Field comparison of intranasal and injectable bovine respiratory disease vaccination on beef calf antibody concentrations, average daily gain, and bovine respiratory disease morbidity

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Abstract

Bovine respiratory disease (BRD) is a multifactorial disease complex that is common in feedlot operations, where it causes major economic loss through: reduction in average daily gain, treatment costs, and mortality. Reducing BRD incidence would increase profits to producers, reduce antimicrobial use, and improve animal welfare. Two studies were performed to compare clinical vaccine response. First, a randomized control study enrolled 75 crossbred heifer calves into an injectable modified live viral (IJ-MLV) group, intranasal homologous boost (IN-MLV) group, or intranasal heterologous boost (IN-KV) group. Vaccines were administered at birth, ‘‘turnout’’ (~2 months of age), and weaning. Blood samples and weights were collected at ‘turnout’, two weeks post ‘turnout’, weaning, and two weeks post weaning, with weights also being collected at birth and 87 days post weaning. Blood samples were analyzed with an ELISA for bovine respiratory syncytial virus (BRSV) and bovine herpes virus type 1 and virus neutralization for bovine viral diarrhea virus types 1 and 2. No differences were observed between the average daily gains of the three groups. The IN-KV group had significantly higher BRSV antibody concentrations than the other groups at all time points except for ‘turnout’ but had lower bovine viral diarrhea virus type 2 concentrations at weaning and two weeks post weaning.

Next, a field study was conducted at two commercial ranches in central Saskatchewan, enrolling 645 calves from one farm and 481 calves from a second farm. The calves were randomly enrolled by vaccine type at branding into either an IJ-MLV group or IN-MLV group. Calves were managed extensively, until weaning when they were moved to a local feedlot. At the feedlot calves were vaccinated and separated into steer and heifer pens and were monitored daily for disease. Weights were collected upon arrival and at 60 days post weaning. Morbidity and

mortality due to bovine respiratory disease and average daily gains were analyzed. The results show no significant difference between the two groups for these outcomes. These two studies show the importance of considering vaccine type and administration route when developing BRD control programs.

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ADG	Average daily gain
BoHV1	Bovine herpes virus type 1
BRD	Bovine respiratory disease
BRSV	Bovine respiratory syncytial virus
BVDV	Bovine viral diarrhea virus
BVDV1	Bovine viral diarrhea virus type 1
BVDV2	Bovine viral diarrhea virus type 2
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immunosorbent assay
FAS	First apoptosis signals
FC	Fragment crystallizable
HIV	Human immunodeficiency virus
ID	Identification
IFN-y	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IJ	Injectable
IJ-MLV	Injectable modified live
IN	Intranasal
IN-KV	Intranasal killed viral
IN-MLV	Intranasal modified live viral
KV	Killed viral

MatAb	Maternal antibody
MatAbs	Maternal antibodies
MLV	Modified live viral
OPD	o-Phenylenediamine dihydrochloride
PCR	Polymerase chain reaction
PI	Persistently infected
PI3	Parainfluenza
RFID	Radio frequency identification

Chapter One

Literature Review

1.1 Introduction

Bovine respiratory disease (BRD) is an important disease complex to the beef cattle industry, impacting cow-calf operations, stockers, and feedlots. Newly weaned calves are highly susceptible to respiratory disease because of the stress of weaning and the mixing that occurs in auction markets and feedlots. In feedlot cattle, morbidity rates averaged 16% in 2011 (Aphis.usda.gov, 2013, a), and mortalities ranged from 1.00 – 1.75% (Miles et al., 2009).

Metaphylactic antibiotics are commonly used in feedlots at calf arrival to help reduce disease in weaned calves that are at high risk of BRD. Increasing public awareness related to antimicrobial use in livestock agriculture has led to Canadian national activities to understand current antimicrobial use in the Canadian Beef Industry, with the goals of promoting antimicrobial stewardship and best practices, while still supporting animal welfare. (Cusack, 2004) (Prescott et al., 2012) (Cameron and McAllister, 2016). Therefore, it is important for feedlot operations to adapt and create new methods of management to help reduce disease pressure on cattle or improve the immune system of the cattle to minimize antimicrobial usage. This chapter will focus on understanding the importance of BRD to the Canadian beef industry and the viruses associated with it, outlining the key aspects of the bovine immune system, and describing vaccination as a control for BRD.

1.2 Respiratory Disease in Pre and Post Weaned Calves

Bovine respiratory disease is a multifactorial disease that affects cattle of all ages but is most prevalent in calves post weaning. Various respiratory pathogens, both viral and bacterial, are

associated with the development of BRD (Murray et al., 2017) (Jones and Chowdhury, 2010). However, high BRD prevalence in recently weaned calves is also associated with risk factors including weaning stress, comingling of source groups, crowding in pens, transport conditions, and weather. While risk factors do not directly cause respiratory disease, they contribute to immune system suppression, or increase contact between animals, therefore, the chance of exposure to disease (Belasco et al., 2015) (Taylor et al., 2010). Bovine respiratory disease is important to the beef industry because it causes production losses through morbidity, mortality and reductions in average daily gain (ADG) (Brooks et al., 2011). This section will discuss the economics associated with BRD, the important pathogens and risk factors associated with BRD.

1.2.1 Economic Loss Due to BRD

Bovine respiratory disease is a disease complex with the largest economic impact on the cattle feeding industry. In the United States, it was found that BRD morbidity averaged 16% across various feedlots (Aphis.usda.gov, 2013, a). Economic losses related to BRD occur through treatment costs, which have been seen as high as $23.60USD/calf, and decreased carcass weight averaging $100/calf (Aphis.usda.gov, 2013, a) (Smith, 2009). Mortality is also an important cause of economic loss. Mortality rates for calves range depending on cattle population type and can vary from 1.0%, among older heavier classes of cattle to 1.75% in younger lighter classes of cattle (Miles et al., 2009). Overall BRD increases the cost of calf production and reduces the value of the calves upon sale (Cernicchiaro et al., 2013).

1.2.2 Pathogens Associated with BRD

Multiple viral and bacterial pathogens are associated with BRD. Viral pathogens associated with BRD include bovine respiratory syncytial virus (BRSV), bovine herpes virus type 1 (BoHV1), and parainfluenza 3 virus (Miles, 2009). Bovine viral diarrhea virus (BVDV) is a major virus

associated with BRD, but does not cause respiratory disease (Ridpath, 2010). Viruses are usually the initial infector causing a weakened immune system in the animal making infection by bacteria more likely (Panciera and Confer, 2010). The major bacterial pathogens associated with BRD are *Manheimia haemolytica, Mycoplasma bovis, Histophilus somni,* and *Pasturella multocida* (Booker et al., 2008).

1.2.3 Bovine Respiratory Syncytial Virus (BRSV)

Bovine respiratory syncytial virus is one of the most important viruses associated with BRD. This virus suppresses the immune system increasing the opportunity for secondary bacterial infections to occur (Brodersen. 2010). The immune system of the calf is triggered by the presence of G and F proteins, major surface proteins of BRSV, detected by Toll-like receptor-4 (Gershwin. 2007) (Gershwin. 2012). This recognition results in the production of inflammatory cytokines, while dendritic cells work to initiate an immune response by T-cells. However, BRSV has been shown to infect dendritic cells, disrupting their function, and thereby weakening the immune response (Gershwin. 2012). The weakened immune response results from decreased interferon gamma (IFN-y) release by dendritic cells, which allows the BRSV virus to evade clearance by cytotoxic immune cells (Bueno et al., 2008). The evasion of clearance by the immune system allows BRSV to multiply and spread.

Bovine respiratory syncytial virus causes clinical signs such as fever, coughing, and nasal discharge which begin approximately four days after infection, and last for about ten days if no secondary infection occurs (Woolums, 1999). Bovine respiratory syncytial virus is spread between calves through nasal secretions and aerosolized droplets (Woolums, 1999).

1.2.4 Bovine Herpes Virus type 1 (BoHV1)

Bovine herpes virus type 1 is a major virus implicated in the development of BRD, and a primary pathogen that suppresses the immune system. Immune suppression occurs by disrupting the function of major histocompatibility complex 1 transporters, causing a decrease in cluster of differentiation 8+ T cell response. The cluster differentiation 4+ T cells also become infected by BoHV1, resulting in cell lysis (Jones and Chowdhury, 2010). In response to BoHV1 infection, the immune system increases the production of cortisol, interferon-y, and white blood cells (Falkenburg et al., 2013) (Babiuk et al., 1996) (Myulkens et al., 2007).

The most common clinical signs involved with BoHV1 infection include fever, coughing, anorexia, nasal discharge, and dyspnea in the larynx, these appear approximately four to five days post infection (Myulkens et al. 2007). Recovery from BoHV1 begins approximately five days after onset of clinical signs. The illness can become worse due to spread of BoHV1, or secondary bacterial infection (Nataraj et al., 1997). Bovine herpes virus type 1 is spread through nasal secretions and aerosolized droplets (Jones and Chowdhury, 2010).

1.2.5 Bovine Viral Diarrhea Virus Types 1 and 2 (BVDV1, BVDV2)

Bovine viral diarrhea virus types 1 and 2 are important factors in the development of BRD, but these viruses are not always viewed as a direct cause respiratory disease. However, BVDV 1 and 2 cause immunosuppression, resulting in calves to be at higher risk for developing BRD. Mechanisms of immunosuppression from BVDV have been established, linked to both innate and acquired immunity. These include downregulation of major histocompatibility complex II and interleukin 2, causing suppression of T-helper cell responses, and decreasing the production of interferons (Van Wyk, 2016) (Ridpath, 2010).

Feedlot calves are commonly exposed to BVD 1 and 2 through exposure to persistently infected (PI) calves. Calves become PI when the dam is infected with BVDV between 45 and 120 days with BVDV, and the virus spreads to the fetus. At this time, the fetus' immune system is just starting to develop and will recognize not recognize BVDV as a pathogen; therefore, it will not create antibodies against it (Van Wyk, 2016). Persistently infected calves act as vectors and spread the virus without showing clinical signs. Bovine viral diarrhea virus is spread by oral or mucosal contact with secretions from other calves containing BVDV (Falkenburg et al., 2018) (Ridpath, 2010). Studies have shown the presence of PI calves in feedlot pens cause an increase in BRD morbidity in their home pen and adjacent pens (Richeson et al., 2012). It has also been found that pens with PI calves had a lower average daily gain from days 14-28, and a higher incidence rate of BRD (Groom et al., 2014).

1.2.6 Parainfluenza 3 Virus (PI3)

Parainfluenza 3 is a direct causative agent of respiratory disease, as it is restricted specifically to the airway (Eberle et al. 2015) (Ogunbiyi et al. 1988). Often the calves that are most at risk include naïve calves that have not received enough colostrum, or received poor quality colostrum (Marshall and Frank, 1975). Clinical signs associated with PI3 infections are mild; however, the virus causes immunosuppression that may lead to a secondary bacterial infection. Infected calves become immunosuppressed through the inhibition of interferon I and interferon III by PI3 resulting in decreased signaling ability of the immune system (Eberle et al., 2015). Viral infection by PI3 also decreases the strength of the innate immune systems response by replicating in macrophages and decreasing their ability to affect foreign invaders (Ellis, 2010, a). Clinical signs associated with PI3 include pyrexia, coughing, nasal discharge, and shallow

breaths (Ellis. 2010, a). Parainfluenza 3 is transmitted through aerosolized particles containing virus (Ellis, 2010, a).

1.2.7 Mannheimia Haemolytica

Mannheimia haemolytica is a bacterial pathogen known to cause a severe bronchopneumonia in calves. However, *M. haemolytica* is part of the normal flora of the upper respiratory tract. The commensal strain of *M. haemolytica* is A2 and is predominantly found in healthy calves. When the calf's immune system is compromised by stress or a primary viral pathogen, the infectious A1 strain becomes dominant (Griffin et al., 2010). *Mannheimia haemolytica* evades detection by the immune system through formation of a biofilm with extracellular polymeric substance (Boukahil and Czuprynski, 2016). The main virulence factor of *M. haemolytica* is leukotoxin. Leukotoxins interact with the B_{2+} integrins of leukocytes, causing apoptosis by upregulating calcium intake, inhibiting the functionality of phagocytosis, inflammation, antigen presentation, and cytotoxicity (Griffin et al., 2010). *Mannheimia haemolytica* has a lipopolysaccharide complex, which acts as an endotoxin that causes recruitment of cytokines into the lung tissue, as well as apoptosis and necrosis (Craddick et al., 2012). Infection due to *M. haemolytica* can cause coagulative necrosis of the lung tissue leading to bronchopneumonia (Griffin et al., 2010).

1.2.8 Other Associated Pathogens

Pasturella multocida is a bacterial pathogen that causes secondary infection in immunosuppressed calves. *Pasturella multocida* is a commensal bacterium of the upper respiratory tract, but when the calf's immune system is compromised, it can migrate to the lower respiratory tract and act as a pathogen (Griffin et al., 2010). Cranioventral bronchopneumonia is typically associated with *P. multocida.* This bacterial pathogen is excreted in nasal secretions and colonizes the respiratory tract after inhalation of the bacteria (Dabo et al., 2007).

Histophilus somni is another commensal bacterium that regularly colonizes the nasopharyngeal region of the upper respiratory tract. This bacterium is mainly involved in secondary infection with BRD. *Histophilus somni* has been associated with several disease manifestations such as fibrinopurulent bronchopneumonia, abscessing laryngitis, septicemic-related cardiovascular necrosis, fibrinous pericarditis and meningoencephalitis (Griffin et al., 2010). This bacterial pathogen is spread through nasal secretions and colonizes the respiratory tract after inhalation of the bacteria.

Mycoplasma bovis is isolated from the upper respiratory tract of healthy calves and is also found on mucosal surfaces of the urinary tract, genital tract, and in milk. This bacterial species has been found to act mainly as a secondary infection, but some evidence suggests it can be a primary pathogen as well (Schibrowski et al., 2014). When the host is immunosuppressed *M. bovis* has the opportunity to become pathogenic, often causing chronic disease. A major symptom of *M bovis* infection is a caseonecrotic bronchopneumonia, with nodules of caseous necrosis within them (Schibrowski et al., 2014). This pathogen is spread mainly through animal to animal contact but can also be obtained through the environment (Griffin et al., 2010).

