virus, also known as bovine herpesvirus-1, is a member of the genus *Varicellovirus* in the subfamily *Alphaherpesvirinae*, which belongs to the *Herpesviridae* family (Nandi et al., 2009). This virus is capable of becoming latent after infection or vaccination with an attenuated strain. Recrudescence has been known to be caused by physiological stress, specifically the influence of cortisol and catecholamines. In addition to common respiratory symptoms, it is most often characterized by lesions and ulcerated areas on and around the nose or in the trachea, hence the terms "red nose" and "stove-pipe trachea". Uncomplicated infections can also cause upper respiratory tract disorders, conjunctivitis, genital disorders, and immunosuppression (Gibbs and Rweyemamu, 1977).

Parainfluenza-3 is a member of the *Paramyxoviridae*, genus *Respirovirus* (Neill et al., 2015). Most infections with PI3V are thought to be subclinical; however, signs include cough, pyrexia and nasal discharge. Vaccination against PI3V has decreased with the increase of commercially-available 3-way vaccines containing BVDV type 1 and 2 and IBRV. In a feedlot veterinary survey (Terrell et al., 2011), only 14 of 23 (60.87%) recommended PI3V antigen be included in vaccines. Mortality due to uncomplicated PI3V infection is rare. The most important role of PI3V is to serve as an initiator that can lead to development of bacterial pneumonia.

Bovine viral diarrhea virus is an RNA virus classified as a *Pestivirus* in the family *Flaviviridae*. The ability to induce a persistently infected (PI) calf makes control of this

virus a challenge. A PI calf is constantly shedding BVDV to fellow pen mates following an in-utero infection between 45 to 125 days of gestation. The presence of a PI calf has been shown to increase respiratory morbidity in a cattle population while producing no clinical sign themselves (Larson et al., 2004). Most BVDV infections are subclinical; however, its immunosuppressive effects allow for subsequent bacterial infection (Liebler-Tenorio et al., 2003).

Bovine respiratory syncytial virus is a widespread, enveloped single negative strand RNA virus in the genus *Pnuemovirus* within the family *Paramyxoviridae*. Much like human respiratory syncytial virus (RSV), BRSV infection is more severe in younger calves (Gershwin, 2007). Infected animals are characterized by pyrexia, anorexia, depression, coughing, and an increased respiratory rate. In severe cases, open-mouth breathing and wheezing are common. Bovine respiratory syncytial virus is one of the most important viruses in the BRD complex because of its frequency of occurrence and ability to predispose the respiratory tract to secondary bacterial infection. Its heterogeneous genome and low fidelity in replication allows for survival in the host. There is additional evidence that at BRSV infection can predispose to allergic pulmonary disease in response to specific antigens (Gershwin, 2007). A more in-depth review of BRSV is in section 1.3.1.

Bovine coronavirus (BCV) is most often recognized as a causative agent in calf diarrhea. However, it can also result in respiratory tract infections and has recently been proposed as another important contributor to BRD (Workman et al., 2017). Primary sites of infection include the epithelial cells of the nasal cavity and trachea. While infections

are typically subclinical, clinical signs can include rhinitis, sneezing and coughing (Clark, 1993).

Respiratory Bacteria

Mannheimia haemolytica, a small gram-negative bacteria, is arguably the most important bacterium associated with BRD. This can be attributed to its virulence factors that induce high mortality rates. *M. haemolytica* produces leukotoxin, a104-kDa protein, during the period of log growth. This cytotoxin interferes with β_2 integrin, which impairs homing, phagocytosis, and antigen presentation of *M. haemolytica* by leukocytes. In addition, this bacteria has an outer membrane protein (OMP) that functions as an adhesin, which is involved in host receptor-specific binding. Additionally, its lipopolysaccharide (LPS) complex can cause hemorrhage, edema, inflammation, and lysis of leukocytes (Griffin et al., 2010). Like many other bacteria, *M. haemolytica* resides within the respiratory microbiome of healthy cattle (Carter et al., 1995). Cattle suffering from *M. haemolytica* infection in the lower respiratory tract may display pyrexia, nasal discharge, respiratory distress, and weight loss (Rice et al., 2007). Fibrinous pleuropneumonia is typically observed in severe cases. For many decades, *M. haemolytica* has been considered the most predominant bacterial pathogen in relation to BRD (Griffin et al., 2010).

Pasteurella multocida is readily cultured in young, freshly weaned calves. Like *M. haemolytica, P. multocida* is a commensal bacteria that may become pathogenic after a period of immunosuppression or viral infection. The proportion of fatal cases of respiratory disease in feedlot cattle attributable to *P. multocida* appears to be increasing

(Welsh et al., 2004). Although serogroup and serotype specificity of *P. multocida* variety is often left undifferentiated, the available data commonly reports serogroup A as the most frequently isolated serotype from BRD cases (Dabo et al., 2008).

Mycoplasma bovis is an often overlooked pathogen within the BRD complex. The lack of a cell wall allows *M. bovis* to be pleomorphic, facilitates resistance to antimicrobials, and interferes with Gram staining (Caswell and Archambault, 2007). Along with respiratory disease, *M. bovis* causes arthritis. *Mycoplasma bovis* infections are responsible for a large percentage of chronic disease occurring in feedlots. No commercial vaccines are currently available for the prevention of *M. bovis* infection.

Histophilus somni (formerly *Haemophilus somnus*) is a commensal, gram-negative bacteria that primary resides in the nasopharyngeal region (Griffin et al., 2010). The major OMP and LPS virulence factors of *H. somni* are similar to *M. haemolytica*; however, *H. somni* also produces a histamine and an exopolysaccharide that play a role in disease pathogenesis (Corbeil, 2007). This bacterium is thought as a "late-day BRD" pathogen that is often associated with sudden death associated with septicemic-related cardiovascular left ventral papillary muscle necrosis and thrombotic meningoencephalomyelitis (Griffin et al., 2010). *H. somni* commonly exists in an asymptomatic carrier state on reproductive and respiratory mucosa. A more in depth evaluation of *H. somni* in regards to pathogenesis and viral synergism is in section 1.3.2.

1.1.3 Pathogenesis of Bovine Respiratory Disease

The pathogenesis of BRD is complex and multifactorial. Stress hormones, including glucocorticoids and catecholamines, are known to alter several components of the immune response (Richeson, 2015). Young calves experience several "stressors" as they transition to the next stage of production. Examples of common stressors in the beef production system include adverse weather conditions, abrupt ration changes, handling, transportation, weaning, and commingling. These stimuli produce coordinated, physiologic responses within the body as it attempts to reclaim homeostasis. This is accomplished through the activation of the hypothalamic-pituitary-adrenal (HPA) axis in addition to the sympathetic nervous system (Carroll and Forsberg, 2007). Chronic stress results in several immunosuppressive mechanisms. For example, it decreases production of secretory IgA, limits the proportion of CD8(+) and CD62L(-) cells, which reduces sensitivity and population of β_2- adrenergic receptors on T cells, and impaires chemotaxis (Roth, 1985). The primary bacteria associated with BRD, *M. haemolytica, P. multocida,* and *H. somni,* are ubiquitous in cattle as they reside as commensal organisms in the nasopharynx. Viral infection causes necrosis of mucosal tissue, programed cell death, secretion of pro-inflammatory cytokines, and mediates multiple immunosuppressive effects. Due to damage of the mucosal epithelial cells and disruption of mucociliary apparatus, bacteria can more easily proliferate and/or migrate to the lower respiratory tract following viral infection or a period of stress (Ellis, 2009). Viral infections, such as IBRV and BRSV, elicit a loss of ciliated cells in the airways (Ellis, 2009). This leads to a disruption in the non-specific mucocilliary defense system, resulting in increased

bacterial population in the lower respiratory tract. Viral infection, often promoted by commingling and increased transmission of novel pathogens, in addition to chronic stress results in immunosuppression that initiates a secondary bacterial infection that ultimately results in bronchopneumonia, pleuritis and extensive lung consolidation from the inflammatory response. Additionally, there may be potential negative consequences when vaccinating cattle during a period of chronic stress.

1.1.4 Antibiotic Resistance

Antibiotic administration is the gold standard for both respiratory disease control and treatment. However, concerns about bacterial resistance have required a more judicious approach to antimicrobial use in food animals. The first publications of research into the mechanisms of AMR are from the early 1960s. Similar AMR publications specific for BRD causing pathogens did not begin appearing in the literature until the early 1980s. Research and documentation of AMR has shed light on the issue, and resistance has steadily increased over time (DeDonder and Apley, 2015). However, AMR results from diagnostic laboratories are consistently more resistant than samples from pre-treated cattle. This bias of un-responsive BRD cases raises questions of monitoring AMR data from the diagnostic lab. The characterization of these isolates in relation to the combination of AMR, virulence, and population remains to be discovered (DeDonder and Apley, 2015). In a broad cross-sectional study of cattle upon entrance of several Canadian feedlots, deep nasal swabs were collected from 2,824 head to evaluate AMR of BRD causing bacteria (Andres-Lasheras, 2021). In this study, *M. haemolytica, P. multocida,* and *H. somni* were all detected and analyzed. Multi-drug resistance was

detected more often in dairy-type cattle than beef. Also, beef cattle that came from backgrounding operations had a greater amount of resistance than auction-derived cattle. Oxytetracycline was the most frequently observed drug to have bacterial resistance across all species and cattle types.

1.2 Respiratory Vaccination

A properly timed and administered vaccine induces immunization, with the goal of protection against infection by stimulating the development of antibodies, long-lived effector cells and memory cells. However, immunization and protection in the field setting can be challenging. During transition from their origin ranch to the feedlot, beef calves experience multiple stressors for several days resulting in chronic stress and immune dysfunction; however, cattle are typically administered a multivalent, modified-live virus (MLV) respiratory vaccine upon feedlot arrival via intranasal (IN), parenteral, or both. A survey of consulting feedlot veterinarians published by Terrell et al. (2011), described the vaccination recommendations for both high- and low-risk cattle. All veterinary feedlot consultants surveyed recommended that high-risk cattle be vaccinated for IBRV and BVDV (types 1 and 2). In addition, 15 of 23 (65.22%) recommended BRSV and 14 of 23 (60.87%) recommended PI3V antigen be included in vaccines. Only 5 (21.74%) recommended vaccination against *H. somni,* 17 of 23 (73.91%) against *M. haemolytica,* and 8 of 23 (34.78%) against *P. multocida.* The timing of respiratory vaccination of beef cattle is critical but often overlooked as vaccination regimens are implemented in the beef industry (Richeson and Falkner, 2020). The USDA vaccine label guidelines (2016) indicate: “This product has been shown to be efficacious in healthy

animals. A protective immune response may not be elicited if animals are incubating an infectious disease, are malnourished or parasitized, are stressed due to shipment or environmental conditions, are otherwise immunocompromised, or the vaccine is not administered in accordance with label directions." Chronic stress is defined as stress enduring >24 h (Hughes et al., 2013), and results in immune dysfunction that can alter the humoral and cell-mediated immune responses to vaccination. Consequences may include a greater rate of replication of the live-attenuated vaccine agents in the immunosuppressed host (Richeson et al., 2019) and/or reversion to greater virulence of attenuated viruses used in modified-live virus vaccines.

Vaccines are most often administered to beef cattle via parenteral route and it is thought that systemic immunity can protect the host primarily through the production of IgG, which is the primary type of humoral immune response to parenteral vaccination. However, most pathogens enter through the mucosal tissue of the nasal or oral surface, making IN administration of respiratory vaccine important (Chadwick et al., 2009). Intranasal vaccines produce a more robust mucosal or local immunity through greater production of secretory IgA and other protective components in mucosal tissues (Holmgren and Czerkinsky, 2005). Furthermore, administering a parenteral vaccine followed by an IN booster resulted in an improved amnestic immune response (Webster et al., 2003). Veterinary and producer interest in IN respiratory vaccines has increased with commercial availability of products; however, the safety and efficacy of IN vaccines administered to high-risk, newly received feedlot cattle is not clear from the current literature comprised of small, unreplicated clinical trials.

