WATER RESTRICTION AND VACCINATION IMPACTS IN BEEF CALVES

by

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ABSTRACT

In the United States beef marketing process, calves are typically weaned from their dam and transported to a local auction market where they are purchased and transported to an order buying facility. They are subsequently transported again to a stocker or feedlot destination such that the potential exists for significant duration of water restriction. Upon feedlot arrival, stressed and dehydrated animals are processed, and this procedure typically includes administration of a modified-live virus (MLV) respiratory vaccine. The objective of this thesis was to review and evaluate the effect of water restriction on hematological, performance, and antibody parameters in beef calves administered a parenteral or intranasal MLV respiratory vaccine. A total of 60 clinically healthy, previously unvaccinated crossbred beef steer (n=28) and heifer (n=32) calves were assigned randomly to 1 of 6 treatments arranged in a 3 × 2 factorial. Three water restriction treatments (Factor A) were applied at the origin ranch: A_1) Control, no water restriction at any time other than during transport (**CON**), A_2) Acute, 48 h water restriction prior to transport (**ACU**), or A_3) Chronic, alternating 24 h periods of water access and restriction over a 7-day period prior to transport (**CHR**). Upon feedlot arrival (day 0), 2 respiratory vaccine treatments were applied (Factor B): B_1) parenteral administration of a pentavalent (IBRV, BVDV type 1 and 2, BRSV, PI3V) MLV respiratory vaccine (2mL subcutaneous; Express 5; **SUB**), or B_2) intranasal administration of a trivalent (IBRV, BRSV, PI3V) MLV respiratory vaccine (1 mL/naris;

Inforce 3; **INT**). Water restriction (ACU or CHR) increased hematocrit ($P < 0.01$) and transiently decreased BW ($P < 0.01$) and ADG ($P = 0.03$) from the time of water restriction treatment application through d 14. Transport caused an increase in white blood cells (WBC; day effect, $P < 0.01$); however, WBC decreased transiently after transport until day 7 (day effect, $P < 0.01$). The SUB group had a decreased total WBC count ($P < 0.01$) with specific decreases in lymphocytes (vaccine × day; $P < 0.01$) and monocytes ($P = 0.01$) and a greater body weight change from day 42 to 56 ($P = 0.01$) compared to INT. The ACU and CHR treatments had reduced ADG from day 0 to 14 compared to CON ($P \leq 0.04$), regardless of vaccine treatment. The INT group tended to have greater overall serum BRSV- and IBRV-specific antibody titer concentrations ($P \leq 0.08$) and BRSV-specific sIgA percent positivity index (vaccine × day; $P < 0.01$). There was a vaccine treatment × day interaction *(*$P < 0.01$) such that the INT groups had increased rectal temperature day 7 and 14 ($P \leq 0.04$), greater BRSV-specific IgA on day 35, 42 and 56 ($P \leq 0.04$), and greater BRSV and IBRV antibody titers on day 14 through 56 and day 21 through 35 respectively ($P \leq 0.04$). These observations suggest greater antigenicity of INT when administered to stressed and water restricted animals. The stress of transport and dehydration may cause immune dysfunction that allows intranasally administered antigens to more effectively penetrate a compromised mucosal barrier in the naris. There was no apparent long-term effect of water restriction on antibody response in either vaccine group; however, the antibody responses to either vaccine in this study were low and may have been impacted by various lengths of water restriction experienced by all animals. Alternatively, the low antibody responses to

vaccines evaluated in the current study could be due to other factors such as lack of a booster vaccination, genetic effects, or other individual animal variations.

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CHAPTER I

INTRODUCTION

Bovine respiratory disease (BRD) is considered to be one of the most complex and costly cattle diseases (Griffin, 2014) that is responsible for the majority of morbidity and mortality in feedlots (Miles, 2009). The complexity of the disease is due to the multiple viral and bacterial pathogens that contribute to its pathogenesis and furthermore make it difficult to diagnose and treat. In addition, many calves that arrive at the feedlot have never received a respiratory vaccine and have experienced the stress of weaning, transportation, and commingling which can increase BRD risk (Taylor et al., 2010). The marketing of beef calves in the United States typically involves the cow-calf origin, the local auction market, an order buyer, and finally arrival to a stocker or feedlot destination. During this lengthy and complex marketing process, calves are often restricted from water, at least during transport, in addition to experiencing other stressors such as weaning, extensive handling, commingling and relocation. Water restriction can result in dehydration if it continues for an extended period of time and initiates the renin-angiotensin aldosterone system, the system responsible for maintaining osmolality and blood pressure and causes other metabolic, physiologic and immunologic alteration (Henderson and Henderson, 1994).

Numerous viral respiratory vaccines are available on the market today including live attenuated and killed versions with various antigen inclusions. However, respiratory

vaccine administration occurs more often at the feedlot than the origin ranch (USDA, 2010; USDA, 2011), yet the efficacy of the procedure hinges on appropriate timing of vaccination before virus challenge occurs and administration to immunocompetent animals (Richeson et al., 2016). Once beef calves reach the feedlot, some have already been exposed to viral pathogens and stress is inherent. Vaccination with a live-attenuated viral vaccine upon feedlot arrival may be ineffective or even detrimental in such a scenario (Callan, 2001; Richeson et al., 2008; Rogers et al., 2016). In other studies with seronegative animals, a serum antibody response following vaccination or challenge was not detected until after day 7, indicating that protection is not immediate (Nobiron et al., 2003; Richeson et al., 2016). An area of growing interest in the vaccine industry is the use of intranasal vaccines in addition to, or in lieu of parenteral vaccines. The majority of the invading viral pathogens gain host access through the nose, mouth, or other mucosal membranes (Chadwick et al., 2009). Intranasal vaccination is known to increase mucosal or local immunity, specifically via production of secretory IgA, to a greater extent than parenteral vaccination (Holmgren and Czerkinsky, 2005). However, determination of the safety and efficacy of various vaccine types is needed in cattle subjected to typical stressors encountered during marketing, such as water restriction and transportation.

CHAPTER II

REVIEW OF LITERATURE

Bovine Respiratory Disease

Pathogenesis of Bovine Respiratory Disease

Bovine respiratory disease (BRD) is a complex and costly disease affecting an estimated 16.2% of all feedlot cattle (USDA, 2013a). Multiple viral and bacterial pathogens are known to be causative agents and stress-induced immunosuppression can have synergistic effects on BRD pathogenesis (Callan and Garry, 2002). Bovine respiratory disease has been studied for over 60 years (Graham, 1953); however, the incidence of BRD mortality in feedlot calves did not change significantly from 1991 to 2013 according to a report by Miles and Rogers (2014). The lack of significant progress in reducing BRD incidence is in part due to the multifactorial etiology, but also due to inability to promptly and accurately diagnose the disease. Clinical BRD signs include fever, rapid or labored breathing, coughing, nasal or eye discharge, and decreased appetite (Snowder et al., 2006; Schneider et al., 2009); however, none of these signs are truly pathognomonic to BRD. Schneider et al. (2009) followed animals from the feedlot to slaughter where lung lesion scores were recorded. In their study, lung lesions were present in 60.6% of calves that were never treated for BRD and 26% of calves that were treated at least once did not have lung lesions suggesting poor diagnosis at the feedlot. In

feedlot calves, BRD is most often detected between day 5 to 80 following arrival and typically peaks around day 14 (Snowder et al., 2006).

Viral Pathogens

The four major viral pathogens associated with BRD are bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV), bovine respiratory syncytial virus (BRSV) and parainfluenza-3 virus (PI3V). In the cascade of events that must occur for an animal to develop BRD, infection by a viral pathogen is typically preceded by stress, physiological alteration and immune dysfunction. The viral pathogens are responsible for causing further immunosuppression that allows bacterial pathogens to invade the host's lungs (Woolums, 2015) resulting in pulmonary infection that develops into bronchopneumonia. Immunosuppression is achieved by several mechanisms including increased apoptosis of cells in the upper and lower airway then in the pulmonary parenchyma (Michel et al., 2008), decreased NK-cell activity and increased pro-inflammatory cytokine production (Hodgson et al., 2005). Each of the viral pathogens have different infection and survival mechanisms, and makes eradication and timely identification of the various BRD viruses difficult.

Bovine viral diarrhea virus survives in the population through persistently infected (PI) calves which represent up to 2% of the population (Fray et al., 2000; Wittum et al., 2001). These PI calves may appear normal but continuously shed infectious virus (Xue et al., 2011) and can only be identified via laboratory analyses from ear-notch tissue samples. After infection, BVDV destroys lymphoid tissue in Peyer's Patches and reduces antigen presenting cell activity (Chase, 2013) causing immunosuppression in the animal. Infectious bovine rhinotracheitis virus can be

transmitted via aerosolization and possesses latent properties due to transcription of latency-related genes (Ellis, 2009). Stress and the associated increase in production of corticosteroids have been shown to induce recrudescence of IBRV following initial infection (Davies et al., 1973; Kapil and Basaraba, 1997) and can account for IBRV outbreaks in the absence of acute infection or in a "closed herd" (Ellis, 2009). Bovine respiratory syncytial virus can also be transmitted via aerosolization or direct contact, but does not contain latent properties. Due to its replication methods, BRSV is very susceptible to genetic changes (Ellis, 2009) with variation of up to 11% reported in samplings from the same herd (Larsen et al., 2000). It is reported that IBRV, BRSV, and PI3V infect via mucosal epithelial cells and alveolar macrophages which can impair local immunity in areas vital for respiratory defense (Bryson, 1985; Wyler et al., 1989) while also destroying cilia which can decrease the body's natural defense to a secondary bacterial infection (Cusack et al., 2003).

Bacterial Pathogens

Following infection with a viral pathogen, otherwise commensal bacterial may exploit the animals' immunosuppressed state. *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* are gram negative, commensal bacteria that can be found in the upper respiratory tract of healthy animals but can become opportunistic (pathogenic) during times of stress or viral infection (Gershwin et al., 2015; Woolums, 2015) and are considered the most common bacterial pathogens involved in BRD. As gram negative bacteria, all three of these bacterial pathogens contain lipopolysaccharide (LPS) endotoxins in their cell wall (Cusack, 2003). These pathogens are initially recognized by innate immune cells via Pathogen Associated Molecular Patterns (PAMPs) and then

engulfed by activated macrophages or neutrophils. Each bacteria have unique defense mechanisms and virulence factors. *Mannheimia haemolytica* produces the ruminant-specific toxin, leukotoxin, which kills macrophages and inhibits phagocytosis (Corbeil and Gogolewski, 1985) while extracellular fractions have been shown to destroy neutrophils (Berggren et al., 1981). On the other hand, the capsule of type A *P. multocida* inhibits neutrophils (Ryu et al., 1984) and allows the bacteria to avoid phagocytosis (Dabo et al., 2007). *Histophilus somni* contains a lipooligosaccharide (LOS) that causes apoptosis of endothelial cells and releases histamines which can result in vascular leakage and bronchoconstriction (Corbeil, 2007). *Mycoplasma bovis* has also been isolated from the lungs of healthy animals (Allen et al., 1992) as well as animals that developed BRD; however, in BRD cases it was often isolated during the later stages of pneumonia (Fulton, 2009). This has caused scientists to question whether *M. bovis* is a primary pathogen or if it infects opportunistically after the animal develops pneumonia and is therefore a secondary pathogen (Fulton, 2009). These differing virulence factors for each bacterial pathogen add to the difficulty of controlling and treating BRD.