1.2.9 Stress as a Factor in BRD development

Psychological and physical stress, such as weaning, comingling with new calves, and animal handling can suppress the immune system of calves. These stresses promote glucocorticoid production by the adrenal gland. Glucocorticoids affect the innate immune system by suppressing white blood cell recruitment and decreasing white blood cells ability to infiltrate the infected area (Schleimer, 2004). Glucocorticoids also act to suppress the acquired immune system by decreasing T-helper cell proliferation, resulting in reduced amounts of cytotoxic T-cells, and decreased B-cell maturation (Pruett, 2003) (Schleimer, 2004) (Priyadarshini and Aich,

2012). Prolonged stress causes a constant influx of glucocorticoids, resulting in prolonged suppression of the immune system (Carroll and Forsberg, 2007).

One of the most important causes of stress in calves is weaning. Weaning is the time period when the calf is separated from its dam, and in the Canadian Beef Industry this is usually followed by transport to a feedlot or backgrounding lot. Transport is also a major stress due to close animal contact within trucks, the potential for extended transport time, and the possibility of exposure to temperature extremes in transit (Bach, 2004) (Sconberg et al., 1993). After arriving at the feedlot, the calves may encounter many more stresses such as: feed changes, extreme weather fluctuations, and adverse pen conditions at times (Arthington et al., 2008) (Smith et al. 2001) (Belasco et al., 2015). This stressful period will increase the level of glucocorticoids released by the calf (possibly over a prolonged period of time), which may result in a weakened immune system. As part of arrival processing procedures, the calf may then be vaccinated (Arthington et al., 2008). Due to the stresses of weaning and transport, the calf's immune system may not be able to respond appropriately to the vaccine(s), resulting in sickness.

1.3 Bovine Immune System

1.3.1 Innate Immune System

The innate immune system is a first response defense against pathogens. It is comprised of different types of defenses that respond to disease challenge rapidly. The innate immune system is comprised of physical barriers, immune cells and chemicals in the blood (Galley and Webster, 1996) (Murphy, 2012). An example of a physical barrier which aids in the defense from disease is the skin. Skin is non-permeable, and regularly sheds epithelium while simultaneously removing bacteria through a process called desquamation. The epidermal surface of the skin is inhospitable to pathogen growth as it lacks the moisture required for colony establishment

(Eyerich et al., 2018). The upper respiratory tract and gastrointestinal systems act as another physical barrier to pathogen invasion and colonization (Murphy, 2012). These systems have a mucociliary lining which captures pathogens and physically removes them. The natural flora of these systems also acts to prevent colonization of foreign bacteria through competitive growth or secretion of toxic substances that inhibit the growth of pathogens (Cha et al., 2010) (Eyerich et al., 2018).

The innate immune system also functions through the activation of the cellular immune response. For example, the complement system is a group of small proteins that aid the cellular immune system by binding to pathogens and marking them for attachment by the immune cells (Ghebrehiwet, 2016). Complement activates the recruitment of inflammatory cells, but also increases the permeability of a pathogen's membrane, resulting in lysis and cell death (Gurao et al., 2017).

The activation of the inflammatory cascade is an important component of the innate immune system as this results in not only a nonspecific cellular response, but also a chemical response (Galley and Webster, 1996) (Tizard, 2013). An example of the inflammatory cascade's function is release of chemicals such as histamine, bradykinin, prostaglandins, etc. which cause vasodilation and subsequently increase blood flow to the area of infection. This increase in local blood supply aids the recruitment of immune cells such as neutrophils (Luster et al., 2005). The inflammatory system is an extensive system which includes many chemical cascades and cellular responses that are a part of the innate immune response. The inflammatory system works synergistically with the nonspecific cellular response of innate immunity. Leukocytes are an important part of the cellular response, and include natural killer cells, eosinophils, basophils and neutrophils. Leukocytes function freely of other parts of the immune system, and circulate

extensively through the body removing cellular debris, foreign particles and invading microorganisms (Bromfield et al., 2011). The innate immune response is an important first barrier to foreign invasion and functions via physical barriers, immune cells, and chemicals in the blood stream to remove or target foreign cells (Tizard, 2013) (Murphy, 2012).

1.3.2 Acquired Immune System

The acquired immune system is the part of the immune system that reacts to specific pathogens that have invaded the body. It clears these pathogens through lysing of the pathogen, neutralization of toxins produced by the pathogen, or lysing the infected host cell (Tizard, 2013). Pathogens and toxins can present specific antigens on their exterior surface that can be recognized by the immune system. Antigen recognition stimulates recruitment of B lymphocytes to an area (Nguyen et al., 2017), where the B lymphocytes mature and multiply into plasma cells. Plasma cells secrete antibodies specific to the recognized antigen. The antibodies work to neutralize the pathogen or toxin through attachment, complement promotion, anti-toxin production, and macrophage recruitment (Ansel et al., 2002) (Murphy, 2012).

The immune system also responds to antigens through recruitment of T lymphocytes. T lymphocytes bind directly to the antigen on pathogens and then multiply and specialize into either cytotoxic T cells or T helper cells. Cytotoxic T cells produce cytotoxins that cause lysis of the pathogen or infected cell. Cytotoxic T cells also act by binding directly to the pathogen (Bedel et al., 2013), releasing first apoptosis signal (FAS) ligands, which lead to apoptosis of both cells. T lymphocytes can also specialize into T helper cells. T helper cells activate cytotoxic T cells, stimulate macrophage recruitment, and stimulate antibody production by B lymphocytes (Tizard, 2013) (Murphy, 2012) (Shedlock, Shen, 2003).

1.3.3 Vaccines and how they affect the immune system

The innate immune system is the first line of defense against pathogens. If this should fail, the acquired immune system is the second defense system. However, the acquired immune system requires time to mount a response, leaving the animal vulnerable for a period of time (Galley and Webster, 1996). If the acquired immune system has encountered an antigen previously, it is able to respond rapidly the next time it is exposed to the same antigen (Ramshaw and Ramsey, 2000) (Lu, 2009) (Kardani et al., 2016). This natural mechanism of priming the immune system for response is the basis for vaccination. Vaccination is the priming of the immune system through exposure to specific pathogen antigens, allowing a more rapid response if natural exposure to the pathogen occurs at a later time point.

Two of the most common types of vaccines used are modified live viral (MLV) vaccines and inactivated vaccines, which are often referred to as killed viral (KV) vaccines. Modified live viral vaccines are produced by growing genetically modified viruses (Arevalo et al., 2015), or growing the pathogen under conditions which reduce the pathogenicity of the pathogen. A major advantage of modified live vaccines is that the virus multiplies in the host, thus requiring a lower initial dose of virus in the vaccine. Killed viral vaccines are produced by growing a pathogen, then neutralizing it through protein cross linking treatments, protein denaturation treatments, or heat treatments (Delrue et al., 2012). These methods of pathogen production decrease the virulence of the pathogen, so it does not cause disease or multiply with in the host. These methods of producing MLV and KV vaccines maintains the viruses' exterior antigens, allowing the B lymphocytes to produce antibodies specific to the pathogen upon vaccination (Ansel et al., 2002) (Murphy, 2012).

1.3.4 Maternal Antibody Interference

An important challenge to vaccinating neonatal calves is interference by maternal antibodies (MatAb). Maternal antibodies are obtained right after birth through the consumption of colostrum (Kehoe et al., 2011). Colostrum is the first milk produced by the dam, and consists of vitamins, minerals, fat, leukocytes and antibodies from the dam's immune system (Lago et al., 2018). During the first 6-12 hours of birth the gastrointestinal tract of the calf is permeable to large proteins, which allows the absorption of full antibodies from the colostrum. These maternal antibodies are absorbed into the blood stream and provide the calf with immune protection (Heinrichs and Elizondo-Salazar, 2009). Absorption of antibodies from colostrum provides the calf with immunity for the first three to four months of life. Maternal antibodies are highly concentrated in colostrum and the antibody half-life is about four months of age (Munoz-Zanzi et al., 2002). Previous research has shown that injectable vaccination prior to four to five weeks of age does not result in a systemic immune response (Platt et al., 2009). High concentrations of maternal antibodies interfere with vaccine effectiveness. The mechanism for this interference isn't definitively known, however, some theories exist. One theory, called epitope masking, suggests that maternal antibodies bind to the vaccine antigen, preventing it from binding to B lymphocytes (Niewiesk, 2014). The second theory suggests that the maternal antibodies bind to the B cell receptors and to the fragment crystallizable (Fc) receptors. The attachment of maternal antibodies to Fc receptors inhibits the B lymphocytes' ability to bind with vaccine antigens. Both theories result in the inability of B lymphocytes to attach to vaccine antigens, so the calf is unable to produce antibodies in response to vaccine exposure (Niewiesk, 2014).

Intranasal (IN) vaccines have shown potential to bypass MatAb interference and provide a priming response (Mahan et al., 2016) through the stimulation of alternate parts of the acquired immune system. Intranasal vaccines prime the mucosal immune system, increasing immunoglobulin A (IgA), whereas maternal antibodies and injectable vaccines primarily affect the systemic immune system, increasing immunoglobulin G (IgG) (Griebel, 2009) (Walz et al., 2017, 1). In calves vaccinated intranasally, an IgA response was detected (based on nasal swab), whereas an IgG response in the blood was not detected (Hill et al., 2012). This suggests a response in the calf's mucosal immune system, but not its systemic immune system.

Priming and boosting is a method used to increase the immune response to vaccination (Ramshaw and Ramsey, 2000) (Lu, 2009) (Kardani et al., 2016). Priming is the first dose of the vaccine, which provides the antigens for the immune system to stimulate the production of antibodies and induce immune memory through B lymphocytes. Boosting is a second dose of vaccine that provides antigens that the immune system recognizes. This recognition of the antigen results in a quicker and more powerful immune response, known as an anamnestic response (Ramirez et al., 2009).

In a previous study, calves were given an initial priming IN vaccine and then a second boosting IN vaccine; after the booster vaccine, IgA concentrations increased even further than those seen after the priming vaccine, indicating an even more effective immune response with the second dose (Hill et al., 2012). This suggests that IN vaccination, in the face of maternal antibodies, may be an effective method to increase a calf's immune protection and offer an increased antibody response upon revaccination.

The prime-boost method of vaccination results in greater immune protection against disease. The most common method of prime-boost vaccination is through homologous vaccination

(Waldner et al., 2018). Homologous vaccination refers to priming and boosting with the same type of vaccine. Heterologous vaccination, rarely used in veterinary medicine, is based on priming and boosting with vaccines that are different, such as priming with a MLV vaccine and boosting with a KV vaccine (Kamble and Lee, 2016). Heterologous vaccination has been researched primarily in human medicine (Lu S. 2009) to offer an increased immune response. An example is heterologous prime-boost vaccination against human immunodeficiency virus (HIV), in which the priming is through a deoxyribonucleic acid (DNA) vaccine, followed by an attenuated viral vector, which usually comes from a modified form of fowl pox virus or adenovirus strains (Ramshaw and Ramsay. 2000). One reason that heterologous prime-boosting is more effective is the ability to stimulate different parts of the immune system. An example is a subunit vaccine stimulating the humoral system, while a DNA or MLV vectors stimulate the cell mediated immune response (Kardani et al. 2016). The cattle industry has yet to develop different vector vaccines, but the heterologous effect is thought to occur between modified live and killed viral vaccines to produce an increased anamnestic response (Bowland and Shewen, 2000).

1.4 Vaccination in Calves

In the perinatal period, the only immunoglobulins present in calves' immune systems are those derived from consumption of colostrum (Lago et al., 2018). The immune system develops and begins creating antibodies as the calf ages and is exposed to antigens. Vaccination is an important method to reduce the effect of natural exposure to disease as vaccines do not cause disease but still stimulate an immune response (Niewiesk, 2014). Most vaccinations are administered through injection with a MLV vaccine at branding (Waldner et al., 2018). Some producers use IN vaccines at branding, with a smaller number using KV vaccines (Waldner et

al., 2018). However, there is still much research to be done to determine the best method of vaccination.

1.4.1 Modified Live Viral (MLV) vaccine

Modified live viral vaccines are produced through an attenuation process. Attenuation is the process of reducing infectivity of pathogens. There are various ways to accomplish attenuation. One method is through changing the growing condition of the target pathogen. This different growing environment disables the virus's ability to cause infection in the host, while maintaining its antigens (Arevalo et al., 2015). This allows the immune system to create antibodies without risk of induction of disease. Genetic modification is also used to develop MLV vaccine; the method of attenuation modifies the virus to be unable to replicate in the host, but retains its antigens (Tizard, 2013).

1.4.2 Inactivated/Killed Viral (KV) vaccine

The production of killed vaccines consist of using killed organisms or inactivated toxoids. The primary method for inactivation or killing is the use of protein cross linking treatments, and protein denaturation treatments (Delrue et al., 2012). This effectively kills the pathogen, removing its pathogenicity, while allowing its antigens to be recognized by the immune system, and antibodies to be created (Tizard. 2013).

1.4.3 Vaccination Route

The most common method of administering vaccines in the beef industry is through injection (Waldner et al., 2018), which includes intramuscular and subcutaneous routes of administration. Subcutaneous vaccination deposits a dose of the viral antigen into the animal's fat layer, where antigen then gets absorbed into the bloodstream (Newcomer and Givens, 2016) (Niewiesk, 2014). In the bloodstream, B lymphocytes will bind to the antigens and specialize, creating

antibodies (Nguyen et al., 2017). Intranasal administration is another route that is used, but it is less common than injectable methods (Waldner et al., 2018). Intranasal vaccines stimulate the mucosal immune system, increasing production of antibodies to the affected area (Griebel, 2009). Oral vaccines are often used in human and companion animal medicine; however, they are not common in food animals. (Woolums et al., 2013) (Kavanagh et al., 2013).

1.5 MLV and Killed Vaccine Trials

Studies have shown that MLV and KV vaccines can offer similar protection between animals. One study compared the blood titer levels of cows after revaccination for BVDV and BoHV1 in dairy cows with a KV or MLV, finding no difference (Walz et al., 2015) (Walz et al., 2017, b. Some vaccines can cause negative reactions in the host, as has been seen with BoHV1 vaccines which have caused abortion in pregnant cows (Walz et al., 2017). It was initially thought that MLV vaccines caused more abortions due to remaining virulence of the vaccine virus. However, studies have shown that there is no difference between the MLV and KV vaccines in regard to disease protection and calving rates (Dubovi et al., 2000). Ellis et al. (2005) observed KV vaccinated calves had increased immune protection from BRSV when challenged compared to unvaccinated control calves, giving similar results as previous MLV vaccine trials (Ellis et al., 2018). This gives evidence that KV and MLV vaccines could offer similar protection against respiratory viruses.