When reviewing vaccine-related literature, it is necessary to differentiate vaccine efficacy and efficiency. Vaccine efficacy is concluded from a controlled vaccination-challenge model with vaccinated compared to a negative control and occurs when the vaccine is proven to be safe and produce an immune response against that specific antigen (Richeson et al., 2015). Ideally, challenge models should be followed with well-replicated field trials that more closely mimic the conditions of their primary use. On the other hand, vaccine efficiency is evaluated by the product's ability to prevent negative outcomes in a desired species (Fedson, 1998). However, the USDA biological approval process is not designed to examine the efficiency. The model of which vaccine approval takes place does not accurately reflect the stages where vaccine recommendations are made (Richeson et al., 2019). If vaccines were as efficient as they are proven to be efficacious, BRD impacts would probably be much less in the feedlot.

1.2.1 Intranasal Vaccination

Recognizing that viruses typically enter the host via the mucosal portal, localized protection has encouraged the development and commercial availability of IN vaccination. Despite the potential preventative success of mucosal immunity via increase in IgA production, effects of IN vaccines remain an active area of research. Immune mediators, both immunoglobulins and effector T cells generated by mucosal vaccination to antigens differ from those generated by parenteral vaccination (Boyaka et al., 2005). The presence of mediators at the mucosal surface is important to prevent an infection. Memory cells generated at mucosal sites home preferentially to other mucosal locations providing a primed response at other potential portals of exposure (Youngman et al.,

2005). In comparison to parenteral vaccination, IN vaccination is thought to provide protection quicker, yield better mucosal immunity, be more effective for calves with maternal antibodies present, and induce greater nasal interferon production, thus protection against any respiratory infection.

Vaccination-Challenge Model

More rapid protection is a common marketing strategy linked to IN vaccines. Todd et al. (1972) examined that calves administered an IN BRSV vaccine 72 to 96 h before an IBRV challenge showed protection, while calves that received an IN IBRV vaccine only 18 to 40 h before the challenge became clinically ill. In another study, 18 calves were administered a virulent IBRV challenge followed by a challenge exposure to *M. haemolytica* four days later (Jericho et al., 1982). Calves were vaccinated either 3, 7, 11, 15, or 19 days prior to viral challenge. Protection against IBR was defined using clinical signs or lackthereof. This was observed before neutralizing antibodies against IBRV were detected in nasal secretions or serum. Protection was concluded to be present from day 3 to 19 after IN vaccination. Roth and Carter (2000) compared four different vaccine regimens for their efficacy in protecting calves from IBRV at 5 and 14 days after vaccination. The four vaccine regimens evaluated were; MLV and killed (KV) IM IBRV, MLV IN IBRV with concurrent administration of a MLV IN IBRV and MLV IM IBRV, and MLV IN IBRV and KV IBRV. Rectal temperatures, clinical scores, and virus shedding was evaluated. Including a negative control, there were 9 treatments of 5 calves in each. Protection was determined by reduced virus shedding during days 2 through 10 following challenge. All vaccine regimens produced protection against the IBRV

challenge 5- and 14-days following vaccination; however, there were differences in the degree of protection across the different vaccine regimens. The MLV and KV IM vaccine resulted in significantly greater protection than the MLV IN vaccine. Regarding IBRV, it was concluded that there is no difference in time of onset of protection for IN vs. SC or IM vaccination.

Mucosal immunity from IN vaccination is best demonstrated when compared to a parenteral vaccination and a negative control. Gutekunst et al. (1969) evaluated IN vaccination vs. intramuscular (IM) vaccination of a MLV PI3V (pre-Nasalgen) in 5 to 7 month old nursing calves (n=109). The two treatment groups were held in separate pastures following vaccination, and five weeks later a subset was challenged while the remainder was sent to the feedlot. Following vaccination, serum neutralizing antibody increased at a faster rate for IN calves. Following challenge, virus shedding was decreased in IN calves. At the feedlot, there was a natural outbreak of BRD approximately one week after arrival. Again, IN vaccinated cattle had reduced virus shedding, and significantly less morbidity (0/32 vs. 13/37). McKercher et al. (1971) conducted a trial with 5- to 9-month-old calves (n=16). Calves were vaccinated either IN or IM with a MLV IBR, then challenged with IBR 28 days later. Vaccinated calves shed IBRV for a shorter period of time; however, there was no difference in clinical signs following the challenge. The authors concluded that IN vaccination did not offer any advantages over IM vaccination. Gerber et al. (1978) evaluated several different immune mechanisms, as they compared IN MLV IBR, IM MLV IBR, and a no vaccine negative control. Cattle were challenged 28 days later. No statistical analysis was reported in this

study; however, IN vaccination numerically increased IFN-γ production post challenge. Intranasally vaccinated cattle had more IgA and IgG in nasal secretions post vaccination and challenge when compared to IM vaccinated cattle. More recently, Palomares et al. (2021) evaluated the effects of beef calves (n=21) given a primary IN vaccine (Inforce 3) at 5- to 6-weeks-old followed by either an IN (Inforce 3) and a subcutaneous (SC) MLV BVDV type 1 & 2 or a SC MLV (Bovi-Shield Gold 5) 60 days later. A SC booster induced greater BRSV-specific serum neutralizing antibody titers and IgA concentrations in nasal secretions, compared to those who received the IN booster. As a whole, IN vaccination decreased viral shedding in most cases and resulted in greater amounts of IgA in nasal secretions post vaccination and challenge. However, there is not a clear consensus as to whether the increase in IgA leads to a difference in disease or health outcomes post challenge.

One of the advantages of IN vaccination is the ability to provide mucosal protection for calves in the face of maternal antibodies (IFOMA). Literature suggest that IFOMA IN MLV vaccinated calves possess the greatest protection after challenge (Woolums, 2007). It was concluded that vaccination of young calves induces the activation of pathogen-specific T cells and activation can occur in the absence of seroconversion following the first vaccination. Hill et al. (2012) demonstrated that calves that received an IN MLV vaccine and were 2 to 3 days of age, followed by a booster 35 days later did not seroconvert but had an increase in BVDV and IBRV- specific IgA in nasal secretions. Vangeel et al. (2007) demonstrated that IN vaccinated calves with

previously existing maternal antibodies against BRSV had a shorter duration of viral shedding post-challenge as compared to negative controls.

Todd et al. (1972) monitored interferon levels in calves following vaccination and subsequent challenge. Seronegative calves (n=4) were divided into two treatments. Two calves received a virulent IBRV virus by IN administration. The other two calves received the same virus intramuscularly. These calves were challenged with IBRV 19 days later. Interferon was detected in IN calves starting 60 to 72 h following vaccination, while IM vaccinated calves did not have detectable interferon at any time. Intramuscularly vaccinated calves possessed interferon in their nasal secretions 24 h after challenge, and IN calves did not. In addition, one of the IM calves shed virus 14 days post-challenge. In another trial conducted by Cummins and Rosenquist in 1980, six calves were inoculated nasally with a vaccinal strain of IBRV and six additional calves served as a placebo control. Four days later, all were challenged with IBRV. All vaccinated calves had detectable interferon in their nasal secretions, as early as 24 h to as late as 7 days following vaccination. Following the challenge, one vaccinated calf and 5 controls shed IBRV in their nasal secretions. All calves had serum antibodies against IBRV at the time of IBRV challenge; however, 3 control calves continued to shed the virus for 6 days, 2 for 10 days, and 1 for 14 days. Conversely, calves vaccinated IN with a MLV 5-way vaccine (Onset 5 IN) had no measurable interferon in their nasal secretions post-vaccination (Hill et al., 2012). More recently, Midla et al. (2021) demonstrated that two different commercially available vaccines (Inforce 3 and Nasalgen- IP) increased nasal interferon following vaccination in calves (n=30) compared to negative controls.

Cortese et al. (2017) were the first to evaluate IN vaccination of mature cows before and after parturition. Cows (n=30) were either vaccinated 2 weeks prior to parturition or 24 h following parturition with a MLV trivalent IN vaccine containing (IBR, BRSV, and PI3; Inforce 3). Nasal IFN-α and IFN-β did not increase following vaccination, but IFN-γ was significantly increased in cows vaccinated after parturition. Interferon is often, but not always reported to increase after IN vaccination. The inconsistencies could result from small sample sizes and limited replication and/or difficulties with assay procedures.

There are many aspects of IN vaccination that are not completely understood including their safety and efficacy under the field conditions which they are used. Current literature is primarily comprised of immune response measurements, challenge studies with a small number of individuals, and poorly replicated clinical trials. It should also be noted that blinding of morbidity observers is rarely disclosed in published literature. Infectious bovine rhinotracheitis virus is the most commonly studied virus in IN vaccine studies, but IBRV-specific outcomes may not be the same for other viruses. In addition, IN vaccine formulation varies among manufacturers, so it inhibits researchers from drawing precise and consistent conclusions from current literature. Important differences in vaccines include the virus strain used, tissue culture infective dose (TCID), and extent of serial passage of the viruses used in MLV vaccines.

Field Evaluation

Curtis and Angulo (1974) conducted one of the first field trials evaluating IN vaccination, specifically a MLV IBRV IN vaccine. Upon arrival, 231 steers were allocated to two treatments: vaccinates and non-vaccinates. Vaccinated cattle received a

single dose of an IN MLV IBRV vaccine during initial processing. Approximately two weeks later, IBRV infection was clinically apparent in both treatments. Morbidity was 17.7 and 8.0% for vaccinated and unvaccinated cattle, respectively. Furthermore, mortality followed the same trend with 10 (5.9%) of the vaccinated cattle and 2 (3.2%) of the unvaccinated cattle dying. The authors concluded that the IN vaccine could have altered the normal body defense mechanisms in combination with stress, thus causing the vaccinated animals to be more susceptible to secondary bacterial infection.

Martin et al. (1983) evaluated IN efficacy against IBRV when given at least 3 week prior to arrival at the feedlot. Cattle (n=849) were allocated into 3 different treatments: negative control (n=278), IN vaccination with IBRV and PI3V (n=335), or IN vaccination with IBRV and PI3V (n=236). Cattle were sold to feedlots across Canada and morbidity and mortality was recorded. There were no differences in morbidity or mortality for vaccinated or unvaccinated cattle. It was concluded that pre-immunization with a MLV IBRV IN had no effect on BRD treatment rate in the feedlot. Marin et al. (1984) repeated a similar trial. Again, IN vaccination of IBRV and PI3V prior to arrival at the feedlot did not affect clinical morbidity or mortality at the feedlot.

Duff et al. (2000) evaluated the efficacy of an IN MLV IBRV and PI3V vaccine against an IM MLV IBRV and PI3 vaccine. Cattle (n=228) were randomly assigned to one of three treatments: 1) no IBRV-PI3 vaccine (Control); 2) an IN MLV IBRV-PI3 vaccine; or 3) an IM MLV IBRV-PI3 vaccine. Vaccines were administered upon arrival at the feedlot. For the first 28 days, intranasally vaccinated cattle had a significantly greater ADG than cattle that received an IM vaccine. In addition, feed:gain (F:G) was

increased for IM cattle. However, no differences were observed for morbidity or mortality for vaccinated and unvaccinated cattle.

Plummer et al. in 2004 conducted a field trial to evaluate the effect IN vaccination against bovine enteric coronavirus. Heifer calves (n=414) were randomly assigned to either receive a MLV vaccine against BCV and rotavirus or saline (negative control) administered in the naris. Vaccination was associated with significantly decreased treatment risk for BRD. However, vaccination appeared to have no effect on BCV-specific antibody titer. Their findings suggest a relationship between BCV and BRD, and it is one of the few field trials to demonstrate decreased treatment rate for IN vaccinated cattle over negative controls.

Field trials are the most typical to the industry conditions that vaccines are used under and provide the best model for veterinarians to make evidence-based vaccine recommendations. Intranasal field trials are limited and report several different conflicting outcomes on morbidity and mortality. The literature is unclear as to the effectiveness of IN vaccination of cattle upon arrival at the feedlot.

1.2.2 Parenteral Vaccination

Parenteral vaccination is most often given subcutaneously, and less often intramuscularly due to concerns with injection site lesions. Parenteral vaccination induces a systemic proinflammatory response within the host, eventually stimulating the production of antibodies from antigen-stimulated B-cells that differentiate into plasma cells. When vaccinating with an attenuated MLV vaccine, it is essentially inoculating the

host with a controlled dosage, less virulent virus. The ability to induce systemic immunity is the greatest advantage for parenteral vaccination.

