Effect of water restriction on performance, hematology and antibody responses in parenteral or intranasal modified-live viral vaccinated beef calves

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Abstract

Crossbred beef steer (n=28) and heifer (n=32) calves were randomly assigned within sex to 1 of 6 treatments to evaluate the effects of water restriction and vaccination. Water restriction was applied at the ranch of origin prior to feedlot shipment and consisted of no water restriction except during transport to the feedlot (**CON**), 48 h water restriction prior to transport to the feedlot (**ACU**), or alternating 24 h periods of water restriction, over a 7 d period prior to transport to the feedlot (**CHR**). Upon feedlot arrival (d 0), 2 different respiratory vaccines were administered; parenteral administration of a pentavalent (infectious bovine rhinotracheitis virus–IBRV; bovine viral diarrhea virus–BVDV, bovine respiratory syncytial virus–BRSV, and parainfluenza-3 virus–PI3V) modified-live virus (MLV) respiratory vaccine (2 mL subcutaneous in the neck; **SUB**) or intranasal administration of a trivalent (IBRV, BRSV, PI3V) MLV respiratory vaccine (1 mL/naris; **INT**). Cattle subjected to each of the water restriction regimens were equally allocated among vaccine treatments applied on d 0. Blood and nasal swabs were collected periodically to determine complete blood count, antigen-specific antibody titer, and BRSV-specific secretory IgA. Rectal temperature and body weight (BW) were recorded concurrent to blood sampling. Total neutrophils tended to increase overall for CONINT and ACUINT ($P \leq 0.09$). Rectal temperature was decreased after transport, but increased beginning on d 5 ($P \leq 0.01$). The CON treatment groups lost an average of 8.2% BW during relocation (d -1 to 0) while the ACU and CHR treatments had a 2.5% increase in BW ($P < 0.01$). Haptoglobin increased numerically following transportation and relocation ($P = 0.22$). The BRSV ($P \leq 0.01$) antibody titers were greatest for CONINT and CHRINT compared to CONSUB, ACUSUB, and CHRSUB ($P \leq 0.03$), and IBRV ($P \leq 0.01$) antibody titers were greater for ACUSUB, ACUINT, and CHRINT compared to CONSUB ($P \leq 0.03$). Water restriction prior to transport altered some hematological variables,

and briefly reduced performance but did not clearly alter antibody responses to either vaccine type.

Key words: beef cattle, water restriction, vaccination

Résumé

Des bouvillons (n=28) et des génisses (n=32) de boucherie de race croisée ont été alloués aléatoirement selon le sexe à l'un de six traitements afin d'évaluer l'effet de la restriction d'eau et de la vaccination. La restriction de la consommation d'eau a pris place au ranch d'origine avant le transport au parc d'engraissement et incluait l'une des conditions suivantes : (1) aucune restriction sauf durant le transport au parc d'engraissement (CON), (2) restriction de 48 h avant le transport au parc (ACU), ou (3) alternance de période de restriction et de non-restriction de 24 h durant une période de 7 jours avant le transport au parc (CHR). À l'arrivée au parc (J0), on a administré deux types différents de vaccins respiratoires : (1) administration parentérale d'un vaccin respiratoire pentavalent à virus vivants modifiés (le virus de la rhinotrachéite infectieuse bovine-VRIB, le virus de la diarrhée virale bovine-VDVV, le virus respiratoire syncytial bovin-VRSB, le virus parainfluenza-3-PI3) (2 ml sous-cutané dans le cou; SUB), ou 2) administration intranasale d'un virus respiratoire trivalent à virus vivants modifiés (1 ml/narine; INT). Les bovins de chaque groupe de traitement de restriction d'eau ont été alloués également dans les deux groupes de vaccination au jour 0. Du sang et des écouvillons nasaux ont été recueillis périodiquement afin de déterminer l'hémogramme complet, les titres d'anticorps spécifiques aux antigènes et la concentration d'IgA spécifique au VRSB. La température rectale et le poids corporel ont été mesurés en même temps que la prise de sang. Les neutrophiles totaux étaient un peu plus élevés pour les traitements CON-INT et ACU-INT ($P \leq 0.09$). La température rectale était moins élevée après le transport mais a augmenté au jour 5 ($P \leq 0.01$). Les bovins du groupe CON ont perdu en