1.6 Intranasal vs. Injectable Vaccines

Intranasal vaccines are used to prime the mucosal immune system and stimulate a localized immune response (Griebel, 2009) (Hill et al., 2012). Intranasal vaccines have been shown to have a protective against BRD pathogens, primarily through increased IgA antibody concentrations for short durations, with minimal effect of IgG antibody concentrations

(Kavanagh et al., 2013). Intranasal vaccines offer protection when administered as the primer and booster, and results in protection similar to that of injectable vaccines (Vangeel et al., 2007) (Vangeel et al., 2009).

An issue that producers face with injectable vaccines is interference due to maternal antibodies (MatAb) (Niewiesk, 2014). These MatAbs inhibit the immune system's ability to respond to antigens, leaving them susceptible to disease after the MatAb concentrations have waned (Vangeel et al., 2007) (Nguyen et al., 2017). Studies have shown that IN vaccines can cause improved protection to the calf, even in the face of MatAb, by bypassing MatAbs (Vangeel et al., 2007) (Ellis et al., 2013). This allows IN vaccines to be used as a primer, even in the face of MatAbs, and elicit an anamnestic response when boosted (Stokka et al., 2016) (Woolums et al., 2013). To properly test for effectiveness of IN vaccines in the face of maternal antibodies, mucosal IgA concentrations should be sampled, though some studies only test for IgG (Xue et al., 2010). This is because IN vaccines elicit a high mucosal IgA response, while responding with no increase, or a small increase in systemic IgG (Kavanagh et al., 2013) (Hill et al., 2012).

Often to determine the effectiveness of a vaccine or immune stimulating activity, researchers will use serology to determine antibody concentrations. With IN vaccines, serology can be a misleading method of determining effectiveness, as IN vaccines do not always show increased systemic antibody concentrations, but rather show increased mucosal IgA antibody concentrations (Kavanagh et al., 2013) (Hill et al., 2012). A study administered BRD vaccines to two groups of calves, one intranasally and one through injection. A polymerase chain reaction (PCR) was done to determine if the antigen from vaccines could be differentiated from natural viral antigens. The results show that both IN and injectable (IJ) vaccines could be detected through PCR performed on nasopharyngeal swabs or deep tracheal washes (Walz et al., 2017, a).

This gives evidence that IJ vaccines can impact the mucosal immune system. There is also evidence that IN vaccines can increase IgG antibodies in the system immune system (Grissett et al., 2014).

Another method to determine effectiveness of IN vaccines in the face of MatAbs is through morbidity and mortality, which is commonly used (Ellis et al., 2010, a) (Vangeel et al., 2007, a) (Ellis et al., 2013) (Vangeel et al., 2007, b). Morbidity and mortality studies have shown that IN vaccines had a disease sparing effect in IN vaccinated calves compared to control calves (Vangeel et al., 2007, a) (Xue et al., 2010). Intranasal vaccines have been observed to reduce the rate of MatAb waning, resulting in a longer lasting protection to the calf (Xue et al., 2010). While antibody concentrations are useful to determine significant differences, they may not be clinically relevant. To determine if vaccination methods have a clinical difference, morbidity data is often used. Multiple challenge studies have shown that IN vaccines are able to reduce the morbidity due to BRD (Vangeel et al., 2007, b) (Xue et al., 2010) (Ellis et al., 2007) (Ellis et al., 2010, b). This contrasts with results from studies that used only IgG concentrations as the determinant for vaccine efficacy (Kavanagh et al., 2013). These studies show that IN vaccines are able to reduce BRD morbidity when compared to control groups and have similar morbidity rates as injectable vaccines (Ellis et al., 2007) (Ellis et al., 2010, b) (Mahan et al., 2016).

Vaccines are administered to help mitigate the effect of a pathogen, but animals may still become infected. Research has shown that IN vaccines can stimulate the immune system in the face of maternal antibodies by stimulating local IgA (Xue 2010). The disease sparing effect of a vaccine is often measured by comparing severity of disease induced by the pathogen. Measurement of disease reduction include amount of virus shed nasally, lung lesion extent and severity, and rectal temperatures. Studies have shown that IN vaccines were able to reduce the severity of these

diseases when compared to non-vaccinated calves (Vangeel et al., 2007, a). For example, it was found that intranasally vaccinated calves had lower rectal temperatures and shed less virus for a shorter period of time (Vangeel et al., 2007, a) (Vangeel et al., 2007, b). Disease reduction has also been tested between IN and injectable vaccines, finding no significant difference between the two groups when comparing viral shedding and rectal temperature (Ellis et al., 2013). These studies suggest that IN and SC vaccines reduce disease severity similarly.

1.7 Conclusions

Bovine respiratory disease is a major cause of economic loss for feedlot operations. Feedlots use many methods to reduce the incidence of BRD, including vaccination and metaphylactic antibiotics. With increasing public awareness about antimicrobial use and resistance in the livestock sectors, the Canadian beef industry continues to explore ways to support antimicrobial stewardship, promote animal welfare, and improve economic margins for cattle producers through modifications in animal health management practices. Current vaccination methods utilize homologous antigen presentation and administration routes. Recently human medicine has begun to test heterologous antigen presentation methods of vaccination, but the cattle industry has little research on this topic. The purpose of this thesis is to add to literature in this area, and to provide a foundation for future research regarding heterologous and IN vaccination in cattle.

1.8 Objective of Research

The overall goal of this study is to compare different calf vaccination protocols for the control of BRD. To accomplish these goals two studies were performed. The first study is outlined in

Chapter 2, and is a smaller scale intensive study. The second study is outlined in Chapter 3 and is a larger scale commercial cow-calf vaccination field study.

The objectives for the study in Chapter 2 are as follows:

- Evaluation and comparison of three vaccine protocols for control of BRSV, BoHV1, BVDV1, and BVDV2 antibody concentrations at branding and weaning. The lab techniques used for BRSV and BoHV1 will be an ELISA, while a virus neutralization assay will be used for BVDV1 and BVDV2.
- Comparison of average daily gains between three vaccine protocols at important time points. The important time points were determined to be birth to 'turnout', birth to wean, and birth to final (end of the study).

These objectives were met through the enrollment of 75 calves into one of three groups. One group received a homologous vaccination protocol, the second group received a heterologous administration route and homologous antigen presentation protocol, and the third group received a heterologous vaccination protocol.

Chapter 3 is a larger scale field vaccination trial that enrolled heifer and steer calves from two different producers at branding. The objectives of this study are as follows:

- Comparison of morbidity and mortality rates between two vaccine protocols on feedlot calves from placement in the feedlot until the second administration of the second hormone implant.
- Comparison of average daily gains between two vaccine protocols on feedlot calves from placement in the feedlot until the second administration of the second hormone implant.

Morbidity rates were determined by experienced animal health personnel and based on standardized and specific diagnostic criteria. The feedlot veterinarian performed post mortem necropsies on animals that died to determine the cause of death. Average daily gains were determined by measuring weights upon placement at the feedlot and at the time of administration of the second hormone implant.

Chapter 2

A COMPARISON OF HETEROLOGOUS AND HOMOLOGOUS VACCINE PROTOCOLS TO INCREASE ANTIBODY CONCENTRATIONS AGAINST BOVINE RESPIRATORY DISEASE ASSOCIATED VIRAL PATHOGENS

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This chapter contains the analysis of antibody concentrations and average daily gains collected from beef calves from the Western Beef Development Center. This chapter reports the comparison of three different vaccination protocols and comparing heterologous and homologous vaccination protocols. This study also tests modified live viral vaccines for BVDV types 1 and 2 against inactivated viral vaccines against BVDV types 1 and 2. This is the first study that compares heterologous vaccination programs for bovine respiratory disease (BRD) in beef cattle. The results of this study show that heterologous vaccination protocols stimulated a significantly higher BRSV antibody response and had similar BoHV1 antibody concentrations as the homologous vaccination protocol. The inactivated viral vaccine resulted in lower BVDV2

antibody concentrations than the modified live viral group. Neither treatment group had an increased immune response to BVDV1, giving reason to rethink the vaccination route or timing of bovine viral diarrhea virus vaccination. Further research should investigate the efficacy of intranasal vaccinations administered as a primer at 'turnout'/branding, as this timing may fit better with cow-calf management of some herds. Development of an intranasal BVDV type 1 and 2 vaccine would be useful to bypass maternal antibodies, to act as a primer. The next chapter compares a homologous intranasal vaccination protocol against a homologous injectable vaccination protocol for protection against bovine respiratory disease.

Author contributions: Erickson was responsible for design of the experiment, design of the data analysis, and manuscript review. Ellis was responsible for assistance in determining type of sample collected and laboratory tests to be used. Lardner, Campbell, and Harding were responsible for manuscript review. Waldner was responsible for design of the data analysis and manuscript review. Berenik was responsible for data collection, the design and analysis of the data, and manuscript preparation.

Contributions: Virus Neutralizations were performed by Prairie Diagnostic Services INC. Enzyme-Linked Immunosorbent Assays were performed by Dr. John Ellis' lab. Calves were provided and cared for by the Western Beef Development Centre.

2.1 Abstract

Bovine Respiratory disease (BRD) is an important disease syndrome in the beef cattle industry, causing morbidity and mortality in calves, as well as large economic losses. Currently the most common vaccination protocols to protect against BRD utilize homologous modified live viral (MLV) vaccines. In this study, a heterologous vaccination protocol was compared, using an intranasal (IN) vaccine as a primer at birth and a MLV or killed viral (KV) vaccine as a booster at 'turnout' and weaning. Bovine viral diarrhea viruses (BVDV) type 1 and 2 were not vaccinated in a heterologous method as commercial IN vaccines do not contain antigens for BVDV types 1 or 2. The enzyme linked immunosorbent assay (ELISA) analyses found that the heterologous protocol had significantly higher BRSV antibody concentrations from two weeks post 'turnout' onwards compared to the other two treatment groups, while no difference was observed in bovine herpes virus type 1 antibody concentrations. Virus neutralization measurements for BVDV type 1 did not show an increased immune response, suggesting that maternal antibodies interfered with antibody induction. The virus neutralization found that KV vaccines resulted in a lower BVDV2 antibody concentrations. These results show that IN vaccines are important for priming the immune system in the face of maternal antibody concentrations, and heterologous vaccination protocols can increase antibody concentrations for BRSV.

2.2 Introduction

Bovine respiratory disease (BRD) is a complex that has a significant economic impact on feedlots across North America through calf mortality, lower average daily gain, and treatment costs (Smith, 2009) (Miles et al., 2009). One strategy to mitigate BRD is for cow-calf producers to vaccinate calves to prime their immune systems for protection against BRD-associated

bacterial and viral pathogens. Calves are often vaccinated against viral pathogens associated with BRD, including bovine respiratory syncytial virus (BRSV), bovine herpes virus type 1 (BoHV1), parainfluenza virus 3 (PI3), and bovine viral diarrhea virus type 1 and 2 (BVDV1 and 2) (Miles, 2009). Bovine respiratory syncytial virus, BoHV1, and PI3 are directly associated with respiratory disease development, while BVDV types 1 and 2 are known to suppress the immune system of calves (Van Wyk, 2016) (Ridpath, 2010). The aim of BRD vaccines is to promote calf health by controlling important respiratory pathogens and supporting immune system function.

On cow-calf operations, injectable vaccination is one of the most common methods of vaccination (Waldner et al., 2018). This type of vaccination frequently occurs at branding to prime the immune system, and at weaning to boost the immune system (Waldner et al., 2018). However, at branding calves may still have high maternal antibody (MatAb) concentrations which can interfere with the immune system's ability to respond to the vaccine by masking antigen binding sites (Munoz-Zanzi et al., 2002) (Niewiesk, 2014). It can be difficult to determine the most beneficial time to vaccinate as MatAbs wane at different rates depending on calf immune function and virus characteristics (Munoz-Zanzi et al., 2002) (Fulton et al., 2004). In recent years, mucosal vaccines have become of interest because they can bypass MatAbs and stimulate the calf immune system to help protect against disease. However, optimal immune response depends on more than the evasion of MatAbs from vaccine priming (Mahan et al., 2016) (Hill et al., 2012).

Current vaccination protocols on cow-calf operations commonly utilize homologous vaccination (Waldner et al., 2018). Homologous vaccination refers to priming and boosting with either the same type of antigen, or with the same route of administration. This method focuses on

stimulating one response type from the immune system (Bolten et al., 2012) (Lu, 2009). Heterologous vaccination protocols, implemented in some aspects of human medicine (Lu, 2009) (Jung et al., 2018), utilize different routes of administration and/or present different forms of antigens to stimulate an increased immune response (Lu, 2009) (Jung et al., 2018). The current study compares the immune responses between beef calves administered an injectable vaccine compared to those primed with a mucosal viral vaccine within 24 hours of birth and boosted with either a homologous or heterologous viral vaccine protocol at 'turnout' and weaning.

2.3 Methods and Materials

2.3.1 Animals

Seventy-five neonatal crossbred heifer calves located at the Western Beef Development Center (WBDC) research facility, near Lanigan Saskatchewan, Canada, were enrolled into this study. This number was selected after reviewing current literature for similar studies, and consulting with Dr. John Ellis. The calves selected for the study were born between late March and early May 2017 to cows with good udders. The cows from this herd were annually vaccinated against disease due to *Clostridium sps*, anthrax, BRD[1] and reproductive diseases prior to breeding. At birth, calves were individually identified by a dangle tag and radio frequency identification (RFID) tag. Calves from all groups were managed on the same pasture, and were moved as a group with their dams to a second pasture at 'turnout', and to the feedlot style pens at the Western Beef Development Center. Only single birth calves born without assistance to multiparous dams were included in the study.

2.3.2 Experimental Design: Randomized Clinical Control Trial

Calves were enrolled in the study within 24 hours of birth and were observed until 60 days post-weaning (Figure 2.1). The calves were enrolled and randomized (Excel[4] randomization formula)

into one of three experimental groups: injectable modified live viral (IJ-MLV) group, intranasal modified live viral (IN-MLV) group, and intranasal killed viral (IN-KV) group. The IJ-MLV group acted as a reference group and received intranasal (IN) administration of sterile water (2 mL) within 24 hours of birth, received a modified live viral (MLV) injectable (IJ) vaccine (Bovishield Gold FP5)[1] at 'turnout', and a MLV IJ vaccine[1] at weaning. The intranasal modified live group (IN-MLV) received an intranasal (IN) vaccine (Inforce 3; 2 mL)[2] within 24 hours of birth, a MLV IJ vaccine[1] at 'turnout' (average age was 47 days, ranging from 34 – 60 days of age), and a MLV IJ vaccine[1] at weaning (average age was 48 days, with a range of 35 to 64 days of age). The intranasal killed viral group (IN-KV) received an IN vaccine (Inforce 3; 2 mL)[2] within 24 hours of birth, a killed viral (KV) vaccine (Triangle 5)[3] at 'turnout' (average age was 48 days, with a range of 34 – 65 days of age), and a KV IJ vaccine[3] at weaning.