Vaccination-Challenge Model

Ellis et al. (2005) evaluated the ability for an inactivated BRSV vaccine to prevent clinical disease. Fourteen 8- to 9-week-old seronegative calves were enrolled. Six calves were vaccinated against BRSV on day 0 and 19. All calves were challenged with virulent BRSV on day 46 and euthanized on day 54. The unvaccinated calves had a significant increase in rectal temperatures and respiratory rate. In addition, viral shedding was only detected in non-vaccinated calves following the challenge. Vaccinated calves had an increase in BRSV-neutralizing antibodies and BRSV-specific IgG. After being euthanized, the percentage of lung that was pneumonic was increased for control calves. This further solidifies that the inactivated BRSV vaccines provided protection against homologous challenge. West et al. (1999) documented the efficacy of a MLV BRSV vaccine. Twenty-seven BRSV seronegative dairy calves were enrolled and assigned to 1 of 4 treatments. Group 1 (n=9) served as a negative control and received no vaccination. Group 2 (n=6) was IM administered a MLV containing BRSV, IBRV, PI3, and BVDV (Bovi-shield 4) followed by a booster 3 weeks later. Group 3 (n=6) was administered the same vaccine as group 2, without a booster. Finally, group 4 (n=6) was administered a single dose of a MLV vaccine containing BRSV, IBRV, PI3, BVDV, and an adjuvant (Pyramid 4). Calves were vaccinated at 2 to 4 weeks of age, challenged with BRSV 3 weeks from their last vaccination, and euthanized 8 days later. Group 1 exhibited increased clinical score on days 5 to 7. Group 2 was the only vaccine group that did not

experience an increase in clinical score during the study. On days 4 to 6, vaccinated calves shed significantly less BRSV than the non-vaccinated controls. Prior to the BRSV challenge, group 2 exhibited the greatest amount of vaccine-induced BRSV-specific serum IgG. There were no differences in IFN-γ from nasal secretions at the time of challenge, implying that vaccination did not clearly induce mucosal antibody responses. However, they did prime for a more rapid post-challenge response, as evident by a greater concentration of IgA and IgG found in the nasal secretions at that time. While there was not any significant difference between vaccine treatments, the authors concluded that a single injection of the unadjuvanted MLV vaccine was least protective against pulmonary pathology and hypoxemia.

Fairbanks et al. (2004) SC vaccinated 33, 6- to 8-month-old steers. Each steer had IBRV-specific serum neutralization antibody titers less than 1:2, with a commercial MLV vaccine (Express 5) containing IBRV, BVDV type 1 & 2, PI3V, and BRSV. Steers were allocated into 3 different treatment groups, with the objective of understanding how rapidly vaccine protection could be provided. Treatment groups were unvaccinated control, or vaccinated 48, 72, or 96 h prior to an IBRV challenge. Steers vaccinated at 96 and 72 h prior experienced fewer clinical signs than the non-vaccinated negative controls. In addition, no difference was observed in the group vaccinated 48 h prior compared to the non-vaccinated group. Rectal temperatures in the groups vaccinated 96 and 72 h prior never exceeded 40°C (104°F) in the 14-day period following the IBRV challenge. There were no statistical differences observed in serology. All animals, vaccinated and non-vaccinated, developed titers against IBRV by day 14 following the challenge. Virus

shedding was reduced on day 3 and 6 for steers vaccinated 96 and 72 h prior to IBRV challenge compared to non-vaccinated negative controls. The authors concluded that a single dose of a multivalent MLV vaccine administered to steers 96 and 72 h prior to challenge provided adequate protection against the development of clinical IBRV.

Peters et al. (2004) sought to define the duration of immunity for calves between 7 weeks and 6 months of age given a quadrivalent vaccine IM. Calves were administered two injections of the vaccine prior to challenge with 1 of 4 viruses, IBRV (n=16), PI3V (n=31), BVDV type 1 (n=16), or BRSV (n=20). Virus challenges occurred either 6 months (PI3), 7 months (IBRV and BVDV type 1) or 12 months (BRSV) following the second dose of the vaccine. Infection caused clinical signs and elevated rectal temperatures in both vaccinated and non-vaccinated calves. This was also followed by an increase in neutralizing antibodies in all treatment groups, vaccinated calves being higher than controls. The authors concluded that the multivalent vaccine provided duration of immunity greater than 6 months for PI3V, IBRV, and BVDV, and 12 months for BRSV. However, it is difficult to determine the efficacy of a viral vaccine to control a multifactorial disease such as BRD in field conditions because of stress-induced immunosuppression, natural exposure and the complication of bacterial infection. In 2007, Salt et al. conducted a similar study with the same vaccine in a more controlled setting. Cattle were 2 to 9 months old at time of treatment administration. They received two doses of a quadrivalent vaccine IM containing IBRV, BRSV, PI3V, and BVDV type 1 three weeks apart. Cattle were challenged 3 to 5 weeks later. In the IBRV challenge study, antibody titers were increased, virus shedding was decreased, and nasal discharge

and rectal temperature was decreased in vaccinated cattle. After PI3V challenge, the vaccinated cattle experienced less virus shedding and fewer days with abnormal respiration and depression. Following the BVDV challenge, BVDV-specific antibody titers were greater, duration of BVDV shedding was reduced (3.1 vs 5.7 days), and rectal temperature was less in vaccinated cattle. The BRSV challenge revealed that vaccinated calves had greater BRSV-specific antibody titer and less BRSV shedding for a reduced amount of time.

Field Evaluation

Van Donkersgoed et al. (1990) conducted 5 different trials to determine the efficacy and efficiency of BRSV vaccines. Focusing on the feedlot arrival stage, trial 4 included 611 auction-derived yearling cattle placed in a 10,000 head capacity commercial feedlot in Canada. Upon arrival, cattle were randomly allocated into vaccinates and negative controls. All calves were administered vitamin A and D, ivermectin, a growth implant, a MLV IBRV and PI3 vaccine, and an 8-way clostridial bacterin upon arrival. Vaccinates also received one dose of a MLV BRSV vaccine at initial processing, but this was excluded for the control group. The overall treatment rate for control cattle was 4% and vaccinates was 2% and this was not statistically different.

MacGregor and Wray (2004) continued to explore the effect of BRSV vaccination of newly received feeder cattle. A total of 50 blocks were allocated involving 19,099 cattle. Cattle were randomly assigned to 1 of 2 vaccine treatments: 1) a multivalent MLV IBRV, BVDV type 1, PI3, and BRSV 2) a multivalent MLV IBRV, BVDV type 1, and PI3 of similar formulation, but did not contain BRSV. Respiratory morbidity did not

differ between treatments. Respiratory mortality was 0.3 and 0.7% for vaccinated and unvaccinated cattle, respectively. No differences were observed in performance or carcass characteristics.

Cravens and Bechtol (1991) evaluated the effect of two vaccines on morbidity and mortality of seronegative beef calves against a negative control. Treatment 1 received a multivalent vaccine consisting of a chemically altered IBRV and PI3V, a MLV BRSV, killed BVDV, and a bacterin containing 5 Leptospira serovars (CattleMaster 4 + L5). Treatment 2 received a killed IBRV, BVDV, PI3V, and BRSV along with a bacterin containing 5 Leptospira serovars (Triangle 5). Cattle were challenged with virulent IBRV and BVDV 30 days following booster vaccination. There was a statistically significant reduction in morbidity and mortality for treatment 1 over treatment 2 and overall vaccinated compared to non-vaccinates.

In conclusion, these results overall demonstrate the clinical efficacy of commercially available parenteral vaccines in challenge and natural models. Vaccine efficacy can be associated with reduced viral shedding, lessened pulmonary pathology, vaccine-induced cell-mediated and antibody responses, as well as increased post challenge antibody response. However, vaccine effectiveness cannot be concluded from vaccination-challenge models previously summarized. A randomized, blinded, and controlled field trial that includes a negative control (unvaccinated) treatment group is required to evaluate vaccine effectiveness.

1.3 Bovine Respiratory Syncytial Virus and *Histophilus somni Synergism*

Within the BRD complex, BRSV has been associated with many secondary bacterial infections. These bacteria include *M. haemolytica, P. multocida,* and *H. somni.* However, BRSV's immunomodulatory effects may foster an environment for increased *H. somni* colonization. Infection with *H. somni* requires bacteria-specific IgG2 (Th1 response) for disease resolution and protection, while IgE antibodies are associated with enhanced pathogenesis of *H. somni*. However, naturally occurring field strains of BRSV induces immunomodulation within the host to favor a Th2 response, eliciting the production of BRSV-specific IgE. Histamine production increases in such a scenario, initiated by *H. somni* with histamine release by IgE cross-linkage of receptors on mast cells (following BRSV immune response) which may enhance lung pathology. Additionally, *H. somni* possesses an antigenically variable major outer membrane protein (MOMP), which is the predominant antigen recognized by IgE. Could the bias to Th2 by BRSV prime the immune system to enhance *H. somni* proliferation by failure to control colonization and result in disease enhancement? Alternatively, other bacteria (*M. haemolytica, P. multocida*) may be less prevalent in such a scenario resulting in differing clinical health outcomes.

1.3.1 Bovine Respiratory Syncytial Virus Infection

Bovine respiratory syncytial virus infection, vaccination, and interaction within the host immune system is comprised of several unique immunomodulatory aspects (Gershwin, 2007). It is closely related to human respiratory syncytial virus, which has been studied extensively. Human strains of RSV are the most important cause of

infectious lung disease in young children and the elderly (Falsey, 2007). Likewise, BRSV is most severe in young cattle and often characterized by pyrexia, anorexia, depression, cough, increased respiratory rate, and severe infections cause open-mouth breathing and wheezing. In fatal cases, lungs typically display large areas of consolidation. Staining sections of the lung for BRSV reveals the presence of the virus in the bronchial epithelial cells and occasionally in type 2 alveolar cells. Viral shedding typically begins on day 3 or 4 following infection and rarely endures beyond day 10. Therefore, BRSV isolation at necropsy is only possible during a short time period following infection (Gershwin, 2007) which can lead to misdiagnosis or misunderstanding of the involvement and impact of BRSV on mortality. Lung consolidation does not correlate with the short duration of the viral infection in the host and the lack of distribution in the entire body. Lung consolidation can be attributed to the host immune response or inflammation in response to BRSV, and other pathogens during a complicated infection. This ability to modulate the immune response away from a traditional virus-induced Th1 response has potential impact on vaccine safety, secondary bacterial infections, and natural infection.

Welliver et al. (1981) discovered that children infected with RSV had RSV-specific IgE antibodies in serum and nasal secretions coupled with increased levels of histamine in nasal secretions. Later, studies began to demonstrate a shift in T-helper cell type in severely infected children (Roman et al., 1997). Interleukin-4 (IL-4) production by lymphocytes supports the shift to a Th2 response. In cattle, the presence of IgE in BRSV-infected calves has been documented (Stewart and Gershwin, 1989). Gershwin et al. (2000) demonstrated clinical severity was positively associated with levels of BRSV-

specific IgE in the serum and lymph. Also, IL-4 from lymphocytes in the lung was temporally related to BRSV infection.

Kim et al. (1969) evaluated vaccine candidates for RSV. This was a formalin inactivated alum-adjuvanted vaccine that killed two children and hospitalized 80% of participants following a natural viral challenge. Disease exacerbation following vaccination has also been documented in cattle. In the Netherlands, a MLV vaccine, created from strain isolated from the population previously, was administered to cattle prior to natural exposure (Kimman at el., 1989). Disease was more severe in vaccinated cattle compared to unvaccinated. Schreiber et al. (2000), in Belgium, reported similar outcomes following the use of a commercially-available killed BRSV vaccine. Gershwin et al. (1998) experimentally reproduced the disease exacerbation following killed BRSV vaccine administration. Nineteen 7 to 8 week old Holstein calves were divided into 3 treatment groups: (n=6) vaccinated against BRSV and sham infected (identical components without the virus), (n=6) sham vaccinated and infected with BRSV, (n=7) vaccinated against BRSV and infected with BRSV. Following a booster on day 14 for all treatment groups, calves were challenged with BRSV on day 44. Necropsies were performed on day 56. The vaccinated and infected calves experienced increased severity and extent of disease (evaluated by clinical signs) and increased histopathological lesions in the lung. Of the two infected groups, the mean clinical score for the vaccinates was significantly higher. Using the serum of these calves, further studies were conducted to demonstrate the correlation between clinical severity and BRSV-specific IgE levels following vaccination with a killed BRSV vaccine. This experiment demonstrated the

correlation between severity of clinical signs and serum BRSV-specific IgE levels. Furthermore, it revealed that vaccination also induced a strong IgE response to several BRSV proteins (Gershwin, 2008).