Stress Impact

In today's beef cattle marketing process, calves that are abruptly weaned and transported to a local auction market experience multiple stressors over several days which can create chronic stress in the animal. Acute stress results from stressors occurring less than 24 hours, such as handling a group of calves through the chute then returning them to their pen. This acute stress is proposed to prime the immune system by increasing the number of cytotoxic T- and NK cells (Naliboff et al., 1991) and increasing secretory IgA production (Bosch et al., 2004). Chronic stress manifests if stressors occur longer than 24

hours, can include more than one individual stressor simultaneously, and causes immunosuppression in the animal (Richeson et al., 2016). The stress that occurs during the typical beef cattle marketing process described above is an example of chronic stress. These calves are experiencing psychological stress from abrupt weaning, transportation, commingling with calves from other cow calf operations, and adaptation to a new environment. Additionally, physical stress manifests from food and water restriction, change in diet, castration, branding and dehorning upon feedlot arrival if applicable, and pathogen exposure (Griebel et al., 2014). Factors such as the duration and type of stressor play into the complex relationship between stress and disease resistance (Hodgson et al., 2005). Another reason for this suppressed immunity is that when the animal is stressed, nutrients are repartitioned and diverted to activities that are imperative for survival (Sapolsky et al., 2000). Multiple stressors simultaneously can cause the animal to become immunosuppressed and increase the risk of developing BRD (Cole, 1996). This immunosuppression from chronic stress is due to the inhibition of L-selectin and consequently reduced translocation of neutrophils as mediated by glucocorticoids such as cortisol (Burton et al., 1995; Burton et al., 2005), reduced NK activity via cytokines (Esterling et al., 1996) and decreased secretory IgA production (Bosch et al., 2004). In previous studies, calves that have not been previously administered a respiratory vaccine and are weaned, transported and commingled with calves from other farms are at the greatest risk of developing BRD (Ribble et al., 1995; Stanton, 2009) and this scenario is realized for a significant number of beef calves in the U.S. production system (Smith, 2004).

Calf Origin

The management that an animal receives at the origin ranch is a critical component of the stress that the calf will experience during relocation to the feedlot. During the relocation process, the animal will undoubtedly undergo stress. One strategy to prepare the calves for this transition is to vaccinate the cows before the breeding season, or vaccinate the calves a minimum of 2 weeks prior to relocation. Vaccinating the cows prior to breeding can enhance maternal antibody production which increases the calves' chance of acquiring passive immunity if colostrum intake is sufficient, while vaccinating the calves boosts their own immune system. According to the Beef 2007- 08 (USDA, 2009) survey, only 53% of operations administered some form of injection to cows; of these injections, 35% were given intramuscular (IM); of those 35% IM, 66% were vaccinations. This passive transfer is critical and it has been suggested that 10 to 25% of calves do not receive adequate colostrum immunoglobulins (Perino, 1997). Wittum and Perino (1995) measured serum IgG and plasma protein in calves 24 hours after birth then followed the calves to the feedlot. Animals that became morbid at the feedlot due to respiratory issues had lower plasma protein concentrations compared to animals that were not morbid; however, serum IgG concentrations did not differ between the morbid and nonmorbid animals. Only 39% of calves received a respiratory vaccine prior to sale (USDA, 2010). Calves that arrive at the feedlot and have never been vaccinated against the BRD associated viruses may have low antibody concentrations to these viral antigens upon feedlot arrival which has been associated with either increased morbidity, increased number of treatments, increased treatments costs or decreased net value to the owner (Fulton et al., 2011).

Weaning

In United States cow calf operations, calves are weaned at an average of 207 days of age (USDA, 2008), a time when maternal antibodies begin to diminish. When calves are weaned, or removed from the dam permanently, multiple weaning methods may be employed such as abrupt, fence-line, and two-step. When calves are weaned, they experience psychological stress due to separation from the dam and deprivation of milk. This separation forces the calf to transition to a forage or concentrate diet and learn to care for itself. In previous research studying the effect of preconditioning prior to feedlot arrival, it was observed that within 24 hours after feedlot arrival, the preconditioned calves were observed to be at the water tank more often than non-conditioned calves (Schwartzkopf-Genswein et al. 2007). However, in this study, the preconditioned calves had access to feed during this time while the non-conditioned calves did not due to a subsequent 2 hour transport that occurred 24 hours after feedlot arrival to mimic production settings. This could account for decreased time at the water tank since feed intake and water intake have been reported to be correlated (MacFarland and Wright, 1969; Wilson, 1970; Bond et al., 1973; Bond et al., 1976). Jarvis et al. (1996) reported that cattle arriving at the slaughterhouse from auction markets consumed water more often and for longer periods than cattle that were transported directly from the farm. The cattle age or weight in this study was not disclosed, however; cattle were being transported to the slaughterhouse so it can be assumed that these were mature animals. Cattle age, and therefore previous acclimation, may factor into their behavior upon arrival and affect their ability to adapt to their environment more quickly. However, recently weaned animals may behave differently upon feedlot arrival.

In a study by Griebel et al. (2014), calves were either weaned abruptly or were weaned using the two-step procedure. Calves in the two-step weaning program had a plastic nose paddle inserted 4 days prior to transportation and were allowed to remain with their dam. On day -1, all calves were transported to the research facility 3.5 hours away and then challenged with BHV-1 on day 0. In this study, the calves that were abruptly weaned had a greater serum haptoglobin concentration on day 3 and 5 compared to the two-step weaned calves. Two-step weaning also reduced peripheral blood mononuclear cell production of TNF-α following the BHV-1 challenge. This study suggests that abrupt weaning, combined with transportation and a viral challenge was more stressful on the animal and increased their inflammatory response in a manner that could compromise the animals' immune response to viral infection.

Transportation

Transportation itself is a stressful event and has been shown to increase serum cortisol (Kegley et al., 1997; Fazio et al., 2005). When feed-restricted calves were compared to calves that were feed-restricted and transported, the feed-restricted and transported group had greater weight loss, nutrient loss (White et al., 2009) and shrink (Marques et al., 2012) compared to fasted only groups. One study evaluating the stress of transportation reported that when calves were transported less than 24 hours, the greatest stressor was the loading and unloading of calves from a truck (Cole et al., 1988). However, the time spent in a trailer may be stressful in itself and the effects can vary depending on the experience of the driver, the age and origin of the animal, heat or cold stress, and the time when cattle were loaded onto the trailer (Gonzalez et al., 2012a). Shrink was increased in

cattle that were transported by drivers with less than 5 years of experience, feeder cattle loaded at farms or feedlots, and cattle that were loaded during the afternoon or evening (Gonzalez et al., 2012a). Cattle were also at greater risk of BRD if they were transported in compartments with more than 15 calves (White et al., 2009), or simply had exposure to other calves during transportation (Phillips et al., 1985). Calves housed in individual stalls had decreased weight loss compared to calves that were not transported in individual stalls and had access to other calves (Phillips et al., 1985).

Commingling

Commingling initially occurs at the auction market, and is maintained or exacerbated when calves are loaded onto the trailer for transport to an order buyer facility or feedlot. Many small farms will market 5 calves at a time which have $6.37/45.45 kg less value than calves that are sold in larger groups (Smith, 2009). Troxel and Gadberry (2013) also reported that calves sold in groups of greater than 6 received a greater price than calves sold in groups of 2 to 5 or individually. In 2010, a greater percentage of cattle sold at Arkansas auctions were marketed as individuals (75%) rather than in groups of 2 to 5 (20%) or groups greater than 6 (5%; Troxel and Gadberry, 2013). Calves that are grouped with calves from other sources suffer from greater BRD morbidity and mortality (Martin et al., 1982) compared to calves that were from a single source ranch and commingled calves were treated more often for BRD, resulting in greater health costs (Step et al., 2008). This increase in morbidity and mortality could be due to increased pathogen exposure (Griebel et al., 2014), immunosuppression caused by stress in calves during establishment of the hierarchy structure of the group or pen, or both. Step et al. (2008) observed single source calves to have greater ADG and G:F from day 15 to 28 compared

to the auction market calves and single source cattle tended to have a greater BW on day 42 and greater overall ADG from day 1 to 42. When comparing morbidity, single source calves had the lowest percentage of BRD morbidity (11.1%), single source and auction market calves that were commingled were intermediate (22.6%), and multiple source auction market calves had the greatest percentage (41.9%). It is recommended that calves not be commingled or minimize the degree of such to reduce stress and the risk of developing BRD, but this is not considered with current marketing strategies.

Castration and dehorning

In 2007, the operation average percent of animals that were castrated and expected to be dehorned at the origin ranch was 55 and 49%, respectively (USDA, 2008). Calves that are not castrated at the origin ranch are typically castrated upon arrival at the stocker or feedlot facility. The stress of castration in addition to the stress of transport, commingling and pathogen exposure further increases the animal's risk. When the morbidity of steers was compared to the morbidity of bulls that were castrated upon arrival at the feedlot, steers had decreased morbidity (Pinchak et al., 2004; Richeson et al., 2013; Ratcliff et al., 2014). Similar to castration, dehorning within two weeks of arrival has also been shown to increase morbidity (Martin et al., 1982). However, Ratcliff et al. (2014) observed no difference in morbidity between animals that were polled or dehorned prior to feedlot arrival and animals that were dehorned on arrival.

Water Restriction

Water as a Nutrient

Water is necessary for survival of all organisms. The amount of water an animal requires varies depending on age, stage of production, moisture and salt intake, and temperature

(Gadberry, 2016). The quality of the water consumed can also affect the amount of water an animal consumes and the health of the animal. Water that contains elevated levels of dissolved salts, nitrates, sulfates, or hydrogen sulfide can create an off-flavor in water which will decrease consumption that can impact performance (Gadberry, 2016). Water is needed to maintain homeostasis of pH, osmotic pressure and acid-base balance in animals (Hogan et al., 2007). The disruption or depletion of water electrolyte and acid base imbalance can rapidly lead to an animals' death (Leal et al., 2012). Cell hydration was also shown to impact viral replication (Offensperger et al., 1994). Offensperger et al. (1994) cultured hepatocytes from ducks infected with duck hepatitis B virus then exposed the cells to a hypotonic or hypertonic medium, which caused cell swelling and cell shrinkage respectively, *in vivo*. Viral replication was decreased by 50% in the hypotonic medium but increased 4-fold in the hypertonic medium. The reason for this is unknown but indicates that cell hydration may be important when it comes to immunity against viral replication.

Industry Contributors

Animals require sufficient feed and water to maintain a state of homeostasis (Rumsey and Bond, 1976). However, when cattle are transported in North America it is very common for them to not have access to hay or water or to be off-loaded for rest periods (Gonzalez et al., 2012b). These conditions can have detrimental effects on the animals; however, in ruminants such as cattle, the rumen acts as a water reservoir which allows them to endure long periods without water (Silanikove, 1994). However, due to the nature of the cattle marketing system in North America, animals may have limited access to water for 24 to 72 hours, which can greatly impact their health and performance.