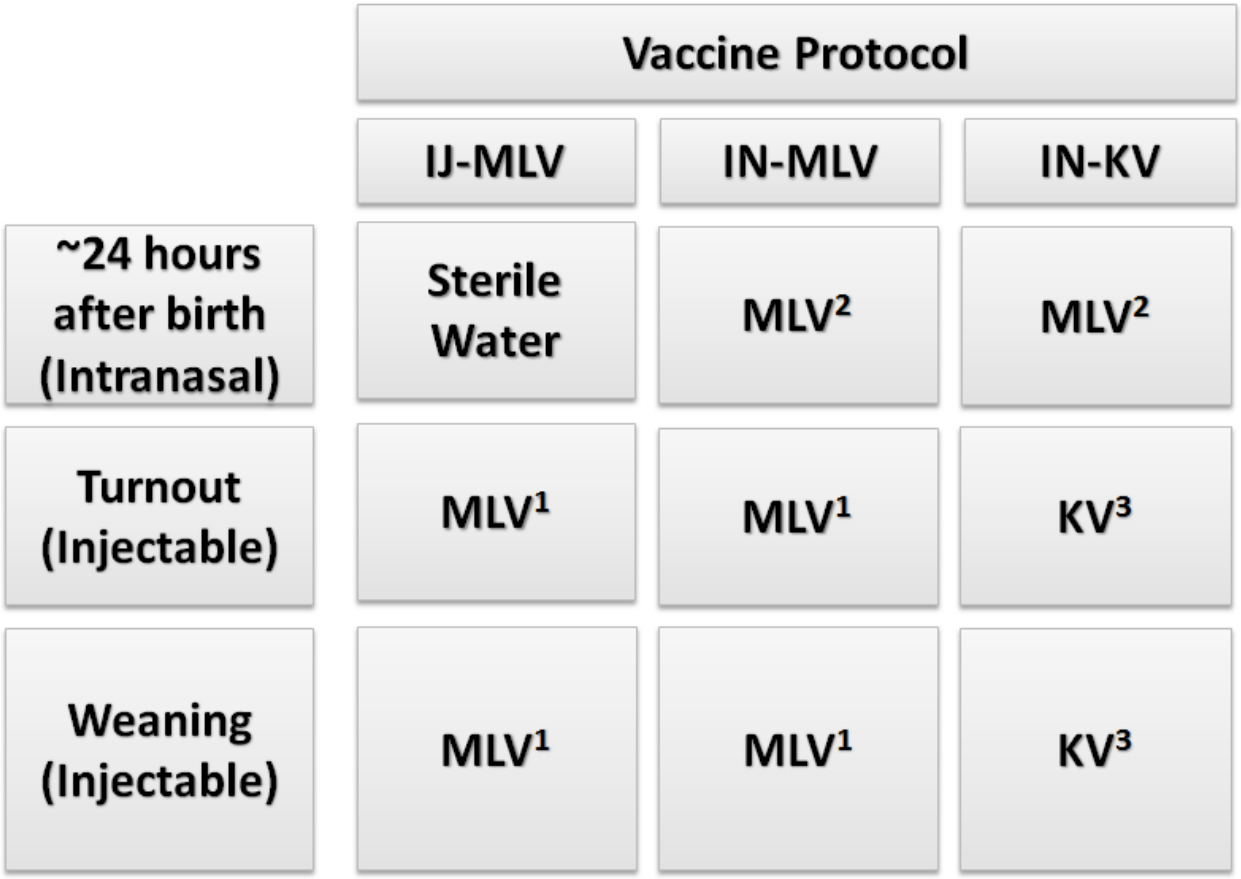

Figure 2.1. A figure showing the type of vaccination each treatment group will receive at each time point

Every three calves were randomized into 1 of the 3 experimental groups based on birth order, and enrollment changed for every set of three calves. At enrollment, calves were administered either an IN vaccine or sterile water, a 10 mL serum tube was used to collect blood samples, and weights were recorded in pounds, using a hanging dial scale. Calves were administered sub-cutaneous booster vaccinations at 'turnout' as per their experimental group protocol, were weighed, and had serum samples collected. 'turnout' occurred when calves were 34-65 days old and involved movement from the birth pasture to a new summer pasture. Two weeks after 'turnout' serum samples were collected, and body weights were recorded.

At weaning calves were moved to the WBDC feedlot operation. At this time 20 calves: 9 from the intranasal modified live viral (IN-MLV) group, 9 from the intranasal killed viral (IN-KV) group, and 2 from the injectable modified live viral (IJ-MLV) group, were removed from the trial to undergo a BRSV challenge study comparing homologous and heterologous vaccine protocols. Less calves were removed from the IJ-MLV group, as they were treated as a sentinel group. The final body weights of the remaining calves were recorded 87 days post-weaning. At weaning, a subcutaneous vaccine was administered as per the experimental group protocols, serum samples were collected, and calves were re-weighed. After weaning, calves were moved to the WBDC backgrounding pen, where groups were comingled. Two weeks after weaning, a serum sample was collected, and body weight was recorded for each calf. The calves were then placed in a single pen for feeding and observation of health for 60 days and a final body weight was collected 87 days post-weaning. The weights were collected when the calves at standard commercial production handling times reduce the number of movements through handling facilities, and to reduce stress to the animals (University of Saskatchewan's Animal Research Ethics Board protocol number 20170003). If a calf died during the trial, a necropsy was performed within 24 hours by a University of Saskatchewan Veterinarian to determine the cause of death.

Calves were observed for BRD morbidity by experienced WBDC animal health staff and treated as required. Clinical BRD disease was reported if a calf had a rectal temperature above or equal to 40°C (103.9 F°) and at least two of the following signs were observed: moderate depression (drooping head and ears), reluctance to move, tucking of the flank/abdomen, rapid shallow breathing or, increased respiratory effort.

2.3.3 Test Procedures

Serum Neutralization

A virus neutralization assay was performed on blood samples collected at 'turnout', two weeks post 'turnout', weaning, and two weeks post weaning, to determine antibody concentrations for BVDV types 1 and 2. Each batch consisted of a virus (BVDV1/SingerB8232) titration and positive (A/S calf 72) and negative (fetal calf serum) controls. Samples were analyzed in duplicate. Prior to testing, serum samples were heat inactivated at 56°C for 30 minutes. Samples were placed into small wells on plates for testing. The first plate of each run utilized a virus back-titration to check that an appropriate virus dilution occurred. A diluted positive and negative control were also included on the first plate, giving a base unit of 6. Samples were diluted by use of 3-fold dilutions to determine antibody cut off values. The virus dilution was added to each of the wells, and the plates were incubated at 37°C in CO_2 for two hours. A cell suspension was prepared with embryonic bovine tracheal cells. The plates were removed from the incubator, and cells were added to each well. The plates were sealed with tape and allowed to incubate at 37°C for seven days. After the incubation period, the plates were observed for cytopathic effects using an inverted microscope (Waldner and Campbell, 2005).

Enzyme-Linked Immunosorbent Assay (ELISA)

An ELISA determined antibody concentrations on samples collected at 'turnout', two weeks post 'turnout', weaning, and two weeks post weaning, for BRSV and BoHV1 (Durham and Hassard, 1990) with minor modifications. Ninety-six well plates (Immulon IV) are coated with a blocking agent for half an hour at 37°C. Positive, negative, and test sera are diluted 1:50 in ELISA

working buffer. Control and test sera are added to duplicate well plates and incubated at 37°C for one hour. After this incubation period, horseradish peroxidase conjugated protein G diluted in working buffer containing 4% polyethylene glycol 8000 and 0.2% gelatin solution is added to all wells, and incubates for an hour at 37°C. The horseradish peroxidase enzyme reaction is visualized with a (2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) substrate and the reaction is stopped with a 1% sodium dodecyl sulphate solution. All wells receive 100 uL of reagents, get washed four with double distilled water containing 0.05% Tween 20. Antibody concentrations are reported as units derived from a calculation of the percentage of optical density of test wells compared to positive controls.

2.3.4 Statistics

Data was imported into the SPSS version 25 statistical software[5]. A Shapiro-Wilk test of normality, with a significance value of $p < 0.05$ was used to determine the normality of the data. The serum concentration data for BRSV and BoHV1 was analyzed using generalized estimating equations using a normal distribution, with an AR1 working correlation matrix, inputting calf as the repeated subject, with treatment group and time as main effects and treatment with time as an interaction term. Data for BVDV1 and 2 were not normally distributed. The serum concentration data for BVDV 1 and 2 was log transformed prior to analysis. General estimating equations using a normal distribution with an AR1 working correlation matrix, inputting calf as the repeated subject, with treatment group and time as main effects and treatment with time as an interaction term. A pairwise comparison was then performed to determine differences between groups of calves at different time points.

Average daily gain (ADG) was calculated using weight values from birth, 'turnout', weaning, and final trial day. Age was included in analysis when birth weights were involved. Average

daily gain was analyzed using a linear regression, with treatment as a main effect, with a pairwise comparison between the groups to determine any significant responses between groups. Differences were considered significant with a p-value of <0.05.

2.4 Results

2.4.1 Bovine Respiratory Syncytial Virus

At each of the four time point's calf mean BRSV antibody concentration was calculated and compared (Table 2.1). At 'turnout', there were no statistically significant differences between any of the treatment groups ($p \geq 0.9$). At two weeks post 'turnout', the BRSV groups antibody concentration for the IN-KV group were significantly higher than the IJ-MLV ($p < 0.01$) or IN-MLV ($p = 0.03$) groups (Table 2.1). The BRSV antibody concentration for the IJ-MLV and IN-MLV groups were not significantly different ($p = 0.2$). At weaning, the IN-KV had a significantly higher BRSV antibody concentration than the IJ-MLV ($p < 0.01$) or IN-MLV groups ($p < 0.01$). The BRSV antibody concentration for the IJ-MLV and IN-MLV groups were not significantly different ($p = 0.3$). At two weeks post weaning the BRSV concentrations for the IJ-MLV and IN-MLV groups were not significantly different from each other ($p = 0.6$) and the antibody concentration of the IN-KV group were significantly higher than the IN-MLV ($p < 0.01$) or IJ-MLV ($p < 0.01$) groups.

Antibody concentration differences within each group were compared between time points to determine whether the calves had antibody responses to the vaccinations (Figure 2.1). Between 'turnout' and two weeks post 'turnout', the IJ-MLV group had decreased BRSV antibody concentration ($p = 0.02$), the IN-MLV group had no significant change in antibody concentration ($p = 0.7$), and the IN-KV group had significantly increased antibody concentration ($p < 0.01$). Between two weeks post 'turnout' and weaning, all three groups had significantly reduced BRSV

antibody concentration ($p < 0.01$) (Figure 2.1). Between weaning and two weeks post weaning, all three groups had significantly increased BRSV antibodies ($p < 0.01$) (Figure 2.1).

2.4.2 Bovine Herpes Virus 1

At each time point, calf mean BoHV1 antibody concentrations were compared between the three groups (Table 2.2). At 'turnout' ($p = 0.7$, $p = 0.7$, $p > 0.9$) and at two weeks post 'turnout' ($p = 0.8$, $p = 0.5$, $p = 0.7$), there were no statistically significant differences in BoHV1 antibody concentration between any of the pairwise comparisons of the groups (Table 2.2). At weaning, the IN-KV group was significantly higher than the IN-MLV group ($p = 0.04$) (Table 2.2), and the IJ-MLV group was not significantly different from the IN-MLV or IN-KV groups ($p = 0.1$, $p = 0.7$). At two weeks post weaning there was no statistical difference between the IJ-MLV and IN-MLV ($p = 0.2$), IJ-MLV and IN-KV ($p = 0.6$), and IN-MLV vs IN-KV ($p = 0.6$).

The antibody concentration differences within group were compared between time points (Figure 2.2). Between 'turnout' and two weeks post 'turnout' all three treatment groups were significantly lower at two weeks post 'turnout' ($p < 0.1$). From two weeks post 'turnout' to weaning, all three treatment groups had significantly lower BoHV1 antibody concentration ($p < 0.1$) (Figure 2.2). Between weaning and two weeks post weaning, all three treatment groups had significantly increased BoHV1antibody concentration ($p < 0.1$).

2.4.3 Bovine Viral Diarrhea Virus 1

The data for BVDV1 were transformed using natural logarithmic function for analysis, and exponentiated to compare the results. At each of the four time points, mean BVDV1 antibody concentrations were calculated and compared in a pairwise manner (Table 2.3). At 'turnout' and two weeks post 'turnout' the relative differences between mean BVDV1 concentrations were not significantly different (Table 2.3). At weaning, the IN-KV group was significantly lower than

the IJ-MLV group ($p = 0.01$), with a relative difference of 2.1 and the IN-KV group was also significantly lower than the IN-MLV group ($p < 0.01$) with a relative difference of 2.2. The IJ-MLV and IN-MLV groups were not significantly different from each other at weaning ($p = 0.8$). At two weeks post weaning, the IJ-MLV group and IN-MLV groups were not significantly different ($p = 0.5$); however, the IJ-MLV group was signficantly lower ($p = 0.04$) than the IN-KV group, with a relative difference of 0.4. The IN-MLV and IN-KV groups were not signficantly different ($p = 0.1$) at two weeks post weaning.

The BVDV1 antibody concentration differences within each group were compared between the time points (Figure 2.3). Between 'turnout' and two weeks post 'turnout', all three treatment groups had decreased BVDV1 antibody concentration levels. Between two weeks post 'turnout' and weaning, all three groups had a decrease in BVDV1 antibody concentration levels. From weaning to two weeks post weaning, the IJ-MLV and IN-MLV groups were not significantly different within their respective groups ($p = 0.2, 0.09$). The IN-KV group had a significantly higher BVDV1 antibody concentration at two weeks post weaning compared to weaning ($p = <0.01$) (Figure 2.3).