The shift to a Th2 response by BRSV vaccination can also be modulated by IFN-γ responses. However, it has been reported that calves vaccinated with inactivated BRSV exhibit reduced IFN-γ secretion after challenge compared to unvaccinated calves (Woolums, 1999). Shifting toward a Th2 response and BRSV-specific IgE cross-linkage on mast cells could be involved in the pathogenesis of disease exacerbation reported in previous literature. Following the observation of BRSV vaccination shifting the host towards a Th2 response, Mapletoft et al. (2006) attempted to create a vaccine that invoked a protective Th1 response. They formulated an inactivated BRSV vaccine with CpG and deoxyoligonucleotides (ODN), which had been proven to stimulate secretion of Th1 cytokines. Calves were either vaccinated with BRSV, BRSV and CpG ODN, or culture medium only. Calves vaccinated with BRSV and CpG ODN showed an increase in IgG2 and IFN-γ levels, indicating that the vaccine successfully induced a greater Th1 response.

Classically, IgE antibodies are produced in response to helminthic parasites, and IgE binds to and activates mast cells and basophils, and cause mast cell degranulation to assist in killing of helminths. Also, IgE antibodies are activated in immediate hypersensitivity reactions such as during allergic responses. Cattle developed IgE antibodies after inhalation of pollens and molds (Olchowy et al., 1995). Gershwin et al. (1994) examined correlations between BRSV infection and the development of IgE

antibodies to *Saccharopolyspora rectivirgula* (causative agent in "farmer's lung"). Previously, BRSV-specific IgE has been associated with increased lung pathology and clinical disease. Furthermore, *S. rectivirgula* aerosolization enhanced development of BRSV-specific IgE. Gershwin and Giri (1992) documented that histamine concentrations were significantly greater in calves infected with BRSV, regardless of *S. rectivirgula* exposure. Dr. Gershwin concluded that these two studies confirm the synergistic effect of allergen exposure and BRSV infection modulate the lung environment for a type 1 hypersensitivity response (Gershwin, 2007).

1.3.2 *Histophilus somni* Infection

Histophilus somni induced pneumonia is often characterized by neutrophilic to fibrinoid vasculitis, degeneration of alveolar macrophages, necrotizing bronchiolitis, suppurative bronchiolitis, lobular necrosis and dilation, and thrombosis of the lymphatics (Gogolewski wt al., 1987). Infection typically endures 6 to 10 weeks in chronic cases. However, severe clinical signs are typically visible for 48 h, and then infection can become subclinical for several weeks. *Histophilus somni* has been described as primarily a northern pathogen due to its previously recorded increased abundance in the Northern U.S. and Canada (Van Donkersgoed et al., 1990); but there is increasing concern that prevalence has increased in the southern U.S. (Groves, 2021).

Histophilus somni possesses several different virulence factors that contribute to the host-pathogen relationship (Corbeil et al., 1991). The 40kDa outer membrane protein (OMP; p40) and Immunoglobulin Binding Proteins (IgBP's) are critical antigenic proteins. The p40 OMP is important in protection because convalescent phase serum and

monospecific bovine antibodies against p40 are passively protective against BRD (Corbeil et al., 1991). However, MOMP was not observed to be recognized by convalescent phase serum in serial dilutions traditionally used for Western blots (1:500 to 1:1000 or more). Following characterization of MOMP, it was shown to be similar to porins of other gram-negative bacteria (Tawanga et al., 2000). Porins are OMP's that are associated with the modulation of cellular permeability. *Histophilus somni* is considered an extracellular pathogen rather than a facultative intracellular pathogen due to its ability to kill macrophages instead of surviving and multiplying in them for periods of time (Corbeil, 2007). Although IgA is typically known as the most important secretory Ig class, IgG2 has been shown to be the most protective in *H. somni*-induced respiratory disease. The production of IgG2 is a result of a Th1 response.

Like BRSV, *H. somni* has been demonstrated to elicit a Type1 (immediate) hypersensitivity (Ruby et al., 2000). Cattle sensitized by exposure to a virulent *H. somni,* treated to eradicate the infection, and then re-challenged following a 4-week rest period developed severe clinical signs consistent with hypersensitivity (Ruby et al., 2000). *Histophilus somni*-specific IgE response following vaccination against *H. somni* plays a role in the host's immediate hypersensitivity reactions (Ruby et al., 2000). Ellis and Jong (1997) reported adverse reactions similar to anaphylaxis after administering a vaccine containing *H. somni* whole cells. Immediate hypersensitivity is caused by an allergic reaction, which is a result of cross-linkage IgE bound to receptors on mast cells and basophils after exposure to the specific pathogen. The balance between IgG2 and IgE

antibodies to *H. somni* is important to understand whether Th1 or Th2 immunopathology or protection dominates at any time during infection.

Histamine can be found within all mammalian tissues, and is a known mediator of Type 1 immediate hypersensitivity. The pathogenesis of *H. somni* also relies on the production of histamine molecules. The synthesis of histamine suggests there is an inflammatory reaction surrounding areas of infection due to histamine production from the invading bacterial pathogen. Histamine on the surface of *H. somni* could bind to histamine receptors located on the epithelial cells of the lung. The effects of histamine on the lungs is mediated by the amount of histamine receptors expressed, constriction of smooth muscle, stimulation of vagal afferent nerve endings and mucus glands, and increased permeability of bronchial epithelium (Ruby et al., 2000). In addition, the presence of a subclinical viral infection (i.e., BRSV) would result in epithelial desquamation, causing a greater amount of exposed histamine receptors present in the submucosa that could provide synergism of the inflammatory response in the lung (Ruby et al., 2000). Although, *H. somni* is known to elicit antigen-specific IgE, 41 kDa MOMP is the most predominant antigen recognized by IgE (Corbeil, 2007). The discovery that MOMP is antigenically variable (Tagawa et al., 2000), and that IgE antibodies are correlated with worsened pneumonia likely explains the disease enhancement during a combined BRSV and *H. somni* infection.

1.3.3 Gershwin & Corbeil Mechanism.

As proposed in Ruby (2000), the combination of *H. somni* and BRSV vaccination of sensitized cattle results in excessive IgE production, increased bronchoconstriction,

edema formation, chemotaxis, and introduction of exogenous histamine that further contributes to IgE production. Vaccination with MLV BRSV is capable of compounding these immune responses even further. Healthy, non-vaccinated calves develop IgE antibodies against *H. somni* and BRSV as a result of constant low levels of natural exposure. The IgE antibodies will bind to their receptors on immune cells (basophils, mast cells, and eosinophils). Simultaneously, cytokines and chemokines are being released in response to IgE binding. Specifically, IL-4 is released and stimulates the production of additional IgE. This signaling cascade results in enhanced IgE production. Therefore, by the time of vaccination with BRSV or natural disease outbreak in the production setting, it is likely calves have already been sensitized to both pathogens. Vaccination against BRSV could then serve as a catalyst. If subsequently infected with BRSV or *H. somni,* a hyperactive immune response is likely to occur. During a BRSV infection, desquamation of respiratory epithelium and cell damage occurs and predisposes the animal to a secondary bacterial infection. *Histophilus somni* produces histamine which further activates the production of IgE antibodies and binds to histamine receptors within the lung. At this point, clinical signs are likely to become evident. Excessive IgE production following vaccination or natural exposure to BRSV with the introduction of exogenous histamine to fuel the production of more IgE antibody can result in disease exacerbation.

Following the work of Dr. Ruby, Gershwin et al. (2005) designed a controlled experiment to further evaluate the *H. somni/BRSV* theory. Eight conventionally-raised, 9-week-old, Holstein bull calves were used. All calves had low levels of antibodies against

BRSV and *H. somni* prior to enrollment. There were 3 treatments; BRSV-infected only (*n*=2), *H. somni*-infected only (*n*=2), and co-infected with BRSV and *H. somni* (*n*=4). Calves were challenged with BRSV on day 0 by aerosol and *H. somni* on day 6 via intratracheal route. On day 28, a necropsy was performed on all animals. Clinical scores for co-infected calves were higher and were maintained for a longer duration, through the end of the 28-day observation period. At necropsy, the co-infected calves possessed significant gross lesions and large areas of pulmonary consolidation, calves that were challenged with BRSV only had no gross lesions observed in the lung, and calves challenged with *H. somni* had minimal focal atelectasis. In addition, the presence of IgE antibodies against *H. somni* in co-infected calves was significantly greater than those infected with *H. somni* only. These results strongly support the hypothesis that BRSV primes the immune system for an environment that increases *H. somni* colonization. In combination with *H. somni*-induced histamine production and MOMP in the midst of a Th2 response, this ultimately results in disease exacerbation.

In addition to the previously described hypothesis, enhanced pathology could be attributed to mechanisms at the alveolar barrier that result in increased invasion of *H. somni* following BRSV infection (Anges, 2013). Bovine respiratory syncytial virus and *H. somni* IbpA increase BAT2 cells reaction and paracellular migration, this allows for *H. somni* to more readily cross the alveolar epithelial cell layer. The combination of BAT2 cells with BRSV and *H. somni* also increases degradation of the basement membrane. This allows *H. somni* to migrate past the second barrier (Gershwin et al., 2005).

Histamine production by *H. somni* also aids in vasodilation and increased capillary permeability.

Gershwin et al. (2005) demonstrated that dual infection with BRSV and *H. somni* results in disease exacerbation as evidenced by increased duration and severity of clinical signs and increased lung pathology. The authors speculated that BRSV infected bronchial epithelial cells may develop altered surface molecule expression (MOMP) and chemokine production that could enhance *H. somni* proliferation and spread of infection.

1.4 Conclusions from the Literature

Arguably, BRD is the most complex mammalian disease and despite research and technological advances it remains the leading cause of morbidity and mortality in the feedlot. With concerns of AMR, effective preventative medicine has become increasingly important. Respiratory vaccination with commercially-available vaccines is widely practiced in the feedlot setting. However, the true efficiency of these vaccines in commercial environments is not fully understood. In addition, could BRSV's immunomodulatory aspects alter the microbial community in the upper respiratory tract? Could the alteration possibly result in enhanced *H. somni* colonization and disease exacerbation?

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CHAPTER II

CLINICAL EFFECTS AND HISTOPHILUS SOMNI PREVALENCE IN HIGH-RISK CALVES ADMINISTERED INTRANASAL OR PARENTERAL VACCINE

2.1 Abstract

Following discovery of acute bovine respiratory syncytial virus (BRSV) infection enhancing *Histophilus somni* (*H. somni*) clinical disease in calves, further understanding of the safety and efficiency of live-attenuated BRSV vaccines is important. Our objective was to determine the safety, efficiency and immunomodulation of an intranasal (IN), trivalent (infectious bovine rhinotracheitis virus [IBRV], parainfluenza-3 virus [PI3V], and BRSV) respiratory vaccine with parenteral, bivalent bovine viral diarrhea virus (BVDV) and a parenteral, pentavalent (BVDV type I and II, IBRV, BRSV, and PI3V) respiratory vaccine. High-risk beef calves (n=525) were received in 5 truckload blocks and stratified by body weight (213 ± 18.4 kg), sex, and presence of a pre-existing ranch ear-tag. Pens were spatially arranged in sets of 3 and randomly assigned to treatment with an empty pen between treatment groups. Treatments included: 1) no viral respiratory vaccination (**CON**), 2) cattle intranasally administered a trivalent, modified-live virus (MLV) respiratory vaccine with parenteral BVDV type I and II vaccine (**INT**), and 3) cattle administered a pentavalent, MLV respiratory vaccine (**INJ**). Pen was the experimental unit, with a total of 15 pens per treatment and 11 or 12 calves per pen in this

70-d receiving study. Performance, morbidity, mortality, BRSV, *H. somni, Mycoplasma bovis* (*M. bovis*)*, Mannheimia haemolytica* (*M. haemolytica*)*,* and *Pasteurella multocida* (*P. multocida*) prevalence and cycle time in nasal swabs via rtPCR on d 0, 7, 14, and 28 was determined, and BRSV-specific antibody titer and serum IFN-γ concentration via ELISA were evaluated on d 0, 14, 28, 42, 56, and 70. Morbidity ($P = 0.83$), mortality ($P = 0.68$) and average daily gain ($P \geq 0.82$) did not differ; however, feed efficiency from d 0 to 56 was improved ($P = 0.05$) for CON. Serum antibody against BRSV increased with time ($P < 0.01$), and was numerically greatest for INT. There was a treatment × time interaction ($P < 0.01$) for *H. somni* present in nasal swabs; on d 14 and 28, INT (21.1 and 57.1%) more frequently ($P < 0.01$) became *H. somni* positive than CON (3.6 and 25.3%) or INJ (3.4 and 8.4%). Also, INT had reduced ($P = 0.03$) cycle time of *H. somni* positive samples on d 28. There was a tendancy ($P = 0.06$) for a treatment effect for BRSV cycle time; CON had a reduced mean (30.80) than INT (33.53) and (32.93). No treatment × day interaction ($P \geq 0.17$) existed for *M. bovis, M. haemolytica,* or *P. multocida* prevalence and cycle time. No treatment differences ($P = 0.55$) were detected for serum IFN-γ concentration. There was a treatment effect ($P < 0.01$) for the rate of *M. haemolytica* positive culture from lung tissue specimens; INT had less (0.0%) *M. haemolytica* positive lung tissue cultures than INJ (45.5%) or CON (74.0%). These data indicate MLV vaccination of high-risk calves, either parenterally or intranasally, did not clearly impact health or growth during the feedlot receiving period. However, INT caused increased prevalence of *H. somni* in the naris and resulted in less *M. haemolytica* cultured from lung tissue samples upon necropsy. The intranasal administration of MLV vaccines may

alter the microbial community in the upper respiratory tract of cattle; specifically, we observed that MLV IN increased the prevalence of *H. somni* in high-risk feedlot calves.