moyenne 8.2 % de leur poids corporel durant la relocalisation (jour -1 à 0) alors que les bovins du groupe ACU et CHR avaient un gain de 2.5% ($P < 0.01$). Le taux d'haptoglobine a augmenté numériquement suivant le transport et la relocalisation ($P = 0.22$). Les titres d'anticorps spécifiques au VRSB ($P \leq 0.01$) étaient plus élevés dans les traitements CON-INT et CHR-INT que dans les traitements CON-SUB, ACU-SUB et CHR-SUB ($P \leq 0.03$). Les titres d'anticorps spécifiques au VRIB ($P \leq 0.01$) étaient plus élevés dans les traitements ACU-SUB, ACU-INT et CHR-INT que dans le traitement CON-SUB ($P \leq 0.03$). La restriction d'eau avant le transport a eu un impact sur certains paramètres hématologiques et a réduit temporairement la performance sans toutefois clairement altérer les réponses humorales suite à la vaccination avec l'un ou l'autre des deux types de vaccins.

Introduction

The cow-calf sector of the US beef production system is widespread and dynamic, with operations present in all 50 states.[20] On many of these operations, calves are weaned from their dam at approximately 207 days (d) of age,[16] then immediately sold via auction market. In a USDA report, more than 60% of calves that permanently left the cow-calf operation were transported to an auction market for sale; whereas, 17% were sold directly to a feedlot, and 12% were sold directly to another type of beef operation.[17] Once calves are transported to an auction market, they often have limited access to water and feed and can become dehydrated. The calves must then be transported to the feedlot or stocker facility, and feed and water are not available during transit. Feedlot shipment is 339 miles (545 km), on average.[18] From the time a calf leaves its ranch origin and arrives at the feedlot, several days of limited feed and water may have elapsed[4] and dehydration, commonly referred to as shrink within the cattle industry, is likely.

Upon feedlot arrival, nearly all calves are vaccinated against at least 1 or more respiratory viruses, including bovine viral diarrhea virus (BVDV; 95.1%), infectious bovine rhinotracheitis virus (IBRV; 93.2% parenteral, 13.4% intranasal), bovine respiratory syncytial virus (BRSV; 61.4%), and parainfluenza-3 virus (PI3V; 55.1%).[19] 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. 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 stimulate systemic IgG[11] and IgM production.[8] There is very little information on the impact of dehydration and the response of dehydrated calves to different routes of vaccine administration. Therefore, the objective of this study was to mimic water restriction and transportation of the previously described marketing scenario and determine effects on systemic and mucosal antibody and other immunological responses following parenteral or intranasal respiratory vaccine administered to beef calves.

Materials and Methods

Cattle Procedures

Animal methods and procedures were approved independently by the IACUC committees at the University of Arkansas and West Texas A&M University (WTAMU), depending on the location of study. 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 d of age with an average body weight (BW) of 395 lb (179 kg) on d 0. Appropriate vaccine handling procedures were closely followed and single doses of vaccines were administered following Beef Quality Assurance guidelines. The label for the parenteral pentavalent modified-live virus (MLV) vaccine[a] used in this study recommends a BRSV booster 14 to 28 d following initial vaccination; however, a booster dose was not administered in this study.

A total of 60 clinically healthy, crossbred beef steer (n=28) and heifer (n=32) calves were used. The calves had not been previously administered a respiratory vaccine. They were confirmed seronegative for IBRV-, BVDV type 1a-, and PI3V-specific antibodies through pre-trial serum samples collected on d -21 that were tested at the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) in Amarillo via virus neutralization assay. Calves were then stratified by d -21 BW and assigned randomly to 1 of 6 treatments with gender evenly dispersed across treatments. Animals were not blocked by any variable in this study. Water restriction was applied at SWREC and consisted of: no water restriction at any time other than during transport (**CON**); 48 h water restriction prior to transport (**ACU**); or alternating 24 h periods of water access and restriction, over a 7 d period and 48 h water restriction prior to transport (**CHR**) intended to mimic the marketing process and induce a greater degree of dehydration. Calves were housed in pens at SWREC according to the water restriction regimen with equal dispersion to the 2 vaccine treatments that were to be administered post-shipment, resulting in 5 calves assigned to each vaccine type in each of 6 water restriction pens.