2.4.4 Bovine Viral Diarrhea Virus Type 2

The data for BVDV2 were transformed using natural logarithmic function for analysis, and exponentiated to display the results. At each of the four time points, mean BVDV2 antibody concentration were calculated and compared in a pairwise manner (Table 2.4). At 'turnout' and two weeks post 'turnout', there was no significant differences between the three treatment groups ($p \geq 0.5$). At weaning, the IJ-MLV and IN-MLV groups were not signficantly different. The IN-KV group was signficantly lower ($p < 0.01$) than both the IJ-MLV and IN-MLV groups by a relative difference of 6.0 and 9.0, respectively. The IJ-MLV and IN-MLV groups were not

signficantly different (p = 0.2). At two weeks post weaning the IJ-MLV and IN-MLV group were not significantly different (p = 0.2). The IN-KV group was signficantly lower than both the IJ-MLV (p = 0.02) and IN-MLV (p >0.01) groups by a relative difference of 3.4 and 6.5, respectively.

The BVDV2 antibody concentration differences within group were compared between the time points (Figure 2.4). From 'turnout' to two weeks post 'turnout', all three treatment groups had significantly lower BVDV2 antibody concentrations within their respective groups. Between two weeks post 'turnout' and weaning, all three treatment groups had a significant decrease in within group BVDV2 antibody concentration. Between weaning and two weeks post weaning within each of the three treatment groups there was a signficant increase in BVDV2 antibody concentration (Figure 2.4).

2.4.5 Average Daily Gains

Average daily gains were calculated for calves from each group, between the important time points (Table 2.5). No differences in ADG were observed between treatment groups during the birth to 'turnout' time period, IJ-MLV vs IN-MLV (p = 0.7), IJ-MLV vs IN-KV (p = 0.6), IN-MLV vs IN-KV (p > 0.9). From birth to weaning there was no significant differences observed between the treatment groups, IJ-MLV vs IN-MLV (p = 0.6), IJ-MLV vs IN-KV (p > 0.9), IN-MLV vs IN-KV (p = 0.6). The ADG from weaning to 87 days post-weaning was not significantly different between the treatment groups IJ-MLV vs IN-MLV (p = 0.4), IJ-MLV vs IN-KV (p = 0.4), IN-MLV vs IN-KV (p = 0.9).

2.5 Discussion

The purpose of this study was to compare the effectiveness of three vaccination protocols to provide increased antibody concentrations against viruses associated with BRD. A primary

objective of the study was to compare heterologous and homologous vaccination protocols of which there is a lack of literature for beef cattle. This study is unique in that it assessed the efficacy of IN vaccines as immune primers, and the effectiveness of heterologous and homologous vaccine protocols.

In the beef cattle industry, pre-weaned calves most often receive a priming vaccine before 'turnout'/branding, while boosting vaccines are most often administered at weaning (Waldner et al., 2018). A problem with vaccinating at 'turnout'/branding is that the calves are young and there is a possible interference of the priming vaccine due to the presence of high maternal antibodies (MatAbs) (Platt et al., 2009) (Niewiesk, 2014). The current study found that the IN vaccines were able to successfully prime calves when administered at birth, showing IN vaccines ability to bypass MatAbs. This was observed for BRSV, as the IN-KV group had higher antibody concentrations at two weeks post 'turnout' compared to 'turnout', while the IN-MLV group had similar antibody concentrations. The IJ-MLV group had lower antibody concentrations at two weeks post weaning, suggesting initial priming vaccines administered at 'turnout' did not successfully stimulate the immune system. At two weeks post 'turnout', the antibody concentrations of the IN-MLV group did not decrease significantly. This suggests that the boosting was successful at stimulating an immune response or maintaining the existing antibody concentrations. Based on these results, the heterologous vaccination administration did not show an increased antibody concentration compared to homologous administration, whereas heterologous antigen presentation did show a greater response than homologous antigen presentation.

Interference of MatAbs occurred when priming for BVDV1, as the priming vaccine did not result in an increased antibody concentration, in the injectable vaccine groups, as has been shown

in previous studies (Ellis et al., 2001) (Downey et al., 2013). A small difference between weaning and two weeks post weaning antibody concentrations was observed, indicating that the weaning booster vaccine did not successfully boost the calf immune system. The IN-KV had significantly higher BVDV1 antibody concentrations at two weeks post weaning compared to weaning but was extremely low compared to 'turnout' and two weeks post 'turnout'; suggesting a boosting response did not occur. The age at which calves receive the priming vaccination is an important factor in successfully priming the immune system. It has been shown previously that BVDV1 MatAb concentrations wane at a slower rate than BVDV2, BRSV, and BoHV1, supporting our finding that BVDV1 was the only virus to not elicit an increased immune response to a booster vaccine (Munoz-Zanzi et al., 2002) (Fulton et al., 2004). As such, development of an IN vaccine for BVDV should be a priority, as IN vaccines are able to bypass MatAbs and act as a primer (Hill et al., 2012) (Woolums et al., 2013), promoting increased protection.

In this study, blood serum samples were collected from calves to determine systemic (IgG) antibody concentrations, which were used to show the vaccine response. Previous research has shown that IN vaccination has a main effect on mucosal antibody concentration levels, as well as increasing systemic antibody concentrations (Kavanagh et al., 2013) (Hill et al., 2012). The results of this study showed the IN vaccines affected systemic BRSV antibody concentrations. This was seen in the IN groups, as the IN-MLV group did not have waning antibody concentrations two weeks after 'turnout', and the IN-KV group had increased antibody concentrations two weeks after 'turnout'. This provides evidence that the IN vaccines also primed the systemic immune system. Antibody concentrations were analyzed to determine the systemic antibody response to an injectable booster. The IN vaccines were able elicit a strong

mucosal response, and a minor systemic response, effectively priming the immune system (Hill et al., 2012) (Kavanagh et al., 2013) (Raja et al., 2018) (Jung et al., 2018). This study has shown that IN vaccines can act as effective primers with increased antibody response when used in a heterologous vaccine protocol.

The results of the current study show that heterologous vaccination increases BRSV antibody concentrations when compared to homologous vaccination. While there is little research on heterologous vaccination in cattle (Walz et al., 2015), human studies have found that heterologous vaccination can increase protection against disease (Raja et al., 2018) (Jung et al., 2018). Heterologous vaccination is promising for the cattle industry, but further research is needed to develop vaccines for heterologous prime-boosting.

This study found that KV vaccines were not as effective in stimulating an immune response against BVDV2 as MLV vaccines. These results are consistent with previous research (Dubovi et al., 2000) (Reber et al., 2006). This study found that the KV vaccines had increased BVDV1 in contrast to previous studies which found MLV vaccines elicited a greater response (Dubovi et al., 2000) (Reber et al., 2006) (Fulton and Burge, 2000). The lack of difference in antibody concentration levels between the groups was likely due to a lack of immune stimulation. The IN-KV group was significantly higher at two weeks post weaning compared to weaning ($p = 0.04$), whereas the IJ-MLV ($p = 0.5$) and IN-MLV ($p = 0.1$) groups were not significantly different between these time points. Heterologous vaccination was not assessed for BVDV in this study as the mucosal vaccine used to prime the calves did not contain antigens for BVDV types 1 and 2, and currently there is no commercial IN BVDV vaccine available.

While the current study allowed the comparison of multiple different vaccination methods, one weakness study was in the number of vaccines each group received. The IN groups received a

vaccine at birth, while the IJ-MLV group received a dose of sterile water at that time. This could have influenced the overall antibody levels, through bypassing of the MatAbs and stimulating the immune system early (Kavanagh et al., 2013) (Hill et al., 2012). However, if the IJ-MLV group was administered an IJ vaccine at birth there likely would have been no immune response as prior studies have shown that systemic vaccination in the face of MatAbs does not cause immune stimulation (Munoz-Zanzi et al., 2002).

Alternatively, a treatment group primed with an injectable KV or MLV vaccine and boosted with the opposite is a possible future investigation; however, if the initial vaccination occurs at the traditional age of 2 – 3 months a priming response may not occur because of MatAb interference. Currently IN KV vaccines are not commercially available, causing KV priming to be a difficult and potentially fruitless effort due to MatAb interruption of IJ vaccines.

One of the limitations of this study was the type of immune response observed. Only humoral antibody concentration levels were determined, but the cell mediated immune response mounted by the calves were not tested. This study shows that some mean antibody concentrations are higher than others; however, this does not determine level of protection against disease (Walz et al., 2017, 1) (Hill et al., 2012). To observe this, a disease challenge study should be conducted, to determine the disease sparing effect of the vaccine protocol (Ellis et al., 2018). Without doing a challenge study, a large field study using naturally occurring disease is an option for future studies. In future research, it would be important to determine different methods of applying heterologous vaccinations, changing the order that different antigens are applied to the animals. Development of IN vaccines for BVDV types 1 and 2 would be useful to provide protection in the face of MatAbs, as research has shown that IN vaccination against BVDV increase protection of calves, compared to control calves (Xue et al., 2010).

Prime-boost vaccination has become the standard for many producers (Waldner et al., 2018), and this study tests different possible methods to improve the effectiveness of prime-boosting. Heterologous antigen presentation is one such method and resulted in a greater antibody concentration response against BRSV compared to the homologous antigen presentation groups. Homologous route of administration did show some effect, as the antibody concentrations against BRSV of the IN-MLV group did not significantly decrease between two weeks post 'turnout' and 'turnout' where the IJ-MLV group did. This provides evidence that the vaccination method a producer employs could improve the immune response of their calves.

2.6 Tables

Table 2.1. A comparison of the mean difference of BRSV antibody concentration between groups at each of the different time points.

	Mean Difference of BRSV Antibody Concentrations			**p-value**		
	Contrast (CI 95%)					
	Treatment			Treatment		
Time Point	**IJ-MLV vs IN-MLV**	**IN-KV vs IJ-MLV**	**IN-KV vs IN-MLV**	**IJ-MLV vs IN-MLV**	**IN-KV vs IJ-MLV**	**IN-KV vs IN-MLV**
'Turnout'	0.6	-0.3	0.3	0.9	0.9	>0.9
	(-8.7 , 9.9)	(-8.5 , 7.9)	(-9.6 , 10.1)			
2 Weeks Post 'Turnout'	-6.0	19.0	13.0	0.2	**<0.01**	**0.03**
	(-15.7 , 3.8)	(8.6 , 29.3)	(1.4 , 24.6)			
Weaning	1.1	13.3	14.4	0.3	**<0.01**	**<0.01**
	(-0.9 , 3.2)	(8.5 , 18.0)	(9.7 , 19.0)			
2 Weeks Post Weaning	3.3	94.9	98.1	0.6	**<0.01**	**<0.01**
	(-9.1 , 15.6)	(81.3 , 108.5)	(85.3 , 111.0)			

Table 2.2. A comparison of the mean difference of BoHV1 antibody concentrations between groups at each of the different time points.

	Mean Difference of BoHV1 Antibody Concentrations			**p-value**		
	Contrast (CI 95%)					
	Treatment			Treatment		
Time Point	**IJ-MLV vs IN-MLV**	**IN-KV vs IJ-MLV**	**IN-KV vs IN-MLV**	**IJ-MLV vs IN-MLV**	**IN-KV vs IJ-MLV**	**IN-KV vs IN-MLV**
'Turnout'	-1.7	1.8	0.1	0.7	0.7	>0.9
	(-10.4 , 7.1)	(-6.7 , 10.3)	(-9.2 , 9.5)			
2 Weeks Post 'Turnout'	-1.2	2.7	1.5	0.8	0.5	0.7
	(-9.2 , 6.7)	(-5.0 , 10.5)	(-7.0 , 9.9)			
Weaning	0.2	1.1	1.3	0.7	0.1	**0.04**
	(-0.7 , 1.0)	(-0.2 , 2.4)	(0.03 , 2.5)			
2 Weeks Post Weaning	-4.3	1.9	-2.4	0.2	0.6	0.6
	(-11.6 , 3.0)	(-6.3 , 10.2)	(-11.6 , 6.8)			

Table 2.3. A comparison of the relative difference of BVDV1 antibody concentrations between groups at each of the different time points.

	Relative Difference of BVDV1 Antibody Concentrations			**p –value**		
	Contrast (CI 95%)					
	Treatment			Treatment		
Time Point	**IJ-MLV vs IN-MLV**	**IJ-MLV vs IN-KV**	**IN-MLV vs IN-KV**	**IJ-MLV vs IN-MLV**	**IJ-MLV vs IN-KV**	**IN-MLV vs IN-KV**
'Turnout'	1.0	1.5	1.6	0.9	0.2	0.2
	(0.5 , 1.8)	(0.8 , 2.7)	(0.9 , 2.8)			
2 Weeks Post 'Turnout'	1.0	1.2	1.1	0.9	0.6	0.7
	(0.6 , 2.0)	(0.6 , 2.2)	(0.7 , 2.0)			
Weaning	1.0	2.1	2.2	0.8	**0.01**	**<0.01**
	(0.6 , 1.6)	(1.2 , 3.6)	(1.3 , 3.7)			
2 Weeks Post Weaning	0.8	0.4	0.5	0.5	**0.04**	0.1
	(0.3 , 1.8)	(0.2 , 1.0)	(0.2 , 1.2)			

Table 2.4. A comparison of the relative difference of BVDV2 antibody concentrations between groups at each of the different time points.

	Relative Difference of BVDV2 Antibody Concentrations			**p-value**		
	Contrast (CI 95%)					
	Treatment			Treatment		
Time Point	**IJ-MLV vs IN-MLV**	**IJ-MLV vs IN-KV**	**IN-MLV vs IN-KV**	**IJ-MLV vs IN-MLV**	**IJ-MLV vs IN-KV**	**IN-MLV vs IN-KV**
'Turnout'	1.09	1.1	1.01	0.8	0.7	>0.9
	(0.6, 1.8)	(0.6 , 1.9)	(0.6 , 1.7)			
2 Weeks Post 'Turnout'	1.0	0.8	0.8	0.9	0.5	0.5
	(0.6 , 1.6)	(0.5 , 1.4)	(0.5 , 1.5)			
Weaning	0.7	6.0	9.0	0.4	**<0.01**	**<0.01**
	(0.3 , 1.7)	(2.7 , 13.4)	(4.74 , 16.9)			
2 Weeks Post Weaning	0.5	3.4	6.5	0.2	**0.02**	**<0.01**
	(0.2 , 1.5)	(1.2 , 9.3)	(2.4 , 18.1)			

Table 2.5. A comparison of mean difference of the ADG of each group.