2.2 Introduction

Bovine respiratory disease (BRD) is the most important disease affecting feedlot cattle (Woolums et al., 2005). Despite significant research investment and improved antimicrobial and vaccine technologies, BRD has remained the leading cause of morbidity and mortality in the feedlot for several decades. Veterinary feedlot consultants unanimously recommend vaccination against respiratory viruses in high-risk cattle upon arrival at the feedlot (Terrell et al., 2011). However, the percentage of feeder cattle that died of BRD was the same in 2007 as it was in 1991 (Miles, 2009) and anecdotal evidence suggests that BRD morbidity and mortality in the feedlot are increasing rather than improving. Current respiratory vaccination literature is largely comprised of controlled pathogen challenge models designed to evaluate vaccine efficacy compared to unvaccinated controls. However, vaccine efficiency should be determined in the production environment using randomized, well-replicated field trials with a negative control treatment. Unfortunately, the USDA approval process for biologicals is not designed to examine vaccine efficiency (Richeson et al., 2019). Veterinary and producer interest in intranasal (IN) respiratory vaccines to prevent BRD has increased concomitant with commercial availability, but a clear understanding of IN vaccine safety and efficiency is lacking.

Within the BRD complex, bovine respiratory syncytial virus (BRSV) has been associated with predisposition to secondary bacterial infections. These bacteria include

Histophilus somni (H. somni)*, Mycoplasma bovis* (*M. bovis*)*, Mannheimia heamolytica* (*M. haemolytica*)*,* and *Pasteurella multocida* (*P. multocida*)*.* However, BRSV's immunomodulatory effects can foster an environment for increased *H. somni* colonization (Gershwin et al., 2005). Infection with *M. haemolytica, P. multocida,* or *H. somni* requires bacteria-specific IgG2 (Th1 response) for disease resolution and subsequent protection, while *H. somni*-specific IgE class antibodies are associated with enhanced pathogenesis of *H. somni*. Likewise, calves infected with BRSV develop an IgE response to viral proteins in addition to other antigens encountered during infection (Stewart and Gershwin, 1989). Bovine respiratory syncytial virus has the ability to modulate the immune response towards a Th2 response that could impact vaccine safety, secondary bacterial infections, and natural infection. Adaptive immunity (Th2) possesses memory enhanced response to subsequesnt exposure to the same antigen. Ruby (1999) observed that the combination of *H. somni* and BRSV parenteral MLV vaccination of sensitized cattle resulted in enhanced IgE production, increased bronchoconstriction, edema formation, chemotaxis, and introduction of exogenous histamine to contribute to IgE production. Gershwin et al. (2005) further evaluated this hypothesis in a dual BRSV and *H. somni* challenge model. At necropsy, the dually-infected calves possessed significant gross lesions and large areas of pulmonary consolidation, yet calves that were challenged with BRSV only had no gross lesions, and calves challenged with *H. somni* only had minimal focal atelectasis. In addition, the presence of IgE antibodies for *H. somni* in co-infected calves was significantly greater than those infected with *H. somni* only. These results support the hypothesis that BRSV shifts the immune system towards a

Th2 response that results in increased *H. somni* colonization, rather than a Th1 response necessary for protection and response to intracellular respiratory pathogens.

Our primary objective was to explore if BRSV-containing MLV vaccines could influence *H. somni* prevalence in high-risk beef calves housed in a research feedlot setting. A secondary objective was to evaluate the clinical efficiency of an IN, trivalent (IBRV, BRSV, PI3V) respiratory vaccine with parenteral, bivalent BVDV and a parenteral, pentavalent (BVDV type I and II, IBRV, BRSV, PI3V) respiratory vaccine compared to a negative control.

2.3 Materials and Methods

Animal procedures were approved by the West Texas A&M University (WTAMU) Institutional Animal Care and Use Committee before study initiation (IACUC #2020.10.002). This trial was conducted from November 2020 to May 2021 at the WTAMU Research Feedlot, near Canyon, TX.

2.3.1 Arrival Processing

A total of 525 crossbred beef calves (213 ± 18.4 kg), were acquired from an order buyer in central Texas. Upon arrival (day -1), individual body weight (BW), sex (bull [n=129] or steer [n=396]), and presence or absence of a pre-existing ranch tag were recorded. Additionally, an ear tissue sample was collected to test for BVDV persistent infection (PI; Cattle Stats, Oklahoma City, OK), and each animal was affixed with a unique visual and electronic identification ear tag (Allflex Livestock Intelligence, Madison, WI). Cattle were also administered a growth promoting implant containing 200

mg progesterone, 20 mg estradiol benzoate, and 29 mg tylosin tartrate (Component E-S with Tylan, Elanco Animal Health, Greenfield, IN), an injectable clostridial vaccine with tetanus toxoid (Calvary 9, Merck Animal Health, Madison, NJ), and an injectable (Ivermax Plus, Aspen, Greeley, CO) and oral (Valbazen, Zoetis, Parsippany, NJ) antiparasitic. Calves were held overnight in a receiving pen with access to hay (0.5% BW), water and 0.5% BW of a common starter ration. Cattle were blocked by truckload (n=5), stratified by arrival body weight, sex, and presence of ranch tag, and randomly assigned to experimental treatments. Additionaly, a random subset (n=6) per pen were choosen for whole blood and nasal swab sampling. The following day (day 0), cattle were individually weighed, administered a *M. haemolytica* bacterin-toxoid (One Shot Cattle Vaccine, Zoetis), metaphylaxis with tildipirosin (Zuprevo, Merck Animal Health, Madison, NJ; 7-day post-metaphylactic interval), bulls were band castrated (Callicrate, No-Bull Enterprises, St. Francis, KS) and provided 1 mg/kg BW oral meloxicam (Unichem Pharmaceuticals, Hasbrouck Heights, NJ), and administered the appropriate MLV vaccine treatment. Administration and handling of vaccines and other products followed Beef Quality Assurance guidelines.

2.3.2 Experimental Design

This experiment consisted of 3 treatment groups evaluated over a 70-day receiving period: 1) negative control, no respiratory vaccination (**CON**), 2) cattle intranasally administered (1mL/nostril) a trivalent, MLV respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0 (**INT**), 3) cattle administered a pentavalent, modified-live virus

respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0 (**INJ**). Treatments were spatially arranged with an empty pen between vaccine treatment groups to minimize unwanted virus transmission between vaccine treatments and negative controls. Additionally, treatments were processed and sampled starting with CON, INJ, and then INT to mitigate virus transmission during handling procedures. In this generalized complete block design, pen served as the experimental unit. Treatment pens were replicated for a total of 15 pens per experimental treatment.

2.3.3 Cattle Management

Cattle were housed in 20.8 m^2 soil-surfaced pens with 50.6 cm of linear bunk space per animal, and were fed the same starter ration throughout the entire 70-d trial. Cattle were fed once daily at approximately 0730 and feed bunks were visually evaluated at 0630 and 1730 to determine the quantity of feed to offer each pen the subsequent day. Feed bunks were managed according to standard procedure at the WTAMU Research Feedlot, with the goal of little or no residual feed remaining immediately before feeding at 0730. Feed samples were collected twice a week for dry matter (DM) determination and a diet composite was collected every two weeks for nutrient analysis at a commercial laboratory (Servi-tech Labs, Amarillo, TX). The DM analysis was conducted at the WTAMU Research Feedlot and was used to adjust diet formulation during the course of the study. Orts were also collected, weighed, and analyzed for DM to adjust DM intake at the end of each 14-d period.

There were two steers that tested positive for BVDV-PI and these were removed from their study pen on d 1 and quarantined. A clinical illness score (CIS, 1 to 4 scale)

was assigned daily by trained investigators blinded to treatment pen assignment. A CIS of 1 described a “normal” steer with no signs of clinical illness. A CIS of 2 indicated a “moderately ill” steer. Apperance of a CIS 2 included gaunt, nasal/ocular discharge, lags behind others, and cough. Steers with a CIS of 3 were deemed “severely ill”, with purulent nasal/ocular discharge, labored breathing, and severe depression. Finally, CIS 4 corresponded to a “moribund” steer that was unresponsive to human approach and near death. Steers with a CIS 2 were removed from their home pen,brought into the processing barn, and restrained to record rectal temperature using a digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA). If rectal temperature was ≥ 39.7°C, that animal was considered a BRD case, treated with an antimicrobial, and immediately returned to their home pen. Steers assigned a CIS 3 were removed from their home pen and were classified as a BRD case and treated with an antimicrobial regardless of rectal temperature. If an animal was observed to be a CIS of 4, it was euthanized. Steers first diagnosed with BRD (BRD1) received 40 mg/kg BW of florfenicol (Nuflor, Merck Animal Health) and were assigned a 3-day post-treatment interval (PTI). Following expiration of the PTI, steers were evaluated and treated using the same BRD case definition. Steers that qualified for a second BRD treatment (BRD2) received 11 mg/kg BW enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, KS) and were assigned a 3-d PTI. Upon expiration of the PTI, steers were eligible for a third and final treatment (BRD3) with 6.6 mg/kg BW ceftiofur crystalline free acid (Excede, Zoetis, Parsippany, NJ). Steers deemed chronically ill (3 antimicrobial treatments combined with <0.45 kg ADG since day 0 and/or BCS <3) were removed from the study and placed into

a critical care pen. Additionally, days to BRD1, BRD2, and BRD3 and antimicrobial treatment cost were determined for each experimental treatment group.

2.3.4 Data Collection and Analysis

Initial (average of d -1 and 0), interim (d 7, 14, 28, 42, 56), and final (average of d 69 and 70) individual BW were recorded. Dry matter intake was recorded and feed efficiency (G:F) was calculated for each interim period.

Blood and Serum Analyses

Whole blood samples were collected from the designated subset of animals on d 0, 14, 28, 42, 56, and 70. Blood samples were collected via jugular venipuncture into two evacuated serum separator tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ; REF:367861) and centrifuged in the WTAMU Animal Health Laboratory at 1,250 × g for 20 minutes at 4°C. On d 0, 14, 28, and 42, serum was divided into four aliquots, and three aliquots were divided for d 56 and 70. One serum aliquot from each time point was submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL; Canyon, TX) and Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University (Starkville, MS) for analysis of BRSV-specific antibody titer and serum cytokine Interferon-γ (IFN-γ), respectively. The serum aliquot for BRSV titer was stored at -20° C and the serum aliquot for IFN-γ ELISA was stored at -80° C until all samples were collected prior to laboratory analysis. Detection of antibodies against BRSV was conducted using the virus neutralization assay described by Rosenbaum et al. (1970).