Transportation

As mentioned before, stressors can have a multiplicative effect; meaning that as an animal is subjected to multiple stressors, such as transport, water restriction, and commingling simultaneously, they may have a greater stress response than when compared to an animal that is subjected to only one stressor. The effect of transportation and water restriction on cortisol levels are not consistent in the literature. Kegley et al. (1997) collected blood from 48 steers, transported the animals 343 km to a different location where they rested overnight and were given access to water but not feed. The following morning, calves were transported 50 km to the feedlot. Cortisol in the transported calves decreased numerically from the time the animals were loaded at the feedlot the first morning and unloaded at the second location in the afternoon. However, from the time the calves were bled at the second location to the time they were bled at the feedlot the second time, cortisol concentration had increased numerically. Kent and Ewbank (1983) showed an increase in cortisol after loading which peaked at 2 hours post-loading and then decreased at hour 6; however, at hour 6 the cortisol concentration of the transported calves remained greater than cortisol for the nontransported calves. Other studies report conflicting results on cortisol post-transport; Crookshanks et al. (1979) reported increased cortisol at hour 12 and 24 following 12 hour transport of cattle; Fazio et al. (2005) reported no change in cortisol after short distance (45-65 km) or long distance (2200 km) travel; and Parker et al. (2004) reported increased cortisol in *Bos indicus* steers 60 hours following water restriction.

Packed cell volume (PCV) is the volume percentage of red blood cells in whole blood, which is often used as an indicator of dehydration in cattle. In studies that

evaluated PCV after transportation of cattle, Kegley et al. (1997) and Kent and Ewbank (1983) observed that PCV decreased after transportation while Schaefer et al. (1990) and Rumsey and Bond (1976) observed an increase in PCV as time of feed and water restriction increased. However, Vogel et al. (2011) observed no difference in PCV when Holstein cows were restricted water access for 36 hours. The effects of transport and water restriction on cortisol or PCV have not been consistent, but it is generally acknowledged that transport and water restriction will increase both cortisol and PCV. The inconsistent results in the literature may be due to other factors, such as breed and age of the animal, sampling criteria, laboratory error or previous stressors encountered before study initiation.

Order Buyer System

In the United States, there are two primary marketing channels that beef calves may go through to reach their feedlot destination. Calves are either sold directly to the stocker or feedlot operation, or they are sold at a local auction market. At the local auction market, the calves are purchased by an order buyer who is assembling groups of calves for stocker or feedlot customers. Customers contact the order buyer and inform them of the price, weight, color, sex, and other desirable features in a group of calves. The order buyer then travels to local auctions and purchases as many calves as possible that meet the customer's criteria. The day of or after purchase, the calves are often transported to the order buyers' facility where they are commingled with calves from other auction markets that may be sorted further and shipped to the customer's stocker or feedlot operation. During transport and sometimes at the local auction market and order buyer facility, these calves may be restricted from feed and water access. Calves may remain at

the order buyers' facility as little as one day and up to one week. The holding time depends on factors such as how long it takes the buyer to assemble the appropriate number and type of calves that were requested or overall demand from buyers. Once the order is filled, the calves are commingled again and transported to their stocker or feedlot destination.

Physiological Effects of Dehydration

During homeostasis, the chief cations present in extracellular fluids and intracellular compartments are sodium and potassium (Thornton, 2010). Periods of dehydration or hypohydration result in decreased fluid volume and corresponding increased blood osmolality (Silanikove, 1994) and sodium concentration. Parker et al. (2003) observed that during dehydration, daily urinary sodium levels decreased as total urinary volume decreased. Likewise, periods of hyperhydration result in an increased fluid volume and decreased serum sodium concentration. This increase or decrease in osmolality stimulates the macula densa of the kidney, which senses the amount of sodium present in the distal convoluted tubule fluid, and then initiates the release of renin from the kidney juxtaglomerular cells (Henderson and Henderson, 1994). This change in osmolality initiates the renin-angiotensin system.

The first step in the cascade of events that define the renin-angiotensin system is the release of renin from the macula densa. This enzyme then interacts with the glycoprotein angiotensinogen which is secreted from liver cells (Peach, 1977). Renin cleaves a zymogen from the glycoprotein, forming the hormone angiotensin I. Angiotensin I is then converted to Angiotensin II in the endothelial cells of the lung by way of Angiotensin Converting Enzyme (ACE) which severs 2 amino acids (Peach,

1977). Angiotensin II initiates the synthesis of the hormone aldosterone from cholesterol in the cortex cells of the adrenal gland and anti-diuretic hormone (ADH), also known as vasopressin, from the posterior pituitary. All three of these hormones, angiotensin II, aldosterone and ADH, are responsible for regulating blood pressure through changes in blood volume and osmolality, or blood concentration. Parker et al. (2004) reported increased plasma vasopressin and increased plasma sodium until hour 72 for water restricted animals compared to the animals that had *ad libitum* access to water. Becker et al. (1984) also noticed a five-fold increase in vasopressin in calves that were restricted from water for 54 hours. However, when cattle were transported with access to water via nipple drinkers, there was no change in serum sodium or potassium (Bernardini et al., 2012). Schaefer et al. (1990) reported no difference in white blood cell concentrations of beef steers that were restricted from feed and water up to 48 hours. However, it was reported that red blood cells, hematocrit and hemoglobin increased as the time restricted from water and feed increased.

Stress & Diet Impacts

Glucocorticoids such as cortisol have been illustrated to impact the renin-angiotensin aldosterone axis described above by inhibiting vasoconstriction via vasopressin and increasing the glomerular filtration rate (Wintour et al., 1985). Parker et al. (2003) arranged Merino wethers in a 2×2 factorial treatment structure to evaluate the effect of water/no water and cortisol/no cortisol on urinary output. In this study, cortisol infusion in animals with *ad libitum* water access caused an increase in urinary output; however, cortisol infusion in animals restricted from water resulted in decreased urine

output. Based on this study, an increase in cortisol in animals allowed water access will induce a diuresis of water while an increase in cortisol in water restricted animals may serve to prevent further dehydration by decreasing urine output and decreasing sodium urinary output which would increase blood osmolality.

Parker et al., (2004) used *Bos indicus* steers in the same manner as the 2003 study described above. Calves that were administered cortisol and allowed *ad libitum* water access did not demonstrate increased urine output as the wethers did. The urine output of these animals remained constant until hour 48 then decreased. However, similar to the wethers, urine output of the no water/cortisol animals decreased soon after 24 hours. In Parker et al. (2004) excess cortisol was also reported to decrease vasopressin concentration at hour 72 only, decrease Angiotensin II at hour 24 and 72, and decrease plasma potassium at hour 24; water restriction increased plasma sodium concentrations and plasma osmolality. The decrease in urine output of the no water/cortisol group indicates that cortisol will decrease urine output in the absence of water, which may serve to prevent increased dehydration. These results indicate that cortisol may inhibit hormonal mechanisms that control the renin-angiotensin aldosterone axis, but the body compensates for this by decreasing urine output and increasing sodium retention which corrects plasma osmolality. Differences in responses between the two studies may be due to differences in species and their respective water tolerance.

Diet has also been shown to impact the effect of dehydration. When water from the rumen is utilized during dehydration, sodium levels increased (Silanikove and Tadmor, 1989). When sodium is reabsorbed from the rumen, this combined with vasoconstriction caused by angiotensin results in increased blood pressure which corrects

blood osmolality. Increased blood osmolality is considered a corrective mechanism during dehydration. Ruminants that consumed a high roughage diet experienced an increase in rumen volume which is linked to transient dehydration of the extracellular fluid (Scott, 1975). Decreased extracellular fluid then initiates the renin-angiotensin aldosterone axis. Bond et al. (1976) evaluated the effect of diet on water intake and reported that when steers were fed a diet that contained 0, 30, or 88% forage, animals consumed more water when fed the 88% forage diet, followed by the 0% then the 30% forage diet. These dietary differences could impact how animals subsequently respond to water restriction during transport.

Practical Concerns

Dehydration and simultaneous vaccination is a concern due to the increasing occurrence of drying of mucosa membranes as dehydration increases (Duggan et al., 1996). It has also been observed that cattle are frequently dehydrated during transport then administered a vaccine upon arrival at the feedlot. This leads to an important question, "Will dehydration affect the response of a parenteral or intranasal respiratory vaccine?" The importance of secretory IgA (sIgA) in preventing pathogens from entering the host will be discussed in greater depth subsequently, but if the nasal mucosa is desiccated, and therefore sIgA is impaired, the efficacy of the intranasal route of vaccination may be altered compared to when it is administered to hydrated, non-stressed animals.

Vaccination in Beef Cattle

Respiratory Vaccinations

Vaccines are considered both efficacious and safe when they serve to activate an adaptive immune response in the host by mimicking an infection without adverse effects

(Chadwick et al., 2009). Since the 1960's when Coggins et al. (1961) proposed attenuated BVDV strains for vaccine purposes, numerous modified-live virus and killed virus vaccines have become available (van Oirschot et al., 1999). Each vaccine product possesses unique characteristics, whether it be the combination and concentration of viral or bacterial antigens included, the approved route of administration, the adjuvant selected or excluded, or modified-live or killed virus versions.

Viral Respiratory Vaccines

Many of the BRD viral vaccines available today are trivalent (IBRV, BRSV, PI3), pentavalent (IBRV, BRSV, PI3, BVDV type 1 and 2), contain only one antigen of these respiratory antigens, or contain viral and bacterin antigens (i.e. combination vaccines). Producers will choose their product based on availability, cost, or products that contain the antigens that they believe are the most important for their operation upon recommendation by a consulting veterinarian. There are currently more than 20 commercial parenteral respiratory viral vaccines but only 3 commercial intranasal respiratory viral vaccines available for use in cattle which can also factor into which vaccine the producer selects.

Bacterins

Vaccines that contain bacterial vectors in addition to viral antigens are available due to the multifactorial nature of BRD. However, upon arrival processing at the feedlot, bacterins are more often recommended for high-risk calves than low-risk calves (Terrell et al., 2011). Bacterins most often recommended were *M. haemolytica*, autogenous bacterins, and *P. multocida* (Terrell et al., 2011). These bacterins are not recommended for low-risk cattle by feedlot veterinarians, but in high-risk cattle *M. haemolytica* was

recommended by 74%, autogenous bacterins by 39%, and *P. multocida* by 35% of veterinarians. This may be due to the higher cost associated with bacterins compared to viral antigens and the perceived efficacy and increased disease risk associated with bacterins and high-risk calves, respectively.