	ADG (kg/day)			**Mean Difference**			**p -value**		
				Contrast (CI 95%)					
	Treatment			Treatment			Treatment		
Time Point	**IJ-MLV**	**IN-MLV**	**IN-KV**	**IJ-MLV vs IN-MLV**	**IJ-MLV vs IN-KV**	**IN-MLV vs IN-KV**	**IJ-MLV vs IN-MLV**	**IJ-MLV vs IN-KV**	**IN-MLV vs IN-KV**
Birth to 'Turnout'	1.0	1.0	1.0	-0.0.39	-0.047	-0.0082	0.7	0.6	>0.9
				(-0.25 , 0.17)	(-0.25 , 0.16)	(-0.21 , 0.20)			
Birth to Wean	1.1	1.1	1.1	-0.041	-0.0049	0.036	0.6	>0.9	0.6
				(-0.18 , 0.010)	(-0.14 , 0.13)	(-0.10 , 0.18)			
Birth to Final	0.9	0.9	0.9	-0.055	-0.052	0.0024	0.4	0.4	0.9
				(-0.19 , 0.077)	(-0.18 , 0.079)	(-0.14 , 0.14)			

2.7 Figures

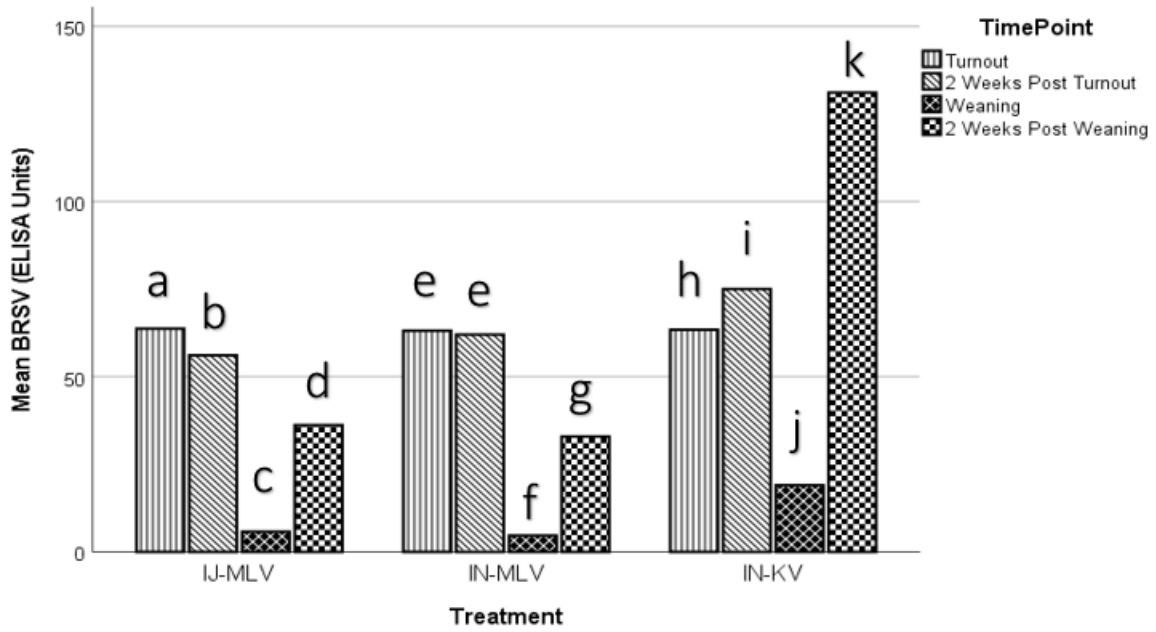

Fig. 2.2. A comparison of mean BRSV antibody concentrations within a treatment group at different time points

*a b c d denote significant differences between time points for the IJ-MLV group.
*e f g denote significant differences between time points for the IN-MLV group.
*h i j k denote significant differences between time points for the IN-KV group.
*IJ-MLV group received an injectable MLV vaccine at 'turnout' and weaning. The IN-MLV group received an IN MLV vaccine within 24 hours of birth, and a MLV injectable vaccine at 'turnout' and weaning. The IN-KV group received an IN MLV vaccine at birth, and a KV injectable vaccine at 'turnout' and weaning.

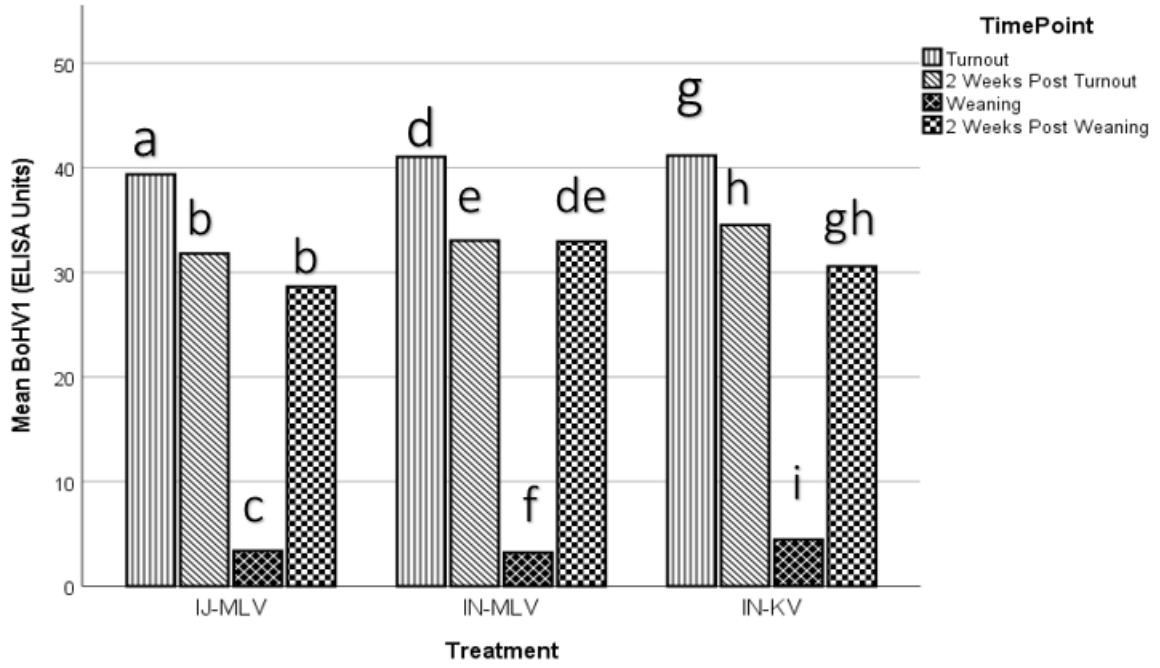

Fig. 2.3. A comparison of mean BoHV1 antibody concentrations within a treatment group at different time points

*a b c denote significant differences between time points for the IJ-MLV group.

*d e f denote significant differences between time points for the IN-MLV group.

*g h i denote significant differences between time points for the IN-KV group.

*IJ-MLV group received an injectable MLV vaccine at 'turnout' and weaning. The IN-MLV group received an IN MLV vaccine within 24 hours of birth, and a MLV injectable vaccine at 'turnout' and weaning. The IN-KV group received an IN MLV vaccine at birth, and a KV injectable vaccine at 'turnout' and weaning.

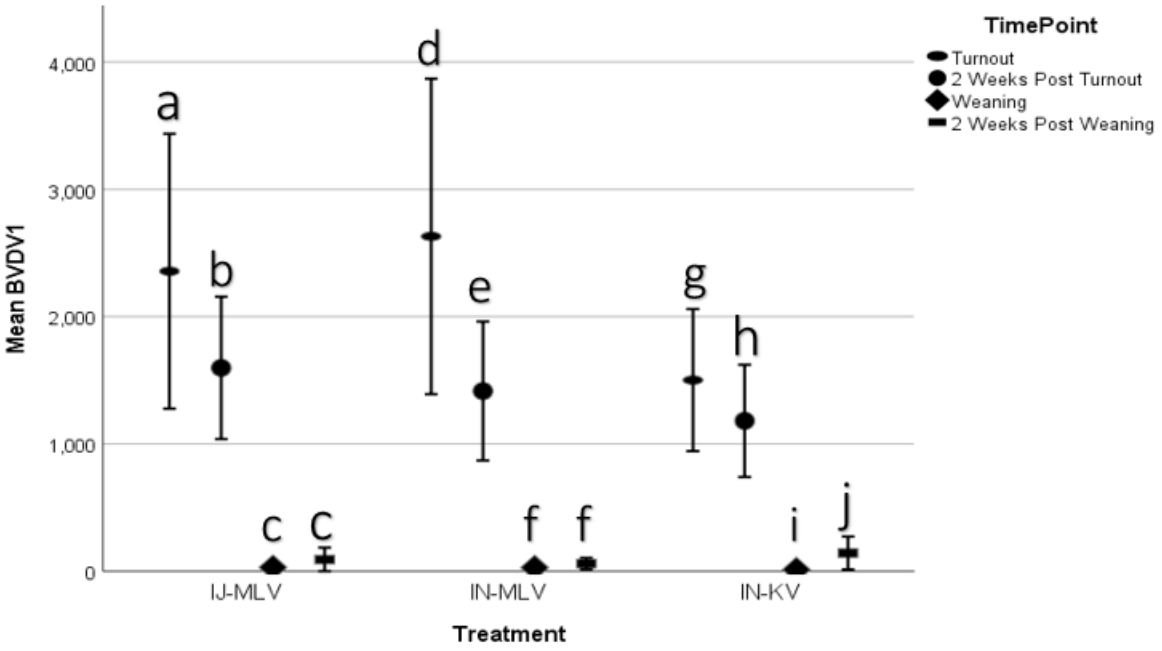

Fig. 2.4. A comparison of mean BVDV1 antibody concentrations within a treatment group at different time points

*a b c denote significant differences between time points for the IJ-MLV group.
*d e f denote significant differences between time points for the IN-MLV group.
*g h i j denote significant differences between time points for the IN-KV group.
*IJ-MLV group received an injectable MLV vaccine at 'turnout' and weaning.
The IN-MLV group received an IN MLV vaccine within 24 hours of birth, and a MLV injectable vaccine at 'turnout' and weaning. The IN-KV group received an IN MLV vaccine at birth, and a KV injectable vaccine at 'turnout' and weaning.

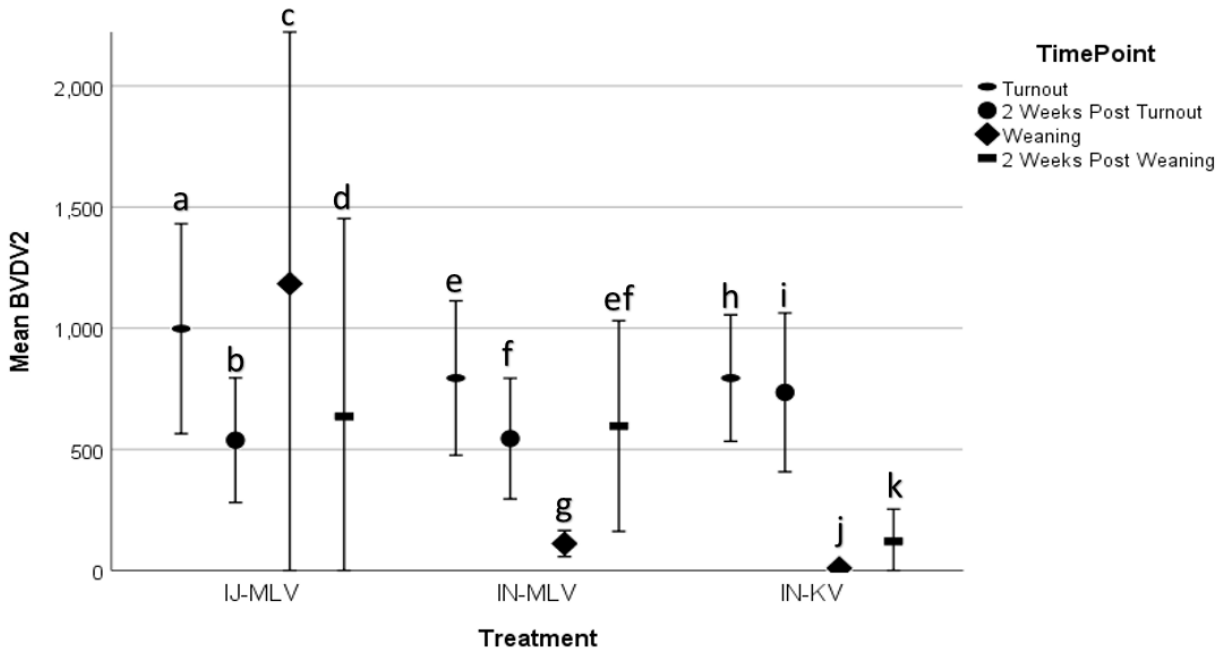

Fig. 2.5. A comparison of mean BVDV2 antibody concentrations within a treatment group at different time points
*a b c d denote significant differences between time points for the IJ-MLV group.
*e f g denote significant differences between time points for the IN-MLV group.
*h i j k denote significant differences between time points for the IN-KV group.
*IJ-MLV group received an injectable MLV vaccine at 'turnout' and weaning. The IN-MLV group received an IN MLV vaccine within 24 hours of birth, and a MLV injectable vaccine at 'turnout' and weaning. The IN-KV group received an IN MLV vaccine at birth, and a KV injectable vaccine at 'turnout' and weaning.

2.8 Endnotes

[1]Bovi-Shield Gold FP5, Zoetis, Kirkland, Quebec

[2]Inforce 3, Zoetis, Kirkland, Quebec

[3]Triangle 5, Boehringer Ingelheim, Burlington, Ontario

[4]Microsoft Corporation, Redmond, Washington

[5]SPSS v.25, IBM, Armonk, New York, United States

Chapter 3

A COMPARISON OF THE EFFECTIVENESS OF INTRANASAL VS INJECTABLE VACCINES AS PRIMERS FOR PROTECTION AGAINST BOVINE RESPIRATORY DISEASE

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Author contributions: Erickson was responsible for design of the experiment, design of the data analysis, and manuscript review. Ellis was responsible for assistance in determining type of sample collected and laboratory tests to be ran. Lardner, Campbell, and Harding were responsible for manuscript review. Waldner was responsible for design of the data analysis and manuscript review. Berenik was responsible for data collection, the design and analysis of the data and manuscript preparation.