Serum concentration of IFN-γ was determined via ELISA with an intra-assay CV of ≤14.91%. Commercially available antibodies and standards were used (Kingfisher Biotech, Saint Paul, MN). Each well was coated with 100 µL of capture antibody working solution (0.6 µl of capture antibody per mL of Dulbecco's Phosphate-Buffered Saline [DPBS]), covered with a plate sealer, and incubated overnight at 4° C. The following morning, plates were aspirated, and 200 µL of blocking buffer (4% BSA [Sigma A7906] in DPBS) was added to each well. Plates were sealed, and left to incubate at room temperature for 2 h. After the plate was aspirated, a 2-fold standard curve (15 ng/mL to 0.23 ng/mL) was plated in duplicates. Vortexed serum was then plated, with 100 µL, in duplicate, in each well and dilutions were performed as needed in blocking buffer. Plates were then covered and left to incubate at room temperature for 1 h. Following incubation, plates were washed 4 times with washing buffer (0.05% TWEEN-20 in DPBS). Plates were then incubated for 1 h at room temperature with 100 µL of detection antibody (0.5 µg/mL of blocking buffer, PBB0267B-050). Plates were washed an additional 4 times with washing buffer, and then incubated for 30 minutes at room temperature with 100 µL of horseradish peroxidase-conjugated streptavidin (0.6 µL/mL of blocking buffer; EMD Millipore, Burlington, MA). Plates were washed 4 times with wash buffer, and 100 µL of TMB substrate (EMD Millipore, Burlington, MA) was added to each well. Plates were incubated in the dark at room temperature for 30 minutes and the enzymatic reaction was stopped by adding 100 µL of stop solution (0.5 M H2SO4) to each well. Absorbance was measured on a plate reader (BioTek, Winooski, VT) at 450 nm.

Whole blood samples were collected via jugular venipuncture into a single evacuated tube containing EDTA (BD Vacutainer K2EDTA; Becton Dickinson). Samples were chilled, transported, and analyzed in < 4 h following collection. Complete blood count was determined on d 0, 14, 28, 42, 56, and 70 using an automated hematology analyzer (Idexx, ProCyte Dx Hematology Analyzer, Westbrook, ME) in the WTAMU Animal Health Laboratory.

Nasal Swab Analysis

Nasal swabs were collected on d 0, 7, 14, and 28 using a single nylon-flocked swab (PurFlock Ultra; Puritan Medical Products, Guilford, ME; 15 cm) and stored in additive-free 14 ml polystyrene tubes (Falcon; Corning, Inc., Corning, NY) at -80° C until submission to TVMDL for rtPCR testing to determine the prevalence and cycle time of BRSV (Boxus et al., 2005), *H. somni* (Moustacas et al., 2015)*, M. haemolytica, P. multocida*, and *M. bovis* (Sachse et al., 2010). *M. haemolytica* and *P. multocida* analysis was adapted from Sachse et al. (2010) using primer probes that were developed in house with the following gene targets: *M. haemolytica* (Superoxidase dismutase [sod]) and *P. multocida* (transcriptional regulator genes, PM076). Cycle times were reported up to 40, with 36 considered the positive cutoff.

Lung Pathology

Lung pathology, bacterial culture (Naikare et al., 2015), and antimicrobial sensitivity (MIC test) were conducted at the TVMDL on lung specimens from all moralities that occurred in the study. Samples were collected using a disinfected knife

following the line of demarcation in the left lung, placed in a whirl-pack sampling bag and immediately transported to TVMDL for culture and antimicrobial sensitivity determination.

2.3.4 Statistical Analysis

This study was a generalized complete block design with experimental unit replication pen within block. Blocks consisted of 5 different truckload arrival groups. Performance outcomes (BW, ADG, DMI, F:G) were analyzed using the MIXED procedure of SAS (SAS Inst., Cary, NC). The fixed effect of treatment was included in the model statement and pen*block was included as the random error term. Binomial health outcomes (morbidity, mortality) were analyzed using PROC GLIMMIX in SAS with the same model inputs. Repeated measures (blood and serum variables, rtPCR) were analyzed using PROC MIXED with repeated measures that evaluated the main effects of treatment, time, and their interaction. Compound symmetry was the covariance structure used for repeated measures analysis. Results from rtPCR were adjusted by removing each animal that had a rtPCR positive sample on d 0. Antibody titers and rtPCR cycle time values were $\log_2$- and $\log_{10}$-transformed, respectively, prior to statistical analysis, and other dependent variables were $\log_{10}$-transformed if it resulted in normal distribution as determined by the Shapiro-Wilk test (PROC UNIVARIATE in SAS). Back-transformed means are reported for cycle times and the $\log_2$ transformed data was reported for antibody titers. Pen served as the experimental unit for all statistical analyses, except lung pathology. Lung tissue cultures were analysed using PROC GLIMMIX, with animal as the experimental unit. Statistical significance was considered using an alpha-level of

0.05. If an F-test was statistically significant, mean separation was performed using the least significant differences test (pdiff in SAS) and treatment means were separated statistically using an alpha-level of 0.05 with a tendency considered for a *P*-value of 0.05 ≥ 0.10.

2.4 Results and Discussion

2.4.1 Feedlot Performance

Performance results are displayed in Tables 1 and 2. Body weight did not differ ($P \geq 0.74$) at any time point during the study. Likewise, there were no differences ($P \geq 0.82$) observed in ADG. These data indicate that MLV respiratory vaccination of high-risk calves upon arrival did not clearly affect performance during the first 70-d in the feedlot. Overall (d 0 to 70) or interim DMI did not differ ($P \geq 0.22$); however, feed efficiency (G:F) from d 0 to 56 was improved ($P = 0.05$) for CON because CON consumed less feed but gained similarly during this time. The inflammatory response following vaccination with a MLV typically elicits cytokines that promote tissue catabolism (Hughes et al., 2013) and the vaccines administered on d 0 may have contributed to the observation of reduced G:F because energy and protein are preferentially utilized by the inflammatory response in favor of growth. Arlington et al., (2013) observed similar results in heifers following vaccination; vaccinates had reduced ADG and G:F ($P < 0.01$) compared to non-vaccinated controls

2.4.2 Clinical Health Outcomes

Health outcomes are reported in Table 3. There were no differences ($P \geq 0.83$) in the overall morbidity rate (BRD1). No statistical differences ($P = 0.17$) were observed for percentage of steers deemed chronically ill; however, CON had 7.43% chronically ill, followed by INJ (5.14%) and INT (2.86%). Mortality was not statistically different ($P = 0.37$); however, mortality rate followed a similar numerical pattern to chronically ill; CON had 10.87% mortality, followed by INJ (7.55%) and INT (5.16%). Days to mortality was not statistically different ($P = 0.61$); INT treatment averaged 26.5 days to mortality, followed by INJ (22.0 d) and CON (21.9 d). There were no differences ($P = 0.99$) in total antimicrobial cost between treatments, which averaged $19.05/animal. These results indicate that respiratory vaccination of high-risk calves upon feedlot arrival had little effect on health outcomes during the receiving period, but additional research with larger sample size is needed to determine if the numerical trends observed in our study are repeatable and meaningful, or random. Our results agree with those of Martin et al. (1983), Duff et al. (2000), and Van Donkersgoed et al. (1990), where respiratory vaccination had no effect on morbidity or mortality in the feedlot setting.

2.4.3 Hematology and Serology

No treatment × day interactions or treatment effects ($P \geq 0.18$) were observed for CBC variables (data not shown). The lack of difference in CBC variables were not surprising because there were no differences in morbidity; however, MLV vaccination can alter CBC variables (Hudson et al., 2020). Hughes et al. (2017) reported an increase in white blood cells from 24 to 48 h post-vaccination followed by a decrease 72 h post-vaccination. There was no treatment × day interaction or treatment effect ($P \geq 0.55$) for

serum IFN-γ concentration (Figure 1). Most viral infections are known to stimulate a more pronounced Th1 immune response for the most effective antiviral effect. The cytokine IFN-γ stimulates natural killer (NK) cells and cytotoxic CD8+ T cells that are both critical for resolving viral infection (Aberle et al., 1999). It is known that BRSV induces immunomodulation within the host to favor a Th2 response (Gershwin et al, 1994) that may result in less IFN- γ production. However, the lack of difference in IFN- γ in the current study could have been confounded by the IBRV, BVDV, and PI3V antigens administered to INT and INJ groups. However, CON did not receive any MLV antigen and it did not differ from INT or INJ regarding serum IFN- γ.

The BRSV-specific antibody titer results are reported in Figure 2. No treatment × day interaction or treatment effect ($P \geq 0.18$) was observed for BRSV-specific antibody titer. As expected, serum antibody against BRSV increased with time ($P < 0.01$). However, this increase with time also existed for CON (d 0 relative to d 14, 28, 42, 56, and 70). The increase in BRSV-specific antibody titers from d 0 to 14 for CON cattle indicates the presence of wild-type BRSV within this population of cattle prior to d 14. It has been previously reported that IN vaccines elicit both mucosal and systemic immune responses, but primarily mucosal; whereas, parenteral vaccines elicit a more robust systemic immune response as measured by serum antibody titer (Medina and Guzman, 2001). However, the BRSV-specific serum antibody response was numerically greatest for the INT (3.11 $\log_2$) cattle than INJ (3.03 $\log_2$) and CON (2.52 $\log_2$) in the current study. Kaufman et al. (2017) also reported an increased BRSV-specific serum antibody response in cattle administered an IN vaccine compared to a parenteral.

2.4.4 Pathogen Prevalence in Nasal Swabs

No treatment × day interaction or treatment effect ($P \geq 0.70$) was observed for BRSV frequency of carriage (Figure 3). With BRSV-positive nasal swabs represented in the unvaccinated CON treatment on d 7 and d 14, it is evident that wild-type BRSV was circulating in this study population. Spatially arranged treatments reduced, but did not eliminate the possibility of vaccine-origin transfer of BRSV. However, these results coupled with increased BRSV-specific antibody titers for CON further support evidence of natural BRSV transmission. There was a tendancy for a treatment × day interaction ($P \geq 0.06$) for cycle time of BRSV positive swabs. Shedding of BRSV typically begins 3 to 4 d following infection and rarely endures beyond day 10 (Gershwin, 2007).

There was a treatment × time interaction ($P < 0.01$) for *H. somni* presence in nasal swabs; on d 14 and 28, INT (21.1 and 57.1%) had more ($P < 0.01$) nasal swab specimens become *H. somni* positive than CON (3.6 and 25.3%) or INJ (3.4 and 8.4%; Figure 4). Also, INT had reduced ($P = 0.03$) cycle time of *H. somni* positive samples on d 28 (Figure 5). Therefore, intranasally vaccinated cattle had increased frequency of carriage and colonization of *H. somni*. It is postulated that a BRSV infection can elicit a Th2 immune response and BRSV-specific IgE production (Gershwin et al., 2005). Additionally, BRSV primes the immune system for increased *H. somni*-specific IgE production following infection. A strong Th1 immune response is needed to resolve *H. somni* infection (Corbeil, 2007). The immune response followed by IN vaccination may have created an environment that allowed for greater *H. somni* colonization. Infection with *H. somni* typically endures 6 to 10 weeks in chronic infections. In addition, severe

clinical signs are only visible for 48 h, and then infection can become subclinical for weeks (Gogolewski et al., 1987). Regardless, the increase in *H. somni* prevalence for INT did not appear to impact clinical health outcomes in our study population and some health variables were numerically improved for INT.

No treatment × day interaction ($P = 0.24$) for *M. bovis* frequency of carriage existed (Figure 6). However, there was a tendency ($P = 0.06$) observed for a treatment effect; parenterally vaccinated cattle had numerically less (45.6%) *M. bovis* present in the naris than CON (53.4%) or INT (58.1%). In addition, no treatment × day interaction or treatment effect ($P \geq 0.27$) was observed for *M. bovis* cycle time value.

There was no treatment × day interaction or treatment effect ($P \geq 0.17$) for *M. haemolytica* prevalence (Figure 7). Numerically, CON were more likely to become *M. haemolytica* positive (11.1%) than INJ (7.2%) or INT (6.4%). Furthermore, *M. haemolytica* cycle time value had no treatment × day interaction or treatment effect ($P \geq 0.77$).