Modified-live Virus Vaccination

Modified-live virus (MLV) vaccines contain an attenuated strain of the desired viral antigen that replicates in the host to mimic natural virus infection (van Oirschot et al., 1999). Attenuation of a virus can be accomplished by "multiple passaging strains in cell cultures from bovine and/or non-bovine origin". "A temperature-sensitive mutant has been selected by treating the strain with nitrous acid, followed by limiting dilutions at permissive and restrictive temperature; this mutant forms the basis for a vaccine" (Lobmann et al., 1984). These vaccines are then able to stimulate cellular, humoral and mucosal immune responses; however, replicating vaccines can cause a too robust immune response which has the potential to cause illness in immunosuppressed individuals (Webster et al., 2003). Richeson et al. (2016) observed this phenomenon in their study where animals were administered dexamethasone to mimic acute or chronic immunosuppression, or no dexamethasone. In their experiment, the chronic immunosuppressed animals had the greatest replicating MLV antigenicity as indicated by increased IBRV and BVDV antibody titers and virus prevalence from nasal swabs, the acute group had intermediate titers and the control group had the lowest titers and only sporadic detection of virus in nasal swabs. This demonstrates that in immunosuppressed animals, the MLV antigen is able to replicate more, which could be detrimental to the animal particularly if analogous acute infection with a wild type virus is present. Another

concern of MLV vaccines is the ability of the BVDV and IBRV fractions to cause infertility in heifers or cows prior to breeding (Perry et al., 2013) or IBRV fractions to cause abortion when administered after breeding (O'Toole et al., 2012). Calves infected with BVDV in utero that are not aborted may become persistently infected (PI) calves that then produce and shed BVDV virus for the remainder of their lives (Fray et al., 2000).

Killed Vaccine

Killed vaccines are developed by growing antigen strains to high titers then applying a chemical treatment to render the strain non-infectious. From then on, the non-infectious strain must be prepared in an adjuvant to elicit a sufficient immune response (van Oirschot et al., 1999). An advantage of utilizing a killed vaccine (KV) is that there is no replication after administration in the host. In healthy animals, this causes a decreased antibody titer compared to animals that received a MLV vaccine (Fulton et al., 2011). In immunosuppressed animals, administration of an inactivated leukotoxoid resulted in decreased antibody titers when compared to non-stressed animals (Richeson et al., 2016). While use of a MLV vaccine will generally produce a greater immune response (Van Donkersgoed et al., 1991), the risks associated with using the MLV vaccine must be considered. Due to the increased safety of KV compared to MLV vaccines, KV vaccines may be the better option especially in stressed or pregnant animals. Further research is needed to elucidate the pros and cons of MLV and KV vaccination in stressed cattle.

Parenteral Vaccination

Parenteral vaccines, which protect the host through the production of immunoglobulins, primarily IgG, have been widely used to protect against respiratory disease (Holmgren

and Czerkinsky, 2005). However, the response of a MLV vaccine administered parenterally to young calves that contain maternal antibodies has been shown to be inhibited by the maternal antibodies (Ellis et al., 2014). Ellis et al. (2014) reported no evidence of immune priming in neonatal Holsteins that were vaccinated with either a MLV pentavalent respiratory vaccine, or a trivalent MLV vaccine that did not contain BRSV or PI3. Calves were challenged with BRSV 11 weeks later with no differences in mortality, virus shedding, cytokines or BRSV-specific antibody responses between the two groups. The reason for this response is not fully understood, but it was suggested that maternal antibodies did have an impact. Disadvantages of parenteral administration include risk of cross contamination if needles are not exchanged, swelling or infection of the injection site, higher delivery cost, increased personnel training for administration, and more pain than mucosal administration (Medina and Guzman, 2001).

Moldoveau et al. (1995) evaluated the effect of a parenteral inactivated influenza virus on immune response in humans. They observed an increase in the number of cells secreting IgA, IgG and IgM on day 6 but the IgG response was the only significant response among the antibody classes evaluated. An increase in primarily IgG antibodies in saliva but IgG or IgM antibodies in nasal lavage fluids was also observed.

Intranasal Vaccination

Mucosal pathogens must bind to the surface of epithelial cells to gain access to the host and create infection (Rochereau et al., 2016). The majority of infections begin at a mucosal surface; therefore, it is believed that a local application of a vaccine is necessary to maximize the protective immune response (Holmgren and Czerkinsky, 2005). Intranasal administration of vaccines is possible due to the lack of an acidic environment,

small surface area and scarcity of secreted enzymes (Rochereau et al., 2016). An improved immune response was observed in vaccination regimens that joined different routes of administration, such as administering a parenteral priming vaccine followed by an intranasal booster (Webster et al., 2003). While intranasal vaccines typically do not elicit as strong of a serum antibody response as parenteral vaccines, they do induce a satisfactory serum antibody response and a significantly greater local sIgA response (Holmgren and Czerkinsky, 2005). Other advantages include stimulation of both mucosal and systemic immune responses, simple administration, and low delivery costs (Medina and Guzman, 2001). Due to the major route of infection mentioned previously and the evidence that intranasal vaccines produce a greater mucosal immune response, interest in intranasal vaccines has increased over the years.

Madic et al. (1995) intranasally inoculated three week old calves with seven different IBRV strains. All inoculated calves elicited an IgM, IgG1 and IgG2 serum response. The first serum antibody response detected was IgM. The mean post-infection day (PID) of first detection was day 9, with peak titer on day 13 and last detection on day 22. IgG1 was the next serum antibody detected on day 13. The peak titer was detected on day 30 and the mean last detection day was day 112. An IgG2 response was not elicited until 10 days after the IgG1 response. The mean peak titer occurred on day 56; however, the response continued until day 112 when the study was concluded. Moldoveanu et al. (1995) noticed an increase in the number of cells producing IgG as well as IgA beginning at two weeks post-vaccination; however, the serum antibody response was significant for IgG only even though an increase in antibodies of all isotypes was observed.

Role of secretory IgA

Two major components of the mucosa-associated lymphoid tissues (MALT) system are the nasopharynx-associated lymphoid tissue (NALT) and the gastrointestinal-associated lymphoid tissue (GALT), both of which are inductive sites where a mucosal immune response is triggered (Kiyono and Fukuyama, 2004). This response is initiated by microfold (M) cells located in the overlying epithelium encountering an invading antigen. Local dendritic cells then process the antigen and present it to B and T cells. T cells differentiate into T helper 1 or 2 cells which travel to local effector sites such as the nasal passage for NALT while IgA^+ B cells also travel to local effector sites. Once at the nasal passage, IgA^+ B cells differentiate into plasma cells due to cytokines produced by the T helper 2 cells. These plasma cells then produce dimeric IgA which is differentiated into secretory IgA (sIgA) when it binds to polymeric Ig receptors (pIgR) as it passes through the mucosal epithelial cells (Kiyono and Fukuyana, 2004). Once it has passed through the epithelial cells, the secretory immunoglobulin binds to mucin and promotes pathogen removal (Biesbrock et al., 1991).

Secretory Immunoglobulin A is the most prominent immunoglobulin present at mucosal surfaces (Holmgren and Czerkinsky, 2005). Secretory IgA has multiple roles in mucosal defense including: inhibiting bacterial adhesion to the mucosa, inhibiting the inflammatory effects of other Igs, neutralizing bacterial toxins and viruses and enhancing innate defense mechanisms (Holmgren and Czerkinsky, 2005). Madic et al. (1995) observed that all calves had a positive detection of sIgA from nasal secretions. Of these animals, the mean post-infection day of first detection was day 13 while peak titer was noticed on day 21 and the mean day for last detection was day 100. It is important to note

that on day 104, sIgA was detected in 23 of the 26 calves used in the study. Moldoveanu et al. (1995) also observed that in humans intranasally administered an attenuated influenza vaccine, antibodies in the nasal lavage fluids were of the IgA class and peak concentrations were observed on day 28.

Vaccination upon Feedlot Arrival

When animals arrive at the feedlot, calves are processed as a group at 97% of feedlots surveyed in North America (USDA, 2012). According to the Feedlot 2011 survey (USDA, 2012), 61% of the calves are processed within 24 hours of arrival with feedlots >8,000 head processing within this time frame more frequently than feedlots with capacity between 1,000 to 7,999 head (63 vs. 49%). Within 25 to 72 hours of arrival, 32% of calves are processed with little difference between small and large feedlots. However, when the percentage of animals processed after 72 hours is considered, feedlots with less than 7,999 animals were more likely to delay processing 72 hours than operations with greater than 8,000 animals (14 vs. 5%). In the survey, it was noted that factors to consider when determining processing time was available labor, weather and the cattle's appearance upon arrival. Regardless of processing time, nearly all (95%) arriving cattle are vaccinated against at least one respiratory virus; BVDV (95.1%), IBRV (93.2% parenteral, 13.4% intranasal), BRSV (61.4%), and PI3V (55.1%) are variably selected (USDA, 2013b). Large feedlots (>1,000 head) were also asked if they altered their vaccination protocols based on certain criteria such as breed, source of cattle, or arrival weight. The factors of greatest concern to these feedlots were source of cattle (40%), preconditioning status (39%), arrival weight (36%), history of previous antibiotic treatment (26%) and distance traveled or percent shrink (22%). Multiple factors affect

animal health and therefore decisions to tailor arrival processing procedures, yet further research in this area is needed including delayed use of vaccines and novel animal health products such as immunomodulators (Rogers et al., 2016).

Conclusions from the Literature

Bovine respiratory disease is a multifactorial complex that is promoted in animals that have experienced chronic stress and are immunosuppressed. Management and marketing practices that are common on many cow calf operations and feedlots are factors that have been observed to cause chronic stress and induce immunosuppression. One of these factors is water restriction, which may hasten infection due to its physiological effects and affect vaccine efficacy. Further research is needed to improve understanding of vaccine safety and efficacy when administered during conditions typically encountered as cattle transition through the beef production system.

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CHAPTER III

EFFECT OF WATER RESTRICTION ON PERFORMANCE, HEMATOLOGY AND ANTIBODY RESPONSES IN PARENTERAL OR INTRANASAL MODIFIED-LIVE VIRAL VACCINATED BEEF CALVES

ABSTRACT

The study objective was to determine if acute or chronic water restriction affected hematology, growth performance or antibody responses in beef calves administered a parenteral or intranasal respiratory vaccination. A total of 60 crossbred beef steer (n=28) and heifer (n=32) calves were used. On d -7, calves within sex were assigned randomly to 1 of 6 treatments arranged in a 3 × 2 factorial. Water restriction treatments (Factor A) were applied at the origin ranch: A_1) Control, no water restriction except during transport to the feedlot (**CON**), A_2) Acute, consisting of 48 h water restriction prior to transport to the feedlot (**ACU**), or A_3) Chronic, consisting of alternating 24 h periods of water access and restriction, over a 7-d period prior to transport to the feedlot (**CHR**). Upon feedlot arrival (d 0), 2 respiratory vaccine treatments were administered (Factor B): B_1) parenteral administration of a pentavalent modified-live virus (MLV) respiratory vaccine (2 mL s.c. in the neck; **SUB**), or B_2) intranasal administration of a trivalent MLV respiratory vaccine (1 mL/naris; **INT**). Blood was collected on d -7, -5, -3, -1, 0, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49 and 56 to determine complete blood count via automated hemocytometer. Rectal temperature (RT) and BW was recorded on the same days blood sampling occurred. Nasal swabs were collected on d 0, 3, 7, 14, 21, 28, 35, 42, 49 and 56 to determine presence of bovine respiratory syncytial virus (BRSV)-specific secretory antibodies (sIgA). Sera were collected weekly from d 0 to 56 for antibody titer analyses. Hematocrit increased for CHR ($P \leq 0.04$) on d -5 and -3 as well as for ACU ($P \leq 0.03$) on

d -1. Transportation (d-1 to 0) increased neutrophils in a manner indicative of stress (day effect, $P < 0.01$). The INT group had increased total white blood cell count ($P < 0.01$); specifically, monocytes were increased on d 3 and 5 ($P < 0.01$). Rectal temperature was increased for INT on d 7 and 14 ($P \leq 0.04$). The BW of ACU and CHR decreased 9.5 and 9.7 kg, respectively, from d -7 to -1; however, the CON treatment gained 5.5 kg during the same time ($P < 0.01$). The CON treatment lost 8% BW during relocation while the ACU and CHR treatments gained 2% BW ($P < 0.01$). The ACU and CHR treatments had reduced ADG from d 0 to 14 ($P \leq 0.04$), regardless of vaccine treatment. The SUB treatment had increased BRSV-specific sIgA on d 3 and 7 ($P \leq 0.03$); however, these were greater for INT from d 35 to 56 ($P \leq 0.05$). The BRSV ($P \leq 0.01$) and IBRV ($P \leq 0.04$) antibody titer was greater for INT vs. SUB from d 14 to 56 and d 21 to 35, respectively. Water restriction prior to transport altered some hematological variables and reduced performance (d 0 to 14) but did not clearly alter antibody responses to either vaccine type. Intranasal vaccination increased some leukocytes, rectal temperature, sIgA and BRSV- and IBRV-specific antibodies compared to SUB.