This chapter contains the analysis of data collected from field study trial conducted at a commercial cow-calf operation. This chapter represents a larger-scale field study to test the effectiveness of intranasal vaccines (IN) in reducing morbidity and mortality of bovine respiratory disease complex (BRD), and the effects the vaccination protocol has on average daily gain (ADG). Small scale disease challenge studies have been conducted with the results showing that IN vaccines can successfully prime the immune system (Xue et al., 2010) (Vangeel et al., 2009). The results of this chapter revealed that the control group, which received an injectable (IJ) vaccine, and the IN group did not have significant differences between morbidity, mortality, and ADG. Further research regarding heterologous vaccination would be beneficial, as this and most other research is performed using a homologous vaccination protocol.

Contributions: Calves were provided and cared for by private producers.

3.1 Abstract

Bovine respiratory disease (BRD) is important cause of morbidity, mortality and economic loss in beef production. Bovine respiratory disease has its greatest impact on lightweight calves due to weaning stress, mixing of source groups at auction marts and feedlot arrival, and transportation; all of which may occur in a short temporal window. This research project compares intranasal (IN) and injectable (IJ) modified live viral (MLV) vaccines as primers for BRD disease-related antibody production. Calves were randomly enrolled at branding into one of two experimental groups: IN or IJ. Both groups received a subcutaneous MLV vaccine at weaning. From weaning to 60 days post weaning, animal health data (morbidity, mortality) were collected, and calf body weights were recorded during standard production handling time points. Morbidity, mortality, and ADG were compared between vaccination groups while controlling for the effect of gender, feedlot pen, and farm pasture. The results showed that morbidity, mortality, and ADG were similar between vaccine groups and between genders ($p \geq 0.05$). These results suggest that both IN and IJ vaccines stimulate the immune system against BRD. This study was limited in its power to detect differences in mortality and therefore differences that are clinically significant may not be statistically significant. Mortality for the IN-MLV group had a strong numerical difference of 1.6% as compared to the IJ-MLV group with 0.3% from weaning to 60 days post weaning.

3.2 Introduction

Bovine respiratory disease (BRD) is an important causes of production losses in all aspects of the cattle industry (Waldner et al., 2018). Bovine respiratory disease results in large economic losses for the producer through cost of treatment, reduction in feed efficiency, loss in carcass value, and death losses (Cernicchiao et al., 2013) (Smith 2009). Research has found that BRD

morbidity is 16% on average across feedlots in the United States, with mortality ranging from 1.0 to 1.75% on average, depending on calf age (Aphis.usda.gov, 2013, b) (Miles et al., 2009).

Bovine respiratory disease is thought to be initiated by 1 or more viral infections that compromise the calf's immune system; allowing a secondary bacterial infection to occur (Panciera and Confer, 2010). Five viruses commonly associated with BRD include: bovine respiratory syncytial virus (BRSV), bovine herpes virus type 1 (BoHV1), parainfluenza 3 (PI3), and bovine viral diarrhea virus types 1 and 2 (BVDV1, BVDV2) (Miles, 2009) (Ridpath, 2010) (Jones, Chowdhury, 2010). The three viruses directly related to BRD are BRSV, BoHV1, PI3 (Miles, 2009). Bovine viral diarrhea virus type 1 and type 2 is not often viewed as a cause BRD, but rather suppresses the calf's immune system, allowing other pathogen infections to occur (Ridpath, 2010). Vaccination is an important method of controlling these viruses (Wildman et al., 2008).

Vaccination presents antigens of one or more pathogens to a host's immune system, without causing disease in the animal. After vaccination, the immune system develops memory cells, allowing a more rapid and powerful immune response when future, similar pathogen exposure occurs. Research has shown the best vaccination method to increase resistance against a virus is through a prime-boost vaccination method (Kardani et al., 2016) (Lu, 2009). This method consists of administering an initial vaccine (primer) followed by a second vaccine (booster). Commonly the prime-boost method is accomplished using an injectable modified live viral (MLV) vaccine for both the primer and the booster (Waldner et al., 2018). A problem that can occur with this protocol is failure of the priming vaccine to successfully stimulate the immune system due to interference from maternal antibodies (Munoz-Zanzi et al., 2002) (Platt et al., 2009) (Niewiesk, 2014), resulting in a poor immune response and leaving the calf susceptible to

disease. Intranasal (IN) vaccines have been shown to bypass maternal antibodies, allowing them to act as effective primers for the immune system (Xue et al., 2010) (Walz et al., 2017, 1) (Vangeel et al., 2009). Intranasal (IN) vaccines have been shown to offer similar protection against disease as injectable vaccines (Ellis et al., 2007) (Ellis et al., 2010, 2) (Mahan et al., 2016).

The current study compares the effectiveness of an intranasal MLV vaccine priming to an injectable MLV vaccine priming for protection against BRD. Effectiveness of the vaccine protocol will be determined through analyses of morbidity and mortality due to BRD, and average daily gains.

3.3 Materials and Methods

3.3.1 Animals

Privately owned crossbred commercial beef calves born to multiparous dams were sourced from two producers, designated Farm #1 and Farm #2, located in Saskatchewan, Canada. Calves at both farms were identified with a unique numbered dangle tag and a radio frequency identification (RFID) tag.

3.3.2 Randomized Controlled Trial

At branding, one to three months of age, calves were allocated into either the IJ-MLV group, or the IN-MLV group by systematic sampling, stratified by pasture. This was accomplished by flipping a coin at the start of the day to determine the order of group enrollment. When the coin flip was heads, the calves were enrolled in the order of one IJ-MLV group calf, then one IN-MLV group repeated until all calves from the pasture were enrolled. When the coin flip was tails, the calves were enrolled in the order of one IN-MLV group calf, then one IJ-MLV group calf, repeated until all calves on the field were enrolled. From Farm #1, 645 calves were enrolled into the two

groups at branding, which occurred in late June of 2017. At branding calves received a BRD vaccine (Bovishield Gold FP5[1], Inforce 3[2]), a multivalent *Clostridium sps.* vaccine[3], and a non-steroidal anti-inflammatory[4]. Calves were managed extensively on 7 pastures, with staff regularly checking calves for signs of disease. Calves were weaned in late September of 2017 and moved to a nearby feedlot. The treatment groups were commingled and housed in four pens by gender after moving to the feedlot. At weaning, the IJ-MLV group had 163 steers and 155 heifers, while the IN-MLV group had 169 steers and 150 heifers. Upon arrival at the feedlot, calves were processed and received a booster vaccine (Bovishield Gold One Shot[5]), a growth implant[6], a multivalent *Clostridium sps.* booster vaccine[3], and a metaphylactic antibiotic[7]. In the feedlot pens, study calves were comingled with non-trial calves, sourced from the same producer. The number of calves from each treatment group were similar within each of the pens (Table 3.1).

From Farm #2, 240 calves were enrolled in the IJ-MLV group, and 241 calves were enrolled in the IN-MLV group. Calves were randomly enrolled at branding, which occurred in Mid-August of 2017. Calves from Farm #2 were castrated at branding and received a dose of a nonsteroidal anti-inflammatory drug[4]. Calves were managed extensively on 3 pastures, with experienced animal health staff blinded to the treatment groups regularly checking calves for signs of disease. Calves were shipped to a feedlot approximately 80 kilometers away in the mid November, 2017. Due to the cattle market at the time, only the steers were shipped to the feedlot. The steers were separated into two pens, comingling with calves from auction marts at the feedlot.

3.3.3 Sample and Data Collection

Calves were monitored on a weekly basis by ranch staff for signs of BRD when on pasture and daily after weaning. Bovine respiratory disease cases were defined as calves that had a rectal temperature above or equal to 40°C (103.9 F°) as well as at least two of the following signs:

moderate depression (drooping head and ears), reluctance to move, tucking of the flank/abdomen, rapid shallow breathing, or increased respiratory effort. If a calf met the BRD case definition criteria, the calf was treated by feedlot staff and morbidity data were recorded.

All calves that died in the feedlot had a necropsy performed and digital photographs recorded within 24 hours to determine cause of death. Standard digital photographs and clinical history were provided to the blinded consulting veterinarian and cause of death was established for each animal. All causes of mortality were recorded; however only BRD mortality was included in the data analysis. Body weights were collected at two time-points for calculation of ADG; weights were collected upon arrival at the feedlot and at reapplication of a growth promoting implant, averaging 68 days on feed, ranging from 52 to 73 days.

3.3.4 Statistics

Statistical analyses were conducted using STATA Version 15. Post weaning mortality and morbidity were analyzed using a generalized linear mixed model equation with a logit link function and binomial distribution. Vaccine and gender were independent variables, while pen and pasture were included as random effects variables.

Average daily gain was determined from weights recorded at arrival to feedlot and reapplication of a growth promoting implant. The dates when weights were collected varied depending on the pasture and pen, and this is accounted for in the statistical calculations. The ADG results were analyzed using a multi-level mixed effects generalized linear model, using a normal distribution. Vaccine and gender were independent variables, while pen and pasture were included as random effects variables. The p-value was considered significant at a value of 0.05.

3.4 Results

3.4.1 Group Information

Farm #2 was originally planned to background all 480 calves, but due to feed prices, the producer sold the calves in mid-November. We were able to retain 196 steers sold to the same feedlot as calves from Farm #1. However, the calves from Farm #2 were not included in analysis because the calves were raised differently from the calves on farm #1, and the total calf numbers were too low for a useful independent analysis to occur.

3.4.2 Average Daily Gain

Average daily gain was compared between the treatment group or gender (Table 3.3). Neither the vaccine group ($p = 0.8$) nor gender ($p > 0.9$) were significant factors in determining ADG of calves. This shows that ADG was not impacted by the vaccine protocol in this study.

3.4.3 Morbidity Due to Bovine Respiratory Disease

Morbidity due to BRD was compared by treatment group or gender. No significant difference ($p = 0.7$) was seen between the treatment groups (Table 3.2). Gender was not a significant factor in determining difference between morbidity due to BRD (Table 3.2).

3.4.4 Mortality Due to Bovine Respiratory Disease

Mortality due to BRD was compared by treatment group or gender. No significant difference ($p = 0.1$) was observed between the treatment groups, though a large numerical absolute difference was found; injectable 0.3% (1) vs intranasal 1.6% (5) (Table 3.2). Gender was not associated with mortality due to BRD ($p = 0.6$).

3.5 Discussion

This study did not show a difference between the IN and IJ priming vaccines related to ADG. In the current study it is difficult to determine if the lack of difference in ADG was caused by vaccine success/failure, lack of disease pressure or reduced power in the study. To understand whether the vaccines successfully protected the animal from disease, antibody concentrations and lung lesion scores would have been useful parameters to measure, as was done in previous field studies (Ollivett et al., 2018) (Cavirani et al., 2016). These parameters allow researchers to more accurately identify respiratory disease in calves (both clinical and sub-clinical disease) (Forbes et al., 2004). This study found that ADG was not different between the IJ and IN groups, which agrees with previous research (Ollivett et al., 2018).

While this study did not measure disease severity, it has been shown in previous studies that IN and IJ vaccines can reduce the severity of disease similarly (Vangeel et al., 2007) (Ellis et al., 2010, b). A reduction in disease severity is beneficial to the producer, as it decreases the time calves spend in sick pens, the number of treatments, and the number of days the calf is on feed (Smith, 2009) (Cernicchiaro et al., 2013). However, vaccination does not guarantee protection to calves. Research in veal calves found that IN vaccines did not have significantly different morbidity or mortality rates between the IN and non-vaccinated groups (Cavirani et al., 2016). This provides evidence that calves with maternal antibodies do not gain a disease sparing effect due to IN vaccination over unvaccinated calves, however, the use of IN vaccine for immune stimulation in calves that have maternal antibodies could be important for protecting calves after the maternal antibodies have waned (Ellis et al., 2013), or as a primer for a booster vaccine (Hill et al., 2012). While disease severity was not determined in this study, it has been done in other field studies (Ollivett et al., 2018), using ultrasounds to determine the level of lung consolidation

in calves. This could be used to help determine the disease reduction each vaccine provides however it is not practical on commercial operations (Ollivett et al., 2018). While disease severity of each treatment group could not be determined, the study was able to compare important production parameters; morbidity, mortality, and average daily gain of the different study groups.

It has been shown previously that male calves have higher ADG and are more susceptible to BRD than heifers (Snowder et al., 2006). However, in this study no difference was found in ADG, or in morbidity, or mortality rates. Steers and heifers were housed in separate pens, comingling both experimental groups together, as well as comingling calves that were not on trial. These factors could have an impact on why no differences were observed between the genders. Part of the reason that no differences were seen could be due to vaccination protocols within the pen and calves being from a single source. These factors could promote herd immunity within the pen, leading to low disease pressure, causing a lack of difference between groups.

In this study, only 6 mortalities due to BRD, roughly 1%, were observed. The mortality rate due to BRD in feedlots has been observed to be between 1 and 2% depending on the risk group (Miles et al., 2009) (Engler et al., 2014), putting this trial on the lower end of average. The calves used in this chapter were relatively low risk, coming from a single producer. Of the 6 deaths, 1 was in the IJ group, and 5 were in the IN group. This difference was not significant ($p = 0.1$) but may be clinically important. To gain appropriate power (0.8) to determine if the mortality was significantly different, the IN-MLV and IJ-MLV groups would require 1021 calves each, given the mortality observed in this study. This information suggests that while a

difference was not seen in this trial, the vaccination protocol could have an impact on calf mortality.

Due to changes in beef calf market conditions the producer from farm #2 sold all enrolled heifer calves to a feedlot and we were unable to track them. Due to potential differences between heifers and steers, gender was accounted for in this study to analyze morbidity, mortality, and ADG. The use of both steers and heifers allowed us to observe how the different vaccine protocols affected the different gender of calves, as previous research has shown gender has an effect on ADG and morbidity (Cernicchiaro et al., 2012) (Snowder et al., 2006). The numbers of heifers and steers were similar between the IN-MLV and IJ-MLV groups, with the IJ-MLV group having 155 heifers and 163 steers and the IN-MLV group having 150 heifers and 169 steers.

The current study compared two homologous methods of antigen presentation in the vaccine protocol, as both were MLV vaccines. In human medicine, heterologous vaccination has been researched and used in practice as it has been shown to offer increased immune response (Bolton et al., 2012). Currently the cattle industry does not use heterologous vaccination methods, rather priming and boosting with the same vaccine type. Research conducted in Chapter 2 compared heterologous and homologous vaccination protocols for antibody concentrations against BRD, showing heterologous vaccination can increase antibody concentrations for BRSV. This is an area that future research should focus on, to determine best vaccination protocols.