No treatment × day interaction or treatment effect ($P \geq 0.50$) existed for *P. multocida* frequency of carriage (Figure 8). The cycle time values for *P. multocida* positive swabs were not impacted ($P \geq 0.30$) by treatment, time, or their interaction.

Overall pathogen prevalence is represented in Figure 9. On d 0, *M. haemolytica* was present in 64.4% of the sub-sampled calves across all treatment groups. For decades, *M. haemolytica* has been considered the most predominant bacterial pathogen in relation to BRD (Griffin et al., 2010). However, *M. haemolytica* was only the most prevalent

pathogen on d 0. The remainder of sample days demonstrated *M. bovis* to be the most prevalent; it was found in 69.1%, 85.8%, and 84.3% of the sub-sampled calves on d 7, 14, and 28, respectively. It is perceived that *M. bovis* is an emerging cause of mortality in the feedlot (Gagea et al., 2006). By d 28, *H. somni* was the second most prevalent of the 4 bacteria quantified from nasal swab samples. Both *M. bovis* and *H. somni* prevalence markedly increased over time ($P < 0.01$). Following administration of metaphylaxis with tildipirosin (Zuprevo, Merck Animal Health) on d 0, *M. haemolytica* and *P. multocida* decreased in prevalence ($P < 0.01$).

2.4.5 Lung Pathology

The most frequent bacterial pathogens isolated from the lungs of respiratory mortalities were *M. haemolytica, P. multocida,* and *H. somni*, respectively. There was a treatment effect ($P < 0.01$) for the rate of *M. haemolytica* positive culture from lung tissue specimens; INT had less (0.0%) *M. haemolytica* positive lung tissue cultures than INJ (45.5%) or CON (74.0%). There was no difference ($P > 0.37$) between treatments for the frequency of *P. multocida* or *H. somni* isolation. Furthermore, *M. haemolytica* had more ($P < 0.01$) isolates resistant to the antimicrobials used for metaphylaxis and treatment of BRD during this trial (tildipirosin, florfenicol, and enrofloxacin) than *H. somni* (Table 4). However, the breakpoints used for ceftiofur resistance are currently under consideration by the Clinical and Laboratory Standards Institute (Luthje and Schwarz, 2006). For many decades, *M. haemolytica* has been considered the most predominant bacterial pathogen in relation to BRD (Griffin et al., 2010). *M. haemolytica* is also commonly reported to be the most antimicrobial resistant respiratory bacteria

(DeDonder and Apley, 2015). This could explain the numerical improvement in mortality rate observed for INT because increased *H. somni* colonization in lieu of *M. haemolytica* may have resulted in predominance of a more antimicrobial susceptible causative agent. We speculate that in certain populations of cattle, INT vaccination might alter the respiratory microbiome, such that it impacts clinical health outcomes.

2.5 Conclusions

These data indicate MLV vaccination of high-risk calves at arrival, either parenterally or intranasally, did not clearly impact health or growth during the feedlot receiving period. However, INT increased the prevalence of *H. somni* in the naris, providing evidence that IN but not parenteral MLV vaccination alters the microbial community in the upper respiratory tract of cattle. Further research is needed to better understand how IN MLV vaccination might impact the respiratory microbiota and the clinical significance of such impact.

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Table 1. Effect of respiratory vaccination and route of administration on performance of high-risk, newly received beef calves

	Treatments[1]				
Item	**CON**	**INT**	**INJ**	**SEM**	***P*-value**
BW, kg					
Initial[2]	212.8	213.0	213.0	2.07	0.99
d 14	223.9	224.4	221.6	3.89	0.74
d 28	239.3	240.1	241.0	2.74	0.91
d 42	264.8	265.1	263.4	3.24	0.92
d 56	291.4	292.1	288.5	4.02	0.79
Final[3]	311.4	312.9	310.4	4.52	0.93
ADG, kg/d					
Initial to d 14	0.57	0.62	0.41	0.21	0.75
Initial to d 28	0.96	0.98	0.99	0.07	0.94
Initial to d 42	1.24	1.24	1.19	0.06	0.82
Initial to d 56	1.41	1.41	1.34	0.05	0.58
Initial to Final	1.41	1.42	1.39	0.05	0.86

[1] CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0.

[2] Initial= average of BW on d -1 and 0.

[3] Final= average of BW on d 69 and 70.

Table 2. Effect of respiratory vaccination and route of administration on dry-matter intake and feed efficiency of high-risk, newly received beef calves

	Treatments[1]				*P*-value
Item	**CON**	**INT**	**INJ**	**SEM**	**TRT**
DMI, kg/d					
Initial[2] to d 14	3.38	3.62	3.37	0.13	0.33
Initial to d 28	4.32	4.62	4.42	0.12	0.22
Initial to d 42	4.98	5.26	5.11	0.13	0.34
Initial to d 56	5.56	5.84	5.67	0.15	0.48
Initial to Final[3]	6.06	6.29	6.13	0.16	0.59
G:F, kg					
Initial to d 14	0.133	0.159	0.097	0.055	0.72
Initial to d 28	0.217	0.211	0.221	0.011	0.83
Initial to d 42	0.246	0.235	0.230	0.007	0.29
Initial to d 56	0.253[a]	0.241[ab]	0.235[b]	0.005	0.05
Initial to Final	0.231	0.226	0.225	0.003	0.37

[a,b] Within a row, means without a common superscript differ, $P \leq 0.05$.

[1] CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0.

[2] Initial= average of BW on d -1 and 0.

[3] Final= average of BW on d 69 and 70.

Table 3. Effect of respiratory vaccination and route of administration on health outcomes of high-risk, newly received beef calves

	Treatments[1]				
Item	**CON**	**INT**	**INJ**	**SEM**	***P*-value**
BRD1[2], %	54.60	58.86	58.41		0.83
BRD2[3], %	30.96	32.27	30.85		0.98
BRD3[4], %	24.69	17.01	21.89		0.47
Chronic[5], %	7.43	2.86	5.14		0.17
Respiratory Mortality, %	10.87	5.16	7.55		0.37
Days to					
BRD1	14.83	14.46	13.80	1.54	0.89
BRD2	18.39	17.79	17.21	1.40	0.83
BRD3	22.57	21.79	20.93	1.72	0.77
Mortality	21.88	26.47	22.00	4.46	0.61
Antimicrobial Costs[5], $/hd					
Nuflor	10.61	11.24	11.14	1.45	0.90
Baytril	4.50	4.53	4.40	1.00	0.99
Excede	3.79	2.70	3.45	0.69	0.53
Total Antimicrobial Treatment Costs[5], $/hd	18.96	19.08	19.12	2.28	1.00

[1] CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0.

[2] Percentage of cattle treated for bovine respiratory disease (BRD) at least once.

[3] Percentage of cattle treated for BRD at least twice.

[4] Percentage of cattle treated for BRD 3 times.

[5] Percentage of cattle removed from study due to chronic respiratory illness.

[5] Antimicrobial cost assumes the following: $0.68/ml for florfenicol (Nuflor, Merck Animal Health, Madison, NJ; first treatment), $0.58/ml for enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, KS; second treatment), $2.27/ml for ceftiofur crystalline free acid (Excede, Zoetis; third treatment).

Table 4. Antimicrobial resistance of bacteria cultured from lung specimens in calves dying of bovine respiratory disease[1]

	Isolate			
Item	***M. haemolytica***	***H. somni***	***P. multocida***	***P*-value**
Resistant outcome, %				
Tildipirosin[2]	94.7[a]	45.0[b]	80.0[ab]	0.03
Florfenicol[3]	78.1[a]	0.0[b]	77.0[a]	0.01
Enrofloxacin[4]	78.1[a]	0.0[c]	48.7[b]	<0.01
Ceftiofur crystalline free acid[5]	5.0	0.0	0.0	0.83

[a,b] Within a row, means without a common superscript differ, $P < 0.05$.

[1] Percent resistant was determined using a minimum inhibitory concentration (MIC) test conducted at TVMDL, Canyon, TX.

[2] (Zuprevo, Merck Animal Health, Madison, NJ; metaphylaxis).

[3] (Nuflor, Merck Animal Health; first BRD treatment).

[4] (Baytril, Bayer Animal Health, Shawnee Mission, KS; second BRD treatment).

[5] (Excede, Zoetis, Kalamazoo, MI; third BRD treatment).

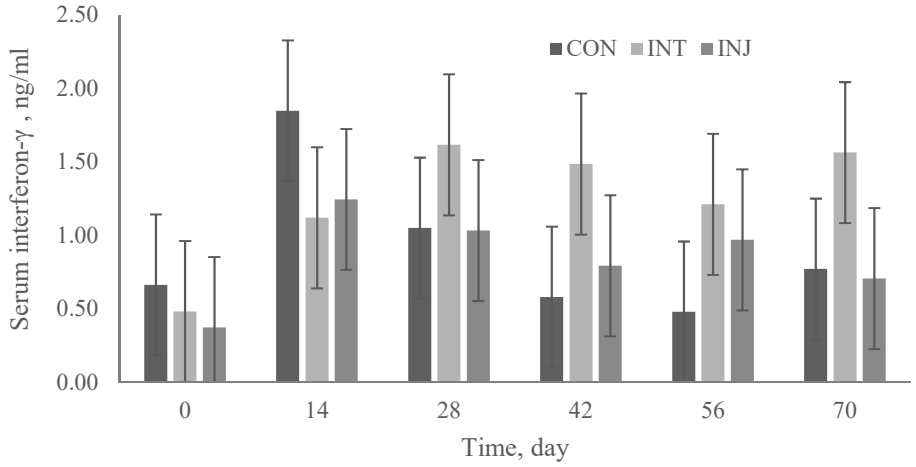

Figure 1. Effect of respiratory vaccination and route of administration on serum interferon-γ (ng/ml) in high-risk, newly received beef calves. Serum concentration of IFN-γ was determined via ELISA. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, P = 0.55; day, P = 0.14; treatment × day, P = 0.75.

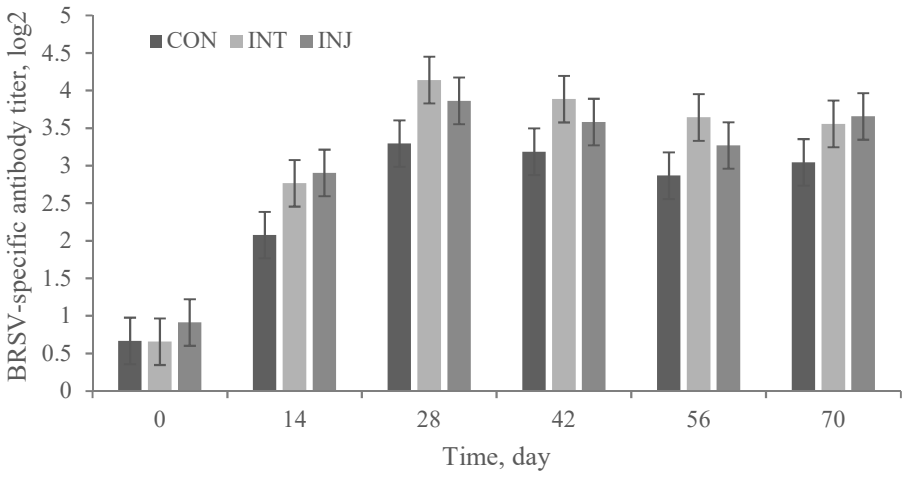

Figure 2. Effect of respiratory vaccination and route of administration on bovine respiratory syncytial virus-specific antibody titer of high-risk, newly received beef calves. Titer results were $\log_2$ transformed prior to analysis. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, $P = 0.18$; day, $P < 0.01$; treatment × day, $P = 0.59$.