Introduction

In the United States (U.S.), beef cattle operations are highly diversified. According to the 2012 Census of Agriculture (USDA NASS, 2012), over 700,000 farms in the U.S. raised beef cows (cow calf operation) while only 26,586 farms had cattle on feed (feedlot operation). The cow calf sector of the U.S. beef production system is the most widespread and dynamic, with cow calf operations present in all 50 states. On many cow calf operations, calves are weaned from their dam at approximately 207 days of age (USDA, 2008) then sold via auction market. According to the Beef 2007-08 survey conducted by the U.S. Department of Agriculture (USDA, 2009), over 60 percent of cattle that permanently left the cow calf operation were transported to an auction market for sale, whereas 17 percent were sold directly to a feedlot and 12 percent were sold directly to another beef operation. For 57 percent of operations, cattle were transported between 10 and 49 miles from their original operation to their initial marketing destination (USDA, 2009). Once cattle are transported to an auction market, they have limited access to water and feed and can become dehydrated. At the auction market, the animals will then be purchased by an order buyer. An order buyer is contracted by a feedlot or stocker operation that is searching for cattle to purchase. The feedlot or stocker informs the order buyer of specific criteria (weight, polled/horned, sex, price, etc.) and the order buyer may purchase cattle for them from multiple auction markets over several days to satisfy specific criteria. The animals must then be transported to the feedlot or stocker facility, where it is common for the animals to not have access to feed or water or be off-loaded for rest (Gonzalez et al., 2012). Stocker operations then background the animals by administering a respiratory vaccine and anthelmintic as well as castrating and

introducing the animals to eating out of a bunk. The calves are then sold to a feedlot. The average distance traveled for a feedlot shipment is 339 miles (USDA, 2011). From the time the animal leaves its cow calf origin and arrives at the feedlot or other final destination, several days have elapsed and the animals have few, if any, opportunity to consume water and feed during this process.

As a result of marketing and transport, at least some animals are certain to become dehydrated. The degree of dehydration can vary depending on transport time and other marketing factors. A dehydrated animal will likely possess an increased percentage of red blood cells and hematocrit, desiccated mucosal surfaces and other physiological alterations that may impact their response to vaccination.

When animals arrive at the feedlot, nearly all (95%) are vaccinated against at least one respiratory virus; the most common respiratory viruses that cattle are vaccinated against are BVDV (95.1%), IBRV (93.2% parenteral, 13.4% intranasal), BRSV (61.4%), and PI3V (55.1%; USDA, 2013). An array of commercially available respiratory vaccines exist that may be administered parenterally (e.g., subcutaneous), with only a few vaccines approved for intranasal administration. The parenteral vaccines are injected into the neck via the recommended route whereas intranasal vaccines are administered into one or both nares. The parenteral vaccines are known to primarily stimulate systemic immunoglobulin (Ig)G1 and secondarily mucosal IgG2 production; whereas, intranasal vaccines primarily stimulate secretory (s) IgA in the mucus membranes of the nose and mouth but also appear to significantly stimulate systemic IgG (Moldoveanu et al., 1995) and IgM production (Madic et al., 1995).

The objective of this study was to mimic the water restriction and transportation of the previously described marketing scenario and determine effects on the systemic and mucosal antibody and other immunological responses to a parenteral or intranasal respiratory vaccine administered to beef calves.

Materials and Methods

Cattle procedures

Animal methods and procedures were approved by the IACUC committees at University of Arkansas and West Texas A&M University (WTAMU). Calves originating from the beef cow herd at the University of Arkansas Southwest Research and Extension Center (SWREC) near Hope, AR, were used for this study. The animals, primarily of Angus and Hereford breeds, were approximately 212 days of age on day 0. Vaccines used in the study were acquired from Hereford Veterinary Supply (Hereford, TX). Appropriate vaccine handling procedures were closely followed and vaccines were administered according to the label using aseptic technique.

A total of 60 (Phase 1, n = 20 calves/water restriction treatment housed in 6 isolated pens containing 10 animals/pen; and Phase 2, n=30 calves/vaccination treatment housed in 2 isolated pens containing 30 animals/pen) clinically healthy, crossbred beef steer (n=28) and heifer (n=32) calves were used. The calves had not previously been administered a respiratory vaccine. They were confirmed seronegative for IBRV-, BVDV type 1a-, and PI3V-specific antibodies through pre-trial serum samples that were tested at the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) in Amarillo via virus neutralization assay. Animals were then stratified by body weight (BW) and assigned randomly to treatments with gender equivalent across treatments. Water

restriction treatments were applied at SWREC and consisted of: A_1) Control, no water restriction at any time other than during transport (**CON**), A_2) Acute, consisting of 48 h water restriction prior to transport (**ACU**), or A_3) Chronic, consisting of alternating 24 h periods of water access and restriction, over a 7 d period prior to transport (**CHR**). Vaccine treatments were applied at WTAMU on day 0 and consisted of: B_1) parenteral administration of a pentavalent (IBRV, BVDV type 1 and 2, BRSV, PI3V) modified-live virus (MLV) respiratory vaccine (2mL subcutaneous; Express 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO; **SUB**), or B_2) intranasal administration of a trivalent (IBRV, BRSV, PI-3) MLV respiratory vaccine (1 mL/naris; Inforce 3, Zoetis, Parsippany, NJ; **INT**).The experimental design resulted in a 3 × 2 factorial arrangement of treatments applied in 2 phases.

Study Phase 1. During the 21-d weaning period, the calves remained as a single cohort group until d -7 when they were sorted into 1 of 6 pens according to water restriction treatment. Water restriction treatments were assigned randomly and began on d -6 (7OCT15) for CHR at SWREC. The 2 pens of CHR calves were initially restricted water access at 0800 h starting on d -6 and followed the water restriction schedule presented in Table 1. The 2 pens of ACU calves were restricted water access from 0800 h on d -2 until feedlot arrival on d -1, whereas the CON pens were only confined from water during transportation on d -1. The animals were loaded in a commercial trailer at 1100 h on d -1 and arrived at the West Texas A&M University (WTAMU) Research Feedlot at 2315 h. The trailer was washed before use to reduce risk of virus transmission. Once the calves arrived at the WTAMU facility, they were unloaded into a holding pen

with access to hay and water overnight; however, water and feed intake were not measured for this study.

Study Phase 2. Beginning at 0500 h on d 0, treatment B_1 was administered 2 mL Express 5 subcutaneously (EX), while treatment B_2 was administered 1 mL/naris Inforce 3 (IN) according to Table 1 (note: applicator tips on the INT syringe were changed after every calf and needles on the EX syringe were changed every 10 animals). The animals were then sorted into 1 of 2 isolated sort pens according to treatment to avoid oral/oral contact immediately following vaccination. On subsequent sample days, the SUB animals were sampled first, the chute and handling equipment were disinfected with a chlorohexidine solution and INT animals were then sampled last. The handling equipment was again washed down and disinfected after each sampling day. This biosecurity protocol was implemented to avoid contact between the two vaccine groups and prevent transmission of intranasal vaccine in parenteral vaccine treatments. The calves remained on study at the WTAMU facility until d 56.

Sample collection and assay procedures

Calves were removed from their pen and briefly restrained on d -7, -5, -3, -1, 0, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 to collect blood or nasal sample specimens. Rectal temperature and body weight were recorded on each sample day.

Blood samples were collected via jugular venipuncture beginning at approximately 0800 h on sample d -7 (6OCT15) through sample d -1 (12OCT15). On subsequent sample days at WTAMU, animals were processed beginning at approximately 0600 h through d 56 (8DEC15). Blood samples via jugular venipuncture were collected on every sample day

through d 56 whereas nasal swabs were collected on d 0, 3, 7, 14, 21, 28, 35, 42, 49, and 56.

Blood was collected into evacuated blood collection tubes with no additive (Vacutainer 10 mL tube, Ref #367985), allowed to clot for at least 30 minutes, centrifuged at 1,250 x *g* for 20 minutes at 4°C and then serum was decanted and stored in triplicate aliquots at -20°C until subsequent analyses. Serum from each animal collected on d 0, 7, 14, 21, 28, 35, 42, 49, and 56 was transported on ice to the TVMDL in Amarillo, TX for determination of serum neutralizing antibody titer concentration against IBRV, BVDV type 1a (Singer strain), BRSV, and PI3V using the virus neutralization assay. Additionally, a serum aliquot from d -7, -5, -3, -1, 0, 1, 3, 5, 7 and 14 was used to determine the acute phase response (bovine-specific haptoglobin; Hp) via ELISA (Immunology Consultants Laboratory, Newberg, OR) at the WTAMU Animal Health Laboratory.

An anticoagulated blood sample was collected via jugular venipuncture, into evacuated tubes containing EDTA (Vacutainer, 7.2 mg K2-EDTA, 4 mL tube, Ref #367861) on d -7, -5, -3, -1, 0, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 and analyzed using an automated hemocytometer (Idexx, ProCyte DX Hematology Analyzer, Westbrook, ME) at the WTAMU Animal Health Laboratory to determine complete blood count. After automated hematology was determined, the blood samples were further used to determine a manual packed cell volume using a capillary tube and microcentrifuge (EKF Diagnostics, HemataStat II, Cardiff, UK) on the same study days as indicated for CBC analysis. Blood samples collected at SWREC on d -7, -5, -3, and -1 were packed on ice once all of the samples were collected for the day, and shipped overnight to the WTAMU Animal

Health Laboratory where they were promptly processed and serum was stored frozen at-20°C.

Nasal swab samples were collected on d 0, 3, 7, 14, 21, 28, 35, 42, 49, and 56 by rotating 3 nylon-flocked swabs (Puritan Medical Products #3306-PN) in the mid-naris region until the swabs were completely saturated. The triplicate nasal swabs were clipped, placed into sealed tubes, and stored at -80°C until subsequent analysis to determine percent positivity of BRSV-specific secretory IgA (sIgA). Briefly, swabs were thawed at room temperature, 300 µL phosphate buffered saline containing 1mM EDTA, and 400 µL prepared Sputolysin Reagent (EMD MILLIPORE chemicals product #560000 10ML) were added to each sample, vortexed and incubated at room temperature for 15 minutes. Supernatant was harvested and analyzed for BRSV-specific sIgA concentration using a commercial ELISA kit specific for BRSV Ab (Svanova, Svanovir BRSV-Ab, Boehringer Ingelheim Svanova, Uppsala, Sweden) with sheep anti-bovine IgA HRP conjugate (Bethyl Laboratories, Montgomery, TX) added to the conjugate contained in the ELISA kits.