On d -1, calves were transported 541 miles (871 km) to WTAMU. Vaccine treatments were applied at WTAMU on d 0 and consisted of parenteral administration of a single dose of a pentavalent (IBR, BVDV types 1 and 2, BRSV, PI3V) MLV respiratory vaccine (2 mL subcutaneous; **SUB**[a]) or intranasal administration of a trivalent (IBRV, BRSV, PI3V) MLV respiratory vaccine (1 mL/naris; **INT**[b]). The calves were housed in 2 pens 48 ft (14.6 m) apart at WTAMU that separated SUB and INT treatments to avoid contact and potential vaccine antigen transfer between groups. This design resulted in the 6 treatments consisting of CONSUB, CONINT, ACUSUB, ACUINT, CHRSUB, and CHRINT.

Study Phase 1. During the 14 d weaning and acclimation period (d -21 to -7), calves were abruptly removed from their dams, placed in a grass pasture, and remained as a single co-hort group until d -7 when they were sorted into 1 of 6 pens (768 sq ft; 71 sq m) according to water restriction treatment to facilitate restriction and availability of water according to the schedule presented in Table 1. Calves were provided ad libitum access to grass hay and supplement. Each pen con-tained an equal number of SUB and INT calves. The 2 pens containing CHR calves were initially restricted water access at 0800 h starting on d -6 (7 OCT 15). The 2 pens containing ACU calves were restricted water access from 0800 h on d -2 until feedlot arrival on d -1, whereas the CON animals were restricted from water only during transportation on d -1. The calves located at SWREC were loaded in a commercial trailer at 1100 h on d -1, and transported 541 miles (871 km) before arriving at the WTAMU Research Feedlot at 2315 h. The trailer was washed with water 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, SUB treat-ments were administered 2 mL of IBRV, BVDV types 1 and 2, BRSV, and PI3V[a] subcutaneously, while INT treatments were administered 1 mL/naris of IBRV, BRSV, and PIV3 vaccine[b] according to Table 1. Applicator tips on the INT syringe were changed after every calf and needles on the SUB syringe were changed every 10 animals. The animals were then sorted into 1 of 2 isolated pens according to treatment to avoid contact between SUB and INT groups following vaccination. On subsequent sample d, the SUB animals were sampled first, the chute and handling equipment were disinfected with a chlorhexidine solution, and INT animals were sampled last. The handling equipment was again washed down and dis-infected after each sampling d. These biosecurity protocols were implemented to avoid contact between the 2 vaccine groups and prevent transmission of intranasal vaccine virus to calves that received parenteral vaccine treatments. The calves remained at the WTAMU facility until the study ended on d 56.

Sample Collection and Assay Procedures

Calves were removed from their pen and briefly re-strained on d -7, -5, -3, -1, 0, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 to collect blood samples, rectal temperature (RT), and BW. Blood samples were collected via jugular venipuncture beginning at approximately 0800 h on sample d -7 (06 OCT 15) through sample d -1 (12 OCT 15). On subsequent sample days at WTAMU, calves were processed beginning at approxi-mately 0600 h through d 56 (08 DEC 15).

Blood was collected into evacuated blood collection tubes with no additive,[c] allowed to clot for at least 30 min-utes, centrifuged at 1,250 × g for 20 minutes at 39°F (4°C), and separated serum was decanted and stored in triplicate aliquots at -4°F (-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 con-centration 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 serum haptoglobin (Hp) concentration via ELISA[d] at the WTAMU Animal Health Laboratory.

An anticoagulated blood sample was collected via jugu-lar venipuncture, into evacuated tubes containing EDTA[e] 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[f] at the WTAMU Animal Health Laboratory to determine complete blood count (CBC). After automated hematology was determined, the blood samples were further used to determine a packed cell volume manually using a capillary tube and microcentrifuge[g] 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 -4°F (-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[h] in

Table 1. Daily water availability schedule during the water restriction period.

Study day*	CON SUB[†] (n=10)	CON INT (n=10)	ACU SUB[†] (n=10)	ACU INT (n=10)	CHR SUB[†] (n=10)	CHR INT (n=10)
-7	Yes	Yes	Yes	Yes	Yes	Yes
-6	Yes	Yes	Yes	Yes	No	No
-5	Yes	Yes	Yes	Yes	Yes	Yes
-4	Yes	Yes	Yes	Yes	No	No
-3	Yes	Yes	Yes	Yes	Yes	Yes
-2	Yes	Yes	No	No	No	No
-1 (Ship to WTAMU)	Yes	Yes	No	No	No	No
0 (Vaccinate)	Yes	Yes	Yes	Yes	Yes	Yes