3.6 Tables

Table 3.1. *Number of calves from each treatment allocated to each pen upon placement at the feedlot. Pen #1 and #2 contained only steers, and pen #3 and #4 contained only heifers.*

	Number of Calves				
	Pen 1	**Pen 2**	**Pen 3**	**Pen 4**	**Total**
Injectable	75	88	67	88	318
Intranasal	78	91	61	89	319

Table 3.2. Comparison of BRD morbidity, mortality, and ADG between treatment groups after placement at the feedlot until re-implantation. Treatment Groups are further subdivided by gender and combined.

	Injectable			Intranasal		
	Steer	**Heifer**	**Combined**	**Steer**	**Heifer**	**Combined**
BRD Morbidity	18 (11%)	11 (7%)	29 (9%)	19 (11%)	13 (9%)	32 (10%)
BRD Mortality	1 (0.6%)	0 (0.0%)	1 (0.3%)	3 (1.8%)	2 (1.3%)	5 (1.6%)
ADG (kg/day)	1.6	1.6	1.6	1.6	1.6	1.6

Table 3.3. The effect of gender and vaccine on ADG

		Coefficient	Confidence Interval		p-value
			Lower	Upper	
ADG (kg/day)	**Gender**	0.0090	-0.6	0.6	>0.9
	Vaccine	0.016	-0.1	0.1	0.8

For comparisons, the group used as the basis for comparison for gender was the steer group, and for vaccine was the injectable vaccine group.

Table 3.4. The effect of gender and vaccine on BRD Morbidity and Mortality

		Odds Ratio	Confidence Interval		p-value
			Lower	Upper	
Post Wean BRD Morbidity	**Gender**	0.84	0.3	2.9	0.8
	Vaccine	1.11	0.7	1.9	0.7
Post Wean BRD Mortality	**Gender**	0.54	0.1	5.9	0.6
	Vaccine	5.16	0.6	45.2	0.1

For comparisons, the group used as the basis for comparison for gender was the steer group, and for vaccine was the injectable vaccine group.

3.7 Endnotes

[1]Bovi-Shield Gold FP5, Zoetis, Kirkland, Quebec

[2]Inforce 3, Zoetis, Kirkland, Quebec

[3]Ultrabac 7, Zoetis, Kirkland, Quebec

[4]Meloxicam, Apotex Inc., Toronto, Ontario

[5]Bovi-Shield Gold One Shot, Zoetis, Kirkland, Quebec

[6]Revalor-G, Merck Animal Health, Kirkland, Quebec

[7]Draxxin, Zoetis, Kirkland, Quebec

Chapter 4

4.1 Objectives

The overarching research objectives of this thesis were to test the effectiveness of IN vaccines as primers to protect calves from BRD and compare homologous and heterologous vaccination protocols. To achieve these objectives two separate studies were conducted. Chapter 2 tests the effectiveness of a MLV IN vaccine primer given at birth, boosted with an IJ-MLV or KV vaccine at 'turnout' and weaning. These two protocols are compared to a standard protocol using an IJ-MLV primer at 'turnout', and an IJ-MLV booster at weaning. The effectiveness of the vaccine protocols was determined through antibody concentrations comparisons and ADG. An ELISA was used to determine the antibody concentrations for BRSV and BoHV1, and a virus neutralization was performed for BVDV types 1 and 2. Chapter 3 was a large-scale field study conducted to determine the effectiveness of IN vaccines as a primer compared to IJ vaccination. The effectiveness of the vaccine protocols was compared by measuring the difference in morbidity, mortality, and ADG.

4.2 General Discussion

The information in the two chapters add new information to the literature, filling in gaps of knowledge regarding vaccination for BRD in beef cattle. The majority of studies focusing on IN vaccines have been small scale challenge studies or lab studies (Ellis et al., 2007) (Ellis et al., 2010) (Vangeel et al., 2009) (Hill et al., 2012). Chapter 2 is a smaller scale study, having 75 animals and performing tests to determine antibody levels, to help determine the effectiveness of different vaccine protocols. It differentiates itself by comparing heterologous methods of vaccination to homologous methods. A small number of large-scale field studies have been conducted testing IN vaccines on veal and dairy calves (Cavirani et al., 2016) (Ollivett et al.,

2018). Chapter 3 is a larger scale field trial, which is rarely done using extensively managed beef cow-calf commercial operations, and it compares IN priming vaccines against IJ priming vaccines in calves destined for a feedlot. These studies are important to assist producers and veterinarians in choosing a proper vaccination protocol.

It has been well documented that IN vaccines are effective at providing calves protection when compared to non-vaccinated calves (Ellis et al., 2010) (Ellis et al., 2007) (Vangeel et al., 2007) (Vangeel et al., 2009). Intranasal vaccines have also been shown to effectively prime a calf's immune system in the face of maternal antibodies (Hill et al., 2012) (Ellis et al., 2013). Chapter 2 adds to this area of literature, by presenting a unique study through the inclusion of heterologous vaccination. Heterologous vaccination has been researched in human medicine (Lu, 2009) (Jung et al., 2018), and in beef cattle when focusing on reproductive disease (Walz et al., 2015). This study observes the effect of heterologous vaccination on BRD. Both chapter 2 and 3 agree that heterologous vaccination route did not increase immune stimulation above that of the homologous vaccination route. As for heterologous antigen presentation (MLV and KV), an increase in antibody concentrations for BRSV was observed in Chapter 2. This creates opportunities for further studies, as the order or vaccination could be important in obtaining the greatest level of protection against disease.

Clinical disease scores and morbidity are frequently used to determine the effectiveness of vaccines (Ellis et al., 2010, b) (Ellis et al., 2007) (Ollivett et al., 2018). If a calf is successfully vaccinated, its immune system will be prepared to respond to natural exposure of the disease. When the immune system responds rapidly, the virus has less chance of replicating in the host and causing illness. In both chapters 2 and 3, the IN and IJ vaccination groups had similar morbidity rates. Chapter 2 showed that both IN and IJ vaccines elicit a similar immune response

to BRSV and BoHV1, when homologous antigen presentation is used. Chapter 3 found that IN and IJ vaccine primers resulted in a similar morbidity rate when calves were placed in a feedlot setting. These results agree with previous research, which has found that both vaccine types can elicit a similar immune response/protection (Ellis et al., 2013) (Vangeel et al., 2007) (Hill et al., 2012).

Mortality is an important parameter when studying BRD. In Chapter 2 mortality was not significantly different between the IN and IJ vaccine groups, however in Chapter 3 a numerical difference was seen, with the p-value trending towards significance. These results agree with previous studies that have found similar results; however, the studies are often challenge studies and are not directly comparable to a field study (Ellis et al., 2010) (Vangeel et al., 2009) (Ellis et al., 2007). One reason that challenge studies are used is because they require a lower number of animals to see a difference in the morbidity and mortality of different groups. In Chapter 3, a larger population was used, compared to the aforementioned challenge studies. The mortality was trending towards significant in Chapter 3, and if a larger population size had been used, a significant difference may have been observed.

In both experimental chapters of this thesis, ADG was observed, to assess differences between vaccination protocols. Average daily gain is an important economic factor for producers. Average daily gain can be influenced by several factors such as: morbidity, genetics, pen condition, weather, and feed consumed (Belasco et al., 2015). Genetics and weather are factors that a feedlot will have difficulty manipulating in a given population of animals, but morbidity is one that can be manipulated and improved through vaccination, and the ability of vaccines to control disease and sub clinical disease could result in an increased ADG (Forbes et al., 2004). Throughout the studies in Chapters 2 and 3, no significant difference was observed in ADG.

However, a small numerical difference was seen between the ADG in the groups, suggesting that further research in vaccine strategies may be of worth to cattle producers.

While differences in vaccination protocols were seen throughout these studies, the way a producer manages their cattle will have an impact on the protocol that they use. The important benefit to IN vaccines is their ability to bypass maternal antibodies (Ellis et al., 2013). If a producer vaccinates their calves after maternal antibodies have waned (3-4 months after birth), then an IJ vaccine may be more effective as it contains antigens against BVDV types 1 and 2, whereas commercial IN vaccines do not (Downy et al., 2013) (Fulton et al., 2004). When choosing a vaccination protocol, the type of diseases that are most common or most impactful to the producer should be considered. For example, if a producer frequently had BRSV-related respiratory disease diagnosed in calves, they may consider a heterologous method, as the study in Chapter 2 showed the heterologous group had higher antibody concentrations against BRSV. Producers should also consider their handling facilities when choosing a vaccination protocol. If a producer does not restrain the calves head appropriately, it can be more difficult to properly administer IN vaccines compared to IJ vaccines, due to the freedom of movement the calf's head. However, with the use of a calf tipping table or ropes, IN vaccines may be just as easy to administer as IJ vaccines. These are a few factors that should be considered while a producer is deciding on a vaccination protocol for their cattle.

The two studies performed in this thesis build upon the knowledge of each other. Chapter 3 was effective in showing that in a commercial calf production system, IN and IJ vaccines offer similar protection against BRD in a feedlot. Chapter 2 was beneficial as it allowed us to measure the effects that the different vaccination protocols had on the immune systems of the calves. Chapter 2 helps explain why the results in Chapter 3 may have occurred. Providing vaccination

at birth helps determine if IN vaccines could effectively bypass maternal antibodies, act as an effective primer, and provide increased immune stimulation over calves not vaccinated at birth. This research shows that IN vaccines successfully prime the immune system, even in the face of maternal antibodies. It also shows that prime-boosting with heterologous antigen presentation can be beneficial depending on the virus.

While the studies in Chapter 2 and Chapter 3 were successful and answered important questions, there were limitations to the studies. Chapter 2 was a smaller scale laboratory-based study, focusing on the immune responses of calves to different vaccination protocols. One limitation of this study was the type of sample collected. It has been shown in previous studies that IN vaccines elicit a mucosal response while IJ vaccines elicit a systemic response. The tests conducted only measured systemic immune responses, minimizing the results of the IN vaccines. Previous research has clearly shown a mucosal immune response to IN vaccination in cattle (Hill et al., 2012). The type of tests used also minimized the immune response BoHV1 may have had, since BoHV1 vaccination results in a strong CMI compared to the IgG response. Using multiple tests to measure all immune responses would have been ideal, however the cost to run all the tests outweighed the benefit for this project. While exact differences between all immune response measures weren't tested, a general idea of immune response trend was observed. Another limitation of this study was the outcome measure itself. Using antibody concentrations is a useful way to determine if an immune response is different between vaccination groups. The limitation is that antibody concentration does not directly translate to disease protection. While it is good to know that antibody differences occur, without knowing how they impact BRD morbidity, antibody concentration do not always reflect clinical differences and this is why larger scale field trials, such as that in Chapter 3, are needed.

Chapter 3 was a larger scale field study, observing morbidity, mortality and average daily gains. One limitation to this study was the use of commercial herds and this presents a challenge because it is difficult to monitor the management of the herds. While using commercial herds is a limitation, it is also one of the most important benefits of this trial because it allowed the comparison of vaccine protocols in a 'real world' situation. The difficulty in managing these larger cow-calf field trials is why they are rarely done, but this makes this trial very unique and will provide useful information to producers and veterinarians. An important example of this limitation is with Farm #2, which had calves that were born and branded later than Farm #1, so they were too different to be analyzed together, as well as being sold where they could not be followed. Another limitation of a larger scale field trial is related to measurement of disease. While morbidity and mortality were able to be measured, it was difficult to be sure that every animal showing signs of respiratory disease was identified due to the non-specific means of BRD identification used here and commonly in commercial production. Also, there were losses of calves that occurred without post mortem diagnosis. Cause of death on pasture was difficult to determine because the calves were often severely scavenged when they were found, and some carcasses were not found at all. However, the available data will help veterinarians better understand the effectiveness of vaccines on commercial cattle. One limitation of the commercial feedlot setting was mixing of trial calves with non-trial calves in the pens; mixing of calves from outside the trial may have provided a greater disease challenge, however there was no way to insure the level of challenge was the same in each pen.

This study has shown that heterologous vaccination is an effective method for the control of BRD. Chapter 2 outlines the need to consider more than one type of immune response, and the need of larger scale field studies. Future studies should measure not only IgG immune

responses, but also IgA when using IN vaccines, or CMI when measuring BoHV1 immune responses. Antibody concentrations are useful for preliminary studies, but do not translate into disease control. Larger scale field studies are important in determining disease control capabilities of vaccines, as they can measure morbidity and mortality in natural environments. Currently larger scale cow-calf field studies are not common, due to the cost and potential challenges. Future research should focus on the use of heterologous vaccination protocols in larger scale field studies, to get a better understanding of how these protocols can impact morbidity, mortality, and average daily gain.

Chapter 2 showed that the response to vaccine antigen for BVDV types 1 and 2 in the face of maternal antibodies was poor. The poor priming response in young calves should be further investigated to find a better method of priming calves in the face of maternal antibodies. One approach would be the use of BVDV antigens mucosally in the face of maternal antibodies rather than using an injectable presentation of the antigen. This work should also observe the difference in heterologous vs homologous boosting.

4.3 General Conclusions

The results of these two studies provide important information for producers, veterinarians, and researchers, through the introduction of a new type of vaccination program to BRD; heterologous vaccination. It confirms that IN vaccinations can have a similar effect on animal health as injectable vaccines, and that heterologous vaccination protocols can be beneficial over homologous vaccination protocols in some cases.

Throughout the two studies, IN-MLV vaccines were delivered as the priming vaccines in the test groups, while injectable vaccines were delivered as the priming vaccine in the control groups. The two chapters of this thesis agreed that IN vaccines were able to successfully prime the

immune system, similar to that of IJ vaccines. In Chapter 2, the IN groups had a similar or higher antibody concentration at two weeks post 'turnout', suggesting that IN vaccines caused an immune response or delayed antibody concentration waning. Chapter 3 observed similar morbidity and mortality rates between the IN and IJ vaccine primers, though mortality for the IN group was numerically higher. However, the overall conclusion of the field study is that IN vaccines show no difference in priming ability compared to IJ vaccines.

Chapter 2 highlights the potential benefits of heterologous vaccination methods. This chapter found that calves receiving a priming MLV vaccine and boosted with a KV vaccine had a higher BRSV antibody concentrations then the homologous MLV groups. Bovine herpes virus type 1 was similar between groups at all time points except at weaning, when antibody concentrations were low. Commercial IN vaccines do not have antigens for BVDV types 1 or 2, therefore these viruses antibody concentrations were tested homologously. The results show that MatAb interference likely occurred with BVDV1, and that the MLV vaccine had a greater antibody response than the KV for BVDV2. These studies provide a solid foundation for the study of heterologous vaccination methods and useful information for bovine practitioners.

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