For BRSV-specific sIgA, the optical density (OD) values in wells coated with BRSV antigen were corrected by subtracting the OD values of the corresponding wells containing control antigen to determine corrected OD. The mean corrected OD was then determined for all control and unknown samples for each plate. Percent positivity index values (PPI) were determined as follows: PPI = corrected OD of sample or control/corrected OD of positive control × 100. The mean PPI for each vaccine group on a given day was determined. A sample was considered positive for BRSV-specific sIgA if the PPI result was ≥ 10.

Concentrations of serum Hp were determined using a bovine-specific ELISA assay (Immunology Consultants Laboratory, Inc., Newburg, OR) with intra-and interassay CV of 7% and 8%, respectively. The Hp present in samples reacts with the anti-Hp antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-Hp antibodies conjugated with HRP are added. These enzyme-labeled antibodies form complexes with the previously bound Hp. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate. The quantity of bound enzyme is directly proportional to the concentration of Hp in the test sample, and is quantified by reading the absorbance at 450 nm. The quantity of Hp in the test sample can be interpolated from the standard curve constructed from the standards, then corrected for sample dilution.

Statistical analyses

This completely randomized design study used animal as experimental unit for all variables. Rectal temperature, complete blood count, serum IBRV, BVDV, BRSV and PI3V antibody titer, bovine-specific Hp, and BRSV-specific sIgA from nasal swab specimens were analyzed using the MIXED procedure of SAS (SAS Inst., Cary, NC) with repeated measures. The repeated statement was day, and effects of water restriction treatment, vaccine treatment, day, and each possible interaction were evaluated. The Kenward-Roger degrees of freedom method was employed for all dependent variables. Serum antibody titer data were $\log_2$ transformed prior to statistical analysis. Other variables were tested for normal distribution using PROC UNIVARIATE. If a normal distribution was not present for a particular dependent variable, that data was $\log_2$

transformed and tested again to determine if normality was improved. The $\log_2$ transformed data was used for statistical analysis if the Shapiro-Wilk *P*-value ≥ 0.05 and back-transformed LSmeans were displayed. A significance level of α=0.05 was used for all possible interactions evaluated using the F-test and if a significant interaction existed, the appropriate treatment mean comparisons were evaluated using the pdiff option in SAS. A tendency was considered for a *P*-value between 0.06 and 0.10.

Results and Discussion

There was no mortality in this experiment; clinical morbidity was observed in one animal that was removed from the study due to development of a severe abscess not related to experimental treatment.

Complete Blood Count

During phase 1, hematocrit for the CHR and ACU calves increased in a manner corresponding to the initial water restriction time (Figure 1). Hematocrit increased on d -5 and -3 for CHR and d -1 for ACU (day effect; $P < 0.01$). After transportation, hematocrit increased in CON (day effect; $P < 0.01$). Hematocrit is the percentage volume of cells in whole blood. As animals become dehydrated and their fluid volume decreases in the blood, the percentage volume of cells increases; therefore, an increased hematocrit indicates that an animal is dehydrated. Hematocrit, or packed cell volume, has been evaluated in cattle after feed and water restriction with conflicting results. Schaefer et al. (1990) reported that hematocrit increased in cattle with increasing time restricted from feed and water; however, Vogel et al. (2011) reported no change in cows restricted for 36 hours from water. On d -3, the CHR hematocrit had increased by 3% ($P < 0.01$) from the

d -7 baseline value while the ACU and CON hematocrit remained static. On d -1, the ACU hematocrit increased 1.5% greater than the baseline value such that CHR and ACU had greater hematocrit than CON ($P < 0.03$). Following transportation on d -1, neutrophils (Figure 2) increased from d -1 to 0 and immediately decreased thereafter (day effect, $P < 0.01$). Stress is known to inhibit L-selectin (Burton et al., 1995; Burton et al., 2005) that assists in translocation of neutrophils from the blood into infected or damaged tissue. This inhibition of neutrophil L-selectin and trafficking results in increased neutrophil concentration in the blood. The increases in hematocrit and neutrophils following water restriction and transport indicate that the animals were dehydrated and stressed and our study model was successful; however, the control group was also subjected to water restriction and stress during transport. During phase 2, the total leukocyte count (Figure 2) for SUB was decreased on d 3, 5, and 7 ($P \leq 0.03$). Specifically, lymphocytes were decreased on d 5 ($P < 0.01$) and monocytes (Figure 2) were decreased on d 3 and 5 ($P \leq 0.04$) for SUB. Eosinophils decreased ($P < 0.01$) by more than 50% from d 0 to d 7 for either vaccine group. After the stress of being transported and relocated to a novel environment, these stressors likely caused eosinopenia induced by glucocorticoids (Hughes et al., 2017). A tendency for water restriction × day interaction was observed for total red blood cell (RBC) count ($P = 0.10$). This was anticipated because RBCs are known to increase during times of dehydration (Schaefer et al., 1990). The CHR group was restricted from water on d -6 and -4 and as a result, on d -3 RBC had increased to 10.37×10^6/ µL. The RBC concentration observed in the CHR group was greater than the CON ($P = 0.06$) and ACU ($P < 0.01$) treatments.

From d 0 to 3, RBC gradually increased overall after the approximately 12-hour transport from SWREC to WTAMU.

Rectal Temperature

There was a vaccine × day effect ($P < 0.01$) and a water restriction × day effect ($P = 0.01$) on rectal temperature. Figure 3 illustrates that vaccine treatments differed on d 7, 14 and 42 with the INT group experiencing greater rectal temperatures on d 7 and 14 ($P \leq 0.04$), yet lower rectal temperature on d 42 ($P < 0.01$). Differences in rectal temperature may be influenced by differences in inflammation which could indicate differences in antigenicity between the vaccine treatments administered under the conditions of the present study. Xue et al. (2010) and West et al. (2000) administered a respiratory vaccine, sterile water diluent, or nothing to seronegative neonatal Holstein calves a minimum of three weeks prior to challenge and recorded rectal temperature following challenge. Compared to the vaccinated animals, both studies reported increased rectal temperatures in the unvaccinated groups indicating a greater inflammatory response in unvaccinated animals exposed to viral antigens. Among dehydration treatments, CHR had reduced rectal temperature compared to ACU and CON ($P \leq 0.05$) on d -5. Conversely, CHR had greater rectal temperature compared to the CON group on d -1 and 5 ($P \leq 0.03$). Rectal temperature was observed to decrease following 48 h deprivation of feed and water in steers (Rumsey and Bond, 1976); however, this was over a continuous 48 h period and the reintroduction of water on d -4 in the current study may explain the subsequent increase in rectal temperature on d -3. Overall, the water restriction × day effect on rectal

temperature suggests an initial reduction in rectal temperature following water restriction and subsequent increase in rectal temperature following water allowance.

Performance

Feed and water deprivation of at least 24 hours has been observed to decrease BW (Vogel et al., 2011), increase percentage shrink, and decrease ADG for 28 days following water restriction (Marques et al., 2012). There was a difference in BW change between the water restriction groups from d -7 to d -1 ($P < 0.01$; Table 2). The CON group gained an average of 5.5 kg during the 6-d period while the ACU and CHR animals lost an average of 9.45 and 9.65 kg respectively, during the same time period. However, shrink from transport was greater for CON compared to ACU and CHR ($P < 0.01$), with the ACU and CHR groups having an increase in BW between the time they were loaded on d -1 until they were weighed on d 0. The increase in BW observed for ACU and CHR during this time may be due to the animals being more aggressive to find feed and water upon arrival at WTAMU and replenish the gut fill that was lost during transport, or perhaps more likely due to previous gut fill loss from the water restriction schedule imposed prior to transport. Water and feed intake were not recorded for this study, so it is not possible to verify this observation. The ADG from d 0 to 14 was greater for CON compared to ACU and CHR ($P < 0.03$) likely due to the CON animals replenishing the lost gut fill during transport and replenishment immediately following transport. There was a vaccine effect observed for change in BW from d 42 to 56 ($P = 0.01$) such that the SUB animals gained an average of 13.77 kg, compared to the 10.35 kg gained by the INT animals. However, there was no difference in ADG from d 0 to 56 for either the water restriction ($P = 0.55$) or vaccine treatments ($P = 0.26$).

Haptoglobin

Haptoglobin is an acute phase protein that is present systemically in response to inflammation and has been observed to increase in response to transport, feed and water deprivation (Marques et al., 2012), and vaccination (Arthington et al., 2013). However, Arthington et al. (2003) reported greater serum haptoglobin concentration in non-transported calves compared to calves transported for 3 hours. There was a water restriction × vaccine × day interaction ($P < 0.01$) detected for serum Hp in the present study. Figure 4 reveals overall increased haptoglobin on d -7, likely due to some unknown and unanticipated stressor experienced immediately prior to study initiation. This confounded the potential serum Hp response due to water restriction or vaccine treatment, yet Hp had declined by d -1, and increased again after transportation and relocation to WTAMU. A difference ($P \leq 0.06$) was observed on d 5 between the CON SUB and the ACU INT treatments with the CON SUB treatment having a greater Hp response.

BRSV-specific secretory IgA

Secretory IgA has been observed to be the most prominent immunoglobulin at mucosal surfaces including the intestinal lumen (Mantis et al., 2011), and naris (Holmgren and Czerkinsky, 2005) that is important for inhibiting bacterial adhesion, reducing the inflammatory effects of other Igs, neutralizing bacterial toxins and viruses as well as enhancing the host's innate immune defense mechanisms (Holmgren and Czerkinsky, 2005). Due to numerous pathogens gaining access into the host via epithelial cells (Rochereau et al., 2016), the secretory IgA response is important to evaluate in

immunological studies. Figure 5 illustrates the vaccine × day effect ($P < 0.01$) on the BRSV-specific sIgA percent positivity index (PPI). It is important to note that the BRSV-specific sIgA assay is semi-quantitative, and PPI values less than 10 are considered negative; whereas, values greater than 10 are considered positive. Furthermore, this assay would also detect any IgG present in nasal swab specimens, in addition to IgA; however, IgA is the primary antibody class present in nasal secretions (Holmgren and Czerkinsky, 2005). The SUB groups had a greater PPI on d 3 and 7 ($P \leq 0.03$); however, the PPI for both vaccine groups was low at this time. In a study by Moldoveanu et al. (1995), there was an increase in IgG following intramuscular administration of an influenza vaccine with antibodies in the serum being present before IgG was detected in mucosal secretions. Because the method used to detect sIgA in our study does not differentiate between IgG and IgA in the nasal swab samples, IgG present in the nasal swabs from SUB may have influenced PPI on d 3 and 7. We speculate that the lack of detectable sIgA for INT during this time suggests that water restriction may have had early impact on the immunological response to INT vaccine with preference for systemic vs. mucosal antibody production. This is supported by previous results (Hughes, 2015); sIgA increased as early as d 4 following INT administration in non-dehydrated calves used in that study. Moldoveanu et al. (1995) recorded peak levels of sIgA on d 28 for humans vaccinated intranasally with inactivated influenza antigen. We did not detect a water restriction effect ($P \geq 0.12$) for sIgA; however, it is likely that CON INT was also dehydrated from the confounding effect of water deprivation during transport and

relocation. On subsequent sample days, PPI for the INT calves was greater on d 35, 42, and 56 ($P \leq 0.05$); however, the PPI index was > 10 on d 56 only.