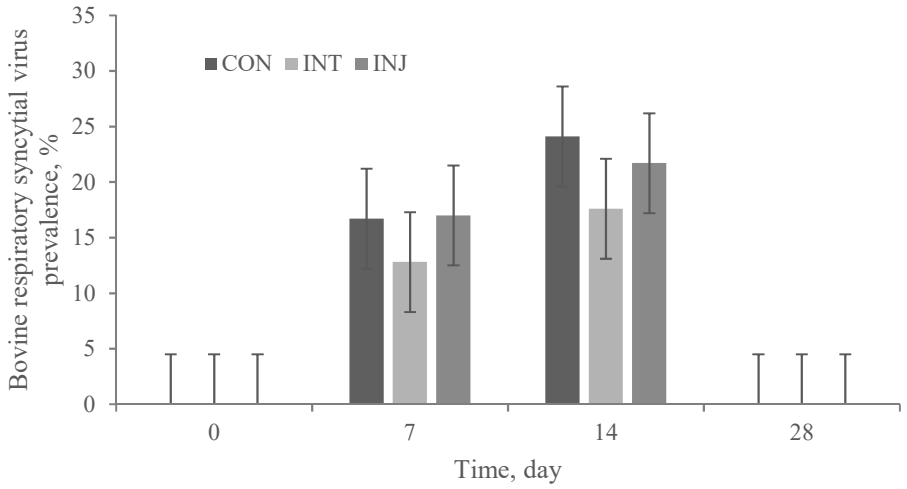

Figure 3. Effect of respiratory vaccination and route of administration on adjusted bovine respiratory syncytial virus prevalence via rtPCR in high-risk, newly received beef calves. Cattle that arrived rtPCR positive were excluded from analysis. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, $P = 0.70$; day, $P < 0.01$; treatment × day, $P = 0.93$.

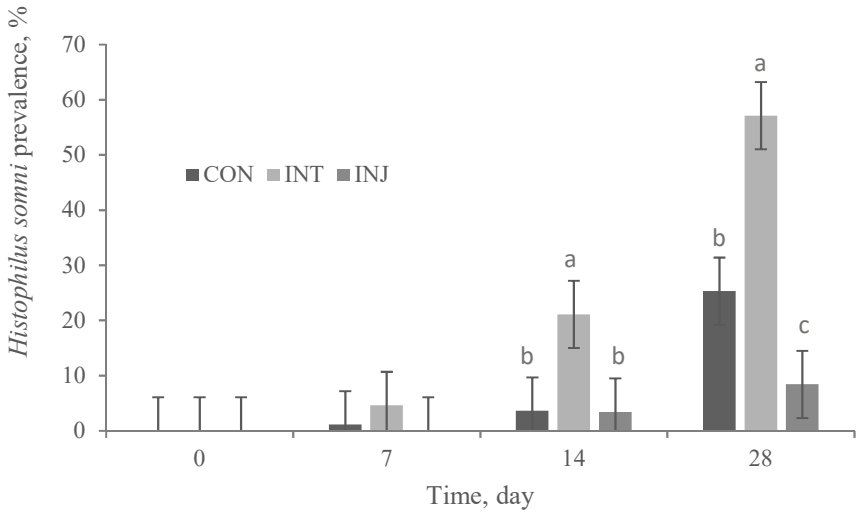

Figure 4. Effect of respiratory vaccination and route of administration on adjusted *H. somni* prevalence via rtPCR in high-risk, newly received beef calves. Cattle that arrived rtPCR positive were excluded from analysis. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, $P = 0.01$; day, $P < 0.01$; treatment × day, $P < 0.01$.

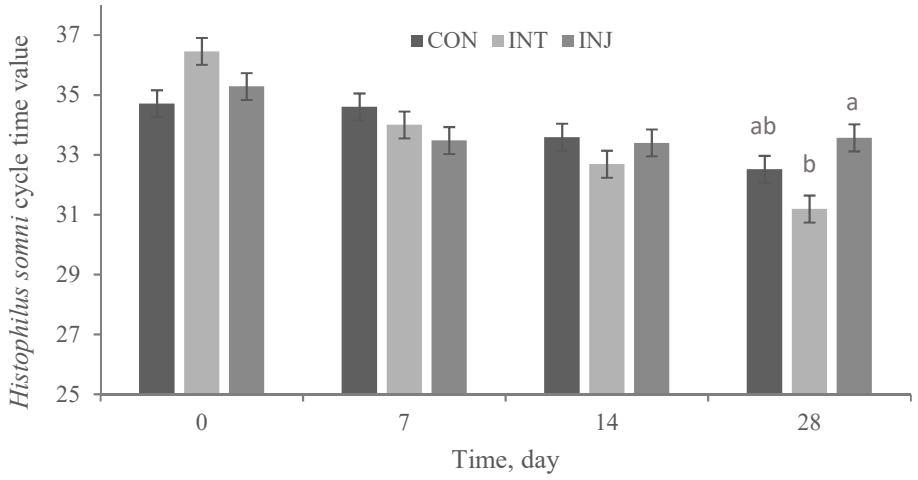

Figure 5. Effect of respiratory vaccination and route of administration on *H. somni* rtPCR cycle time values in high-risk, newly received beef calves. Cycle time values were $\log_{10}$ transformed and only analyzed for rtPCR positive and intermediate samples; back transformed means are shown. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, $P = 0.83$; day, $P < 0.01$; treatment × day, $P = 0.03$.

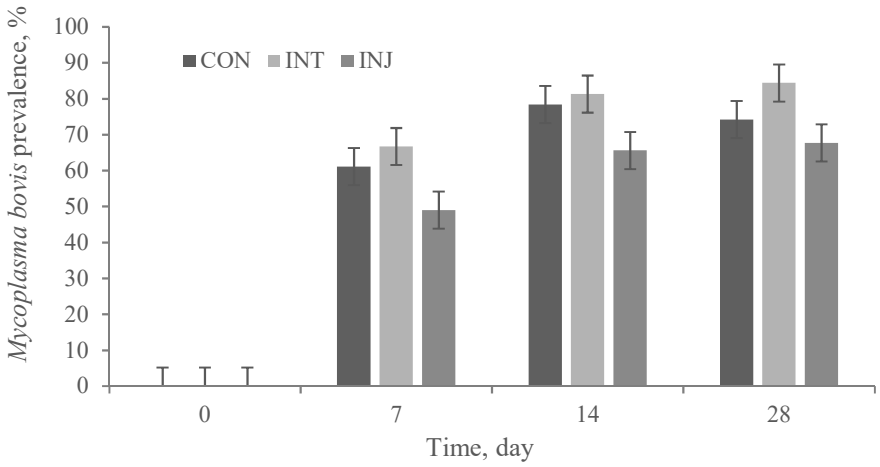

Figure 6. Effect of respiratory vaccination and route of administration on adjusted *M. bovis* prevalence via rtPCR in high-risk, newly received beef calves. Cattle that arrived rtPCR positive were excluded from analysis. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, $P = 0.06$; day, $P < 0.01$; treatment × day, $P = 0.24$.

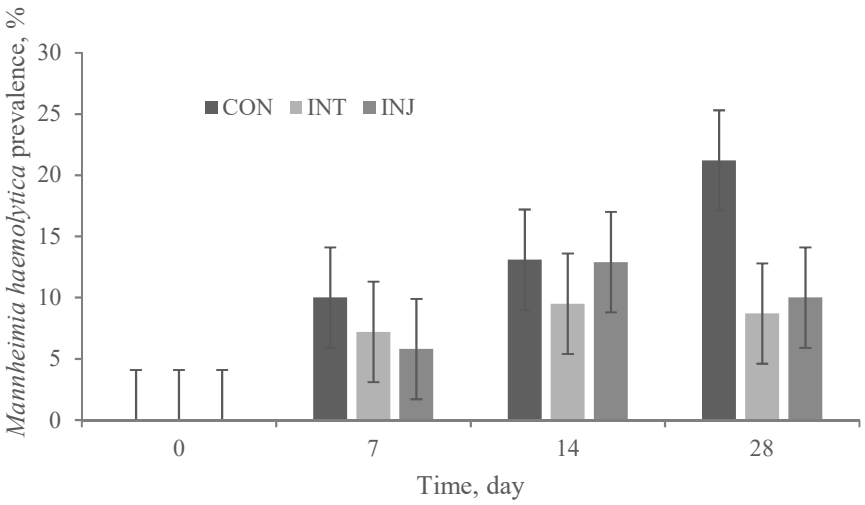

Figure 7. Effect of respiratory vaccination and route of administration on adjusted *M. haemolytica* prevalence via rtPCR in high-risk, newly received beef calves. Cattle that arrived rtPCR positive were excluded from analysis. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, $P = 0.58$; day, $P < 0.01$; treatment × day, $P = 0.17$.

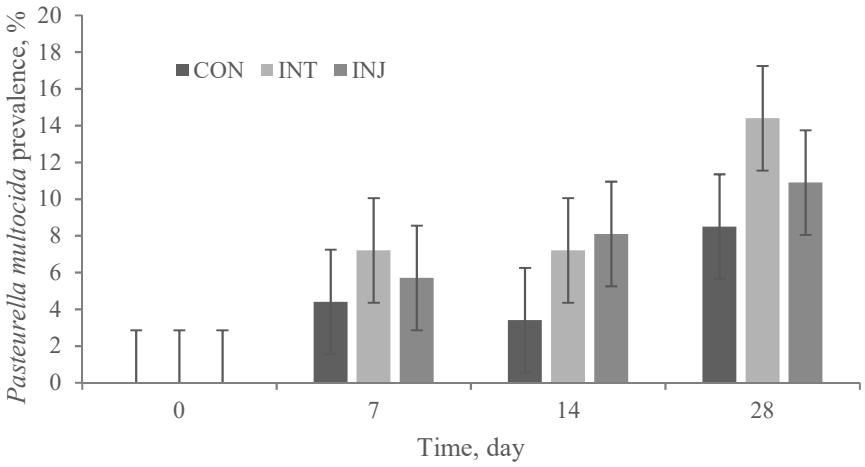

Figure 8. Effect of respiratory vaccination and route of administration on adjusted *P. multocida* prevalence via rtPCR in high-risk, newly received beef calves. Cattle that arrived rtPCR positive were excluded from analysis. CON= Negative control, no respiratory virus vaccination; INT= Cattle intranasally administered (1mL/nostril) a trivalent, modified-live virus respiratory vaccine (Inforce 3, Zoetis, Kalamazoo, MI) with parenteral BVDV type I and II vaccine (Bovi-Shield BVD, Zoetis) on day 0; INJ= Cattle administered a pentavalent, modified-live virus respiratory vaccine (Bovi-Shield GOLD 5, Zoetis) on day 0. Effect of treatment, $P = 0.50$; day, $P < 0.01$; treatment × day, $P = 0.87$.

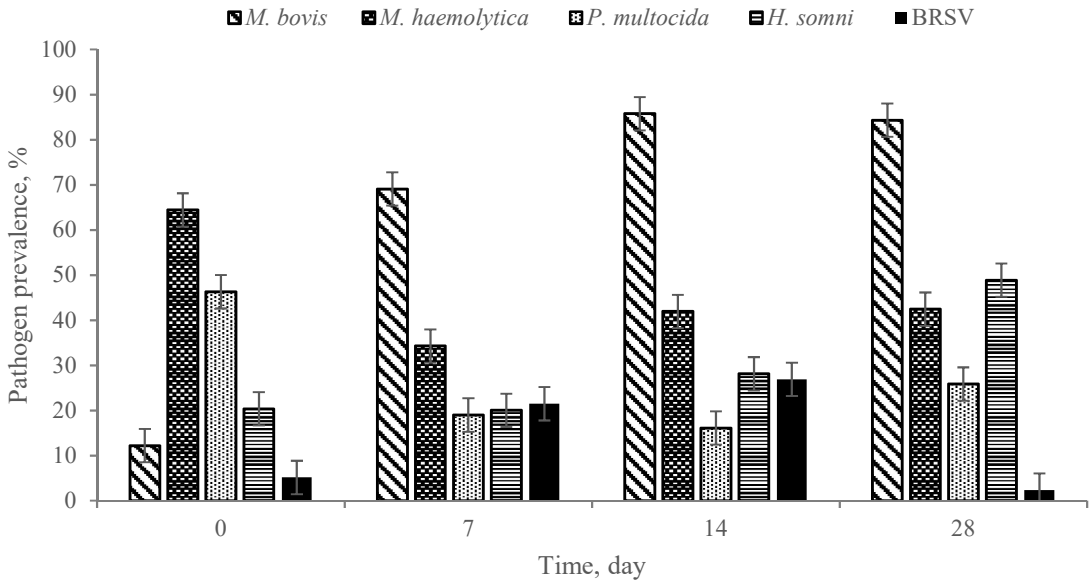

Figure 9. Overall pathogen prevalence in the naris of high-risk, newly received beef calves. All rtPCR results were included in analysis. Total population means are reported, and no statical analysis was preformed.