Serum Antibody Titers

It has been reported that parenteral vaccines elicit a strong systemic immune response while intranasal vaccines elicit a systemic and mucosal immune response although the intranasal systemic response tends to be less robust than the parenteral systemic response (Medina and Guzman, 2001). This is also supported by research conducted by Moldoveanu et al. (1995) and Van Donkersgoed et al. (1991) who reported greater serum antibody responses in systemic vs. intranasal vaccinated humans and cattle, respectively. Richeson et al. (2016) observed that cattle that were chronically immunosuppressed via repeated dexamethasone infusion had greater serum antibody titers to BVDV and IBRV antigens but decreased antibody titers to non-replicating antigens, indicating greater antigenicity of MLV in chronically stressed animals. No water restriction × day ($P \geq 0.15$) or water restriction × vaccine × day ($P \geq 0.62$) effects were observed for any of the serum antibody titer data; however, there was a vaccine × day effect ($P \leq 0.08$) for all four antigen-specific antibody titers evaluated (Figure 6). The SUB group had a tendency ($P \leq 0.07$) for increased PI3V-specific antibody on d 14 and 21 compared to the INT group; conversely, INT had greater PI3V antibody titer from d 35 to 49 ($P \leq 0.06$). On day 56, the PI3V antibody was greater ($P < 0.01$) for SUB. Figure 6 illustrates serum BRSV-specific antibody titer results. From d 14 to 56, INT had greater BRSV-specific antibody titer compared to the SUB group ($P < 0.02$). As expected, INT remained seronegative to BVDV type 1a antibody because INT contains no BVDV

antigen, while the SUB group had a robust antibody response to the BVDV antigens in that vaccine. Serum IBRV-specific antibody was not present for either vaccine treatment until d 14 and concentration was greatest on d 21 for both INT and SUB treatments. From d 21 to 35, the INT group had a greater IBRV-specific antibody concentration in serum ($P \leq 0.04$). On d 56 the INT and SUB treatments once again had similar IBRV-specific antibody concentration. While the INT group had a greater overall BRSV- and IBRV-specific antibody response, both groups had very low antibody responses overall (< 1:8).

Conclusion

Transient alteration in hematologic and performance variables indicates that water restriction during the marketing process impacts beef calves, in a time-dependent manner. Dehydration and transport stress can cause immunosuppression which may allow intranasally administered antigens to penetrate the mucosal membranes more readily, potentially altering the antigenicity of the intranasally administered vaccine and shifting the antibody response towards systemic rather than local. This is supported by increased WBC, monocytes, lymphocytes, and rectal temperature observed for INT vaccinated calves subjected to conditions that were intended to mimic the beef marketing process.

Table 1. Daily water restriction schedule and vaccination regimen.

Study Day[1]	CON[2] (n=20)	ACU[2] (n=20)	CHR[2] (n=20)	SUB[3] (n=30)	INT[3] (n=30)
-7	YES	YES	YES		
-6	YES	YES	NO		
-5	YES	YES	YES		
-4	YES	YES	NO		
-3	YES	YES	YES		
-2	YES	NO	NO		
-1 (Ship to WTAMU)	YES	NO	NO		
0 (Vaccinate)	YES	YES	YES	2mL Express® 5 administered subcutaneous	1mL Inforce® 3 / naris

[1]A study day was from 0800 to 0800 (24 h); water was provided/restricted at 0800 of the appropriate study day.

[2] CON= control, no water restriction at any time other than during transport; ACU= acute, consisting of 48 h water restriction prior to transport; CHR= chronic, consisting of alternating 24 h periods of water access and restriction, over a 7-day period prior to transport.

[3]SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V; Express 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO]; INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V [Inforce 3, Zoetis, Parsippany, NJ]

Table 2. Effect of water restriction and vaccine treatment on performance.

Item	Treatment					SEM	Water restriction *P*-value	Vaccination *P*-value
	CON	ACU	CHR	SUB	INT			
D -7 to -1 BW, kg	5.5[a]	-9.45[b]	-9.65[b]	-5.17	-3.90	1.23	<0.01	0.37
Shrink, %	8.19[a]	-2.34[b]	-2.60[b]	1.01	1.16	0.66	<0.01	0.84
D 0 to 14 ADG, kg	1.61[a]	1.30[b]	1.25[b]	1.40	1.38	0.10	<0.03	0.89
D 0 to 56 ADG, kg	1.30	1.25	1.30	1.29	1.22	0.05	0.55	0.26

Effect of control (CON), acute (ACU) and chronic (CHR) water restriction and subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V; Express 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO]) and intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V [Inforce 3, Zoetis, Parsippany, NJ]) vaccination on performance variables. Within main effect, treatments with unlike letters differ, $P \leq 0.05$.

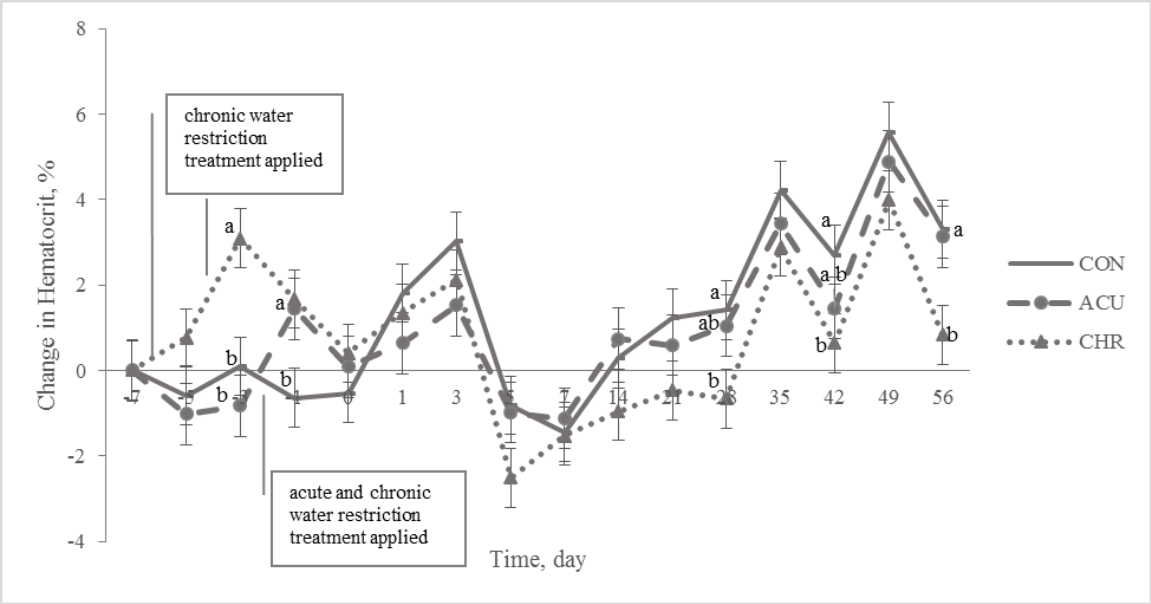


Figure 1. Effect of control (CON), acute (ACU) and chronic (CHR) water restriction treatment on change in hematocrit (water restriction × day; $P < 0.01$). Within day, treatments with unlike letter superscripts differ, $P \leq 0.05$.

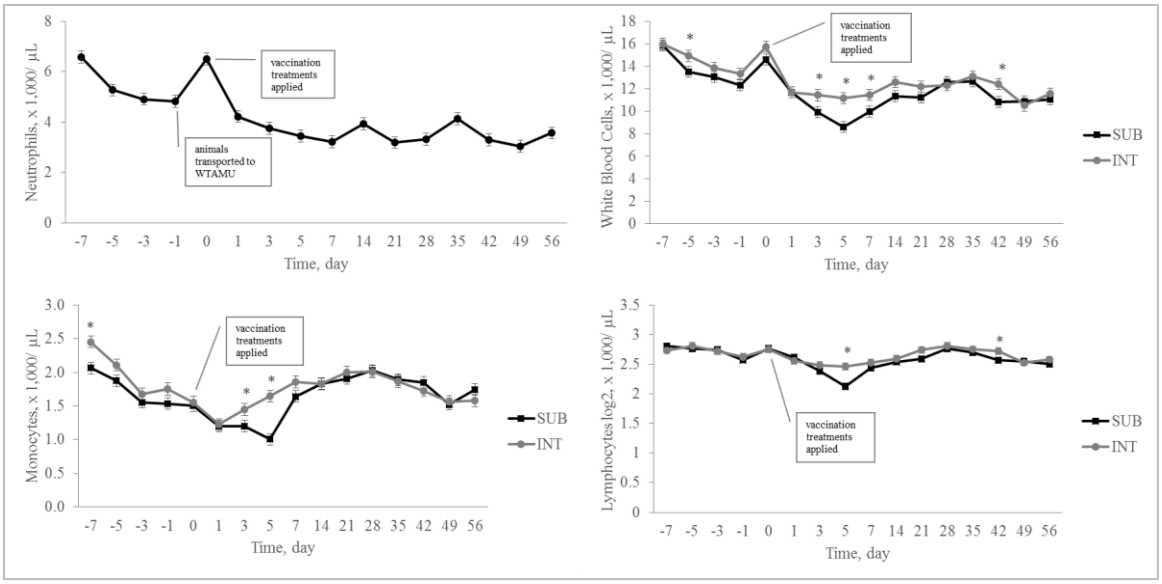
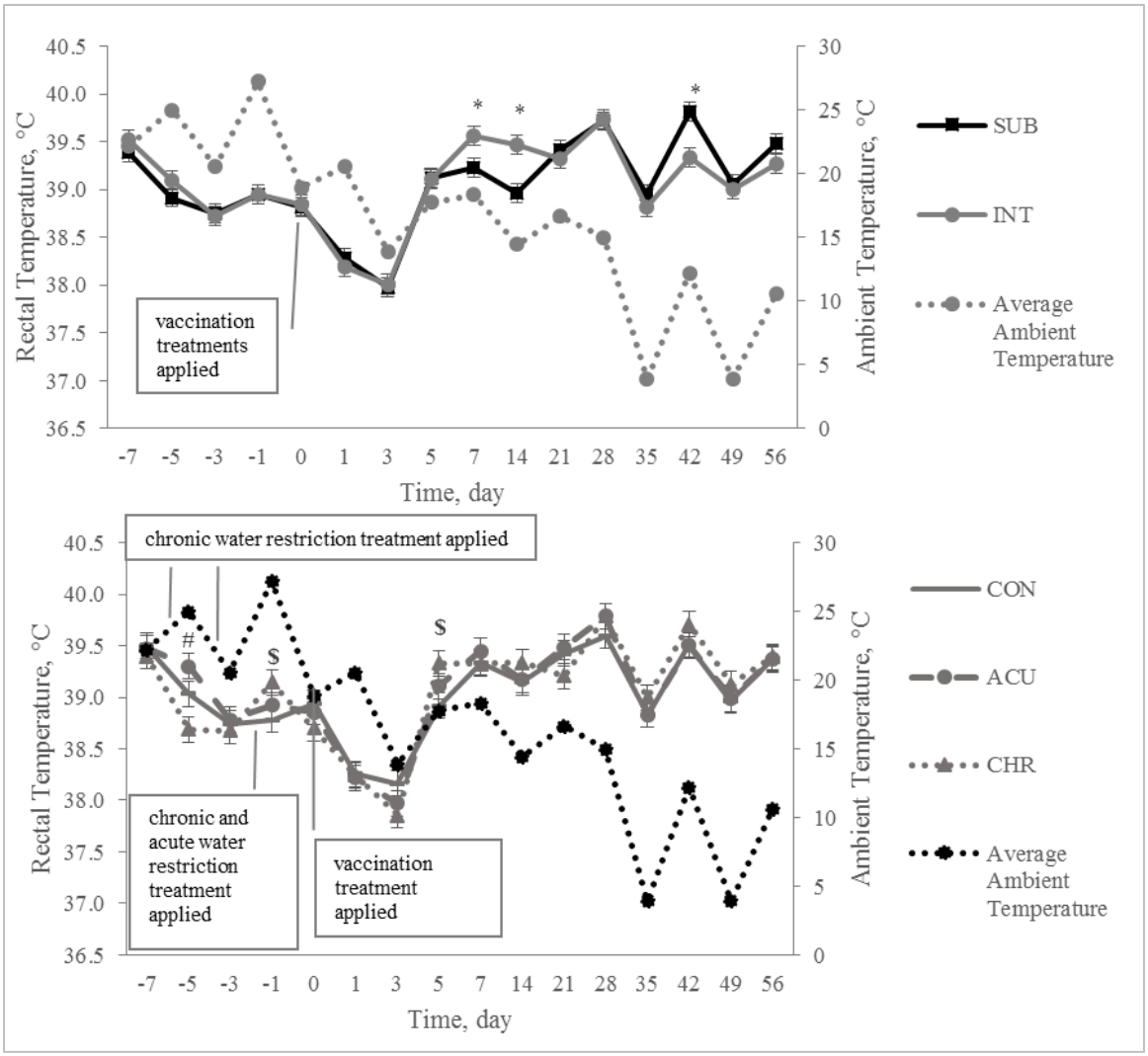


Figure 2. Effect of subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V; Express 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO]) and intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V [Inforce 3, Zoetis, Parsippany, NJ]) vaccination on neutrophil count (day effect; $P < 0.01$), total white blood cell count (vaccine × day effect; $P < 0.01$), monocyte count (vaccine × day effect; $P < 0.01$), and lymphocyte count (vaccine × day effect; $P < 0.01$). * SUB differs from INT within day, $P \leq 0.05$.



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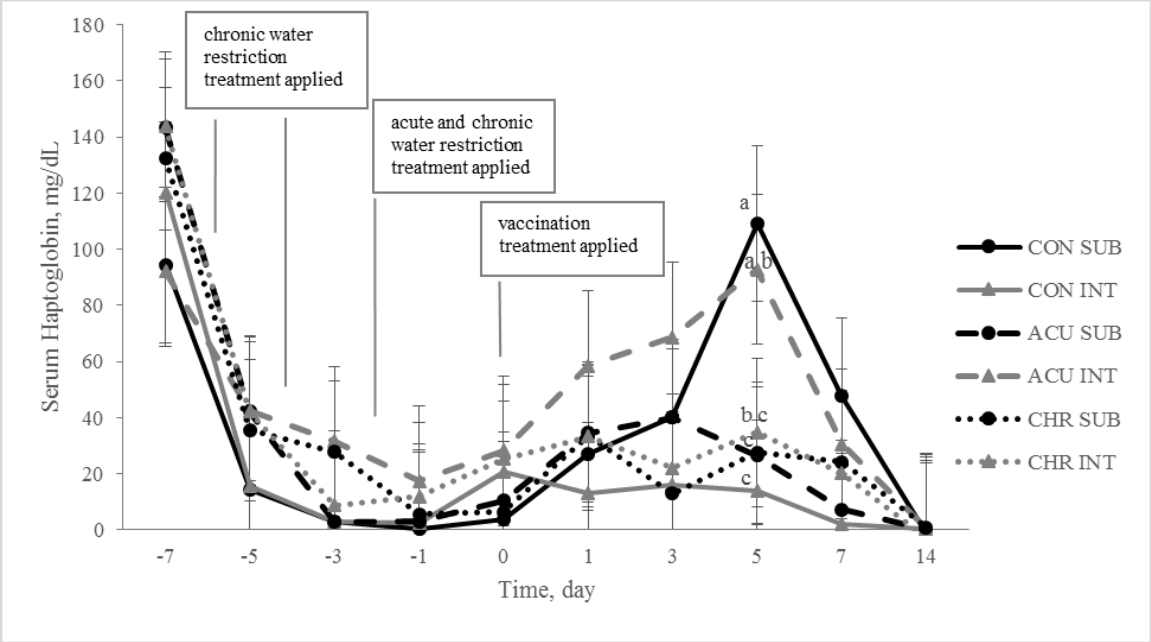

Figure 4. Effect of control (CON), acute (ACU) and chronic (CHR) water restriction and subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V; Express 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO]) and intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V [Inforce 3, Zoetis, Parsippany, NJ]) vaccination on serum haptoglobin ($P < 0.01$). Within day, treatments with unlike letter superscripts differ, $P \leq 0.05$.

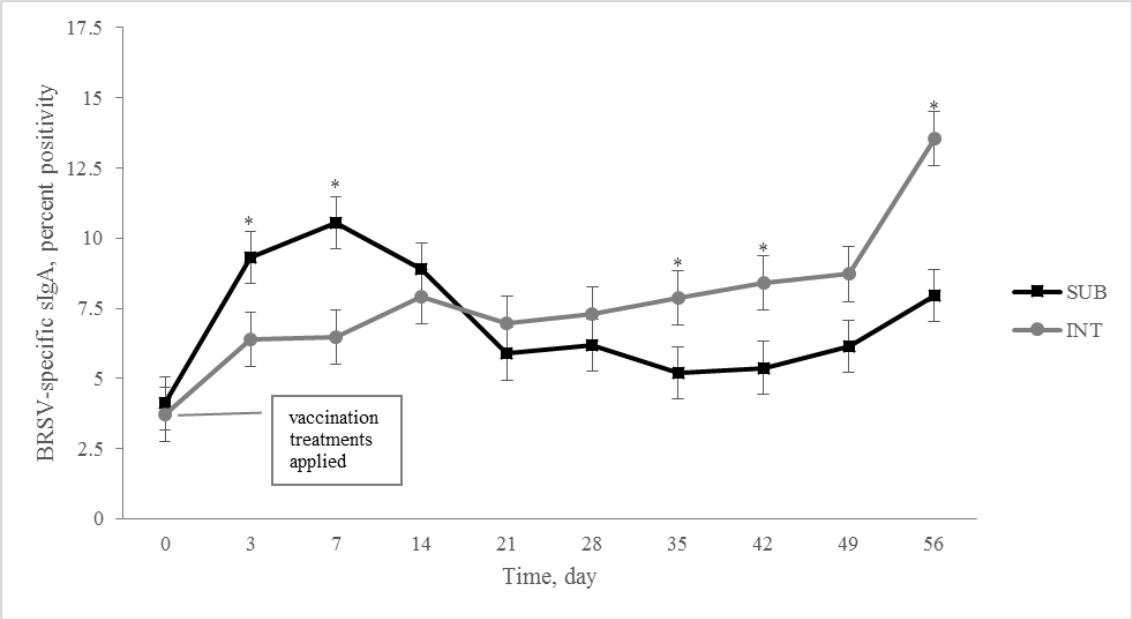

Figure 5. Effect of subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V; Express 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO]) and intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V [Inforce 3, Zoetis, Parsippany, NJ]) vaccination on BRSV-specific sIgA percent positivity index ($P < 0.01$). * SUB differs from INT within day, $P \leq 0.05$.

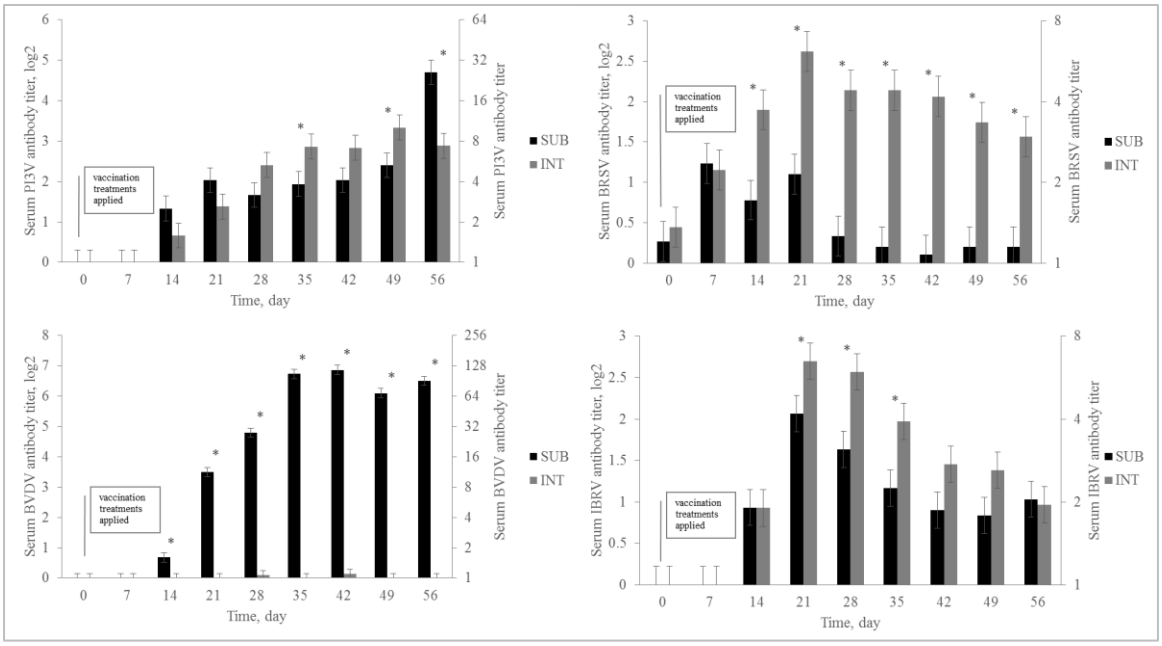

Figure 6. Effect of subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V; Express 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO]) and intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V [Inforce 3, Zoetis, Parsippany, NJ]) vaccination on serum PI3v-specific antibody titer concentration ($P < 0.01$); serum BRSV-specific antibody titer concentration ($P < 0.01$); serum BVDV-specific antibody titer concentration ($P < 0.01$); and IBRV-specific antibody titer concentration ($P < 0.08$). * SUB differs from INT within day, $P \leq 0.05$.

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