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

[†]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. 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).

the mid-naris region, proximate to the nasal concha, until the swabs were completely saturated. The triplicate nasal swabs were clipped, placed into sealed tubes,[i] and stored at -112°F (-80°C) until subsequent analysis to determine percent positivity of BRSV-specific secretory IgA (sIgA). Briefly, swabs were thawed at room temperature, and placed in microtubes prior to adding 300 μL phosphate-buffered saline containing 1mM EDTA and 400 μL prepared Sputolysin Reagent.[j] Samples were then vortexed and incubated at room temperature for 15 minutes. After centrifugation at 3000 × g for 5 minutes at 39°F (4°C) to pellet insoluble mucus, 100 μL supernatant from swabs was harvested using a micropipette, distributed into the 96-well plate and analyzed for BRSV-specific Ig concentration following instructions provided in the commercial ELISA kit specific for BRSV Ab.[k] To capture IgA specific antibody, 5 μL of sheep anti-bovine IgA HRP conjugate[l] was added to the IgG conjugate provided in the ELISA kits.

For BRSV-specific sIgA determination, 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 and analyzed statistically. According to the manufacturer, a sample is considered positive for BRSV-specific Ig if the PPI result is ≥ 10.

Statistical Analyses

This completely randomized design study used animal as experimental unit for all variables. Rectal temperature, CBC, serum IBRV, BVDV, BRSV, and PI3V antibody titer, bovine-specific Hp, and BRSV-specific sIgA PPI were analyzed using the MIXED procedure of SAS[l] with repeated measures. The repeated statement was d, and effects of treatment, d, and their interaction was evaluated. The autoregressive covariance structure was used in the model for all repeated measures variables. Performance variables and percent shrink were analyzed using PROC MIXED. The Kenward-Roger degrees of freedom method was employed for all dependent variables and F-test protected means were separated using the Tukey-Kramer adjustment. 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 were $\log_2$-transformed and tested again to determine if normality was improved. The $\log_2$-transformed data were used for statistical analysis if the Shapiro-Wilk P-value ≥ 0.05 and back-transformed LSmeans were displayed. A significance level alpha less than or equal to 0.05 was used and 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 only observed in 1 animal that was removed from the study due to development of a severe abscess not related to experimental treatment.

Hematocrit and Complete Blood Count

During phase 1, the change in hematocrit for the water restricted calves numerically increased in a manner corresponding to the initial time of water restriction (Figure 1) such that a treatment × d interaction existed ($P < 0.01$). However, the only treatment difference within d was observed on d 56, as CHRSUB was reduced compared to CONINT and ACUINT ($P ≤ 0.05$). As animals become dehydrated and their fluid volume decreases in the blood, the proportion of cells increases; therefore, an increased hematocrit may indicate that an animal is dehydrated. We expected treatment differences in hematocrit to exist early in the study, but only numerical differences were observed. The difference observed on d 56 may be a random artifact, yet the lack of statistical difference during early observations may be a result of increased within-treatment variability in the limited number of subjects available to use in this study. Nevertheless, the numerical pattern of hematocrit increase followed the initial time of water restriction and agrees with other findings. Shaefer and others[15] reported that hematocrit increased in cattle with increasing time restricted from feed and water; however, another study[22] observed no change in hematocrit in cull cows restricted for 36 hours from water prior to slaughter. There was a treatment × d interaction for total white blood cell count (Figure 2). While there was not a treatment × d interaction ($P = 0.35$) for neutrophils, there was a trend for a treatment effect ($P < 0.06$; Figure 2). Overall, CONINT and ACUINT neutrophil counts tended to be greater than CONSUB ($P ≤ 0.09$). Monocytes for CHRSUB tended to be decreased compared to ACUINT on d 5 ($P = 0.08$) and lymphocytes were decreased numerically for CHRSUB vs ACUINT on d 5 ($P = 0.27$). Eosinophils decreased (day effect, $P < 0.01$) by more than 50% from d 0 to d 7 regardless of treatment (data not shown). The stress of being transported and relocated to a novel environment likely caused eosinopenia induced by glucocorticoids.[6] There was a treatment × d interaction observed for total red blood cell (RBC) count ($P = 0.02$) such that RBCs increased numerically following water restriction and transportation (data not shown). This was anticipated as RBC concentration is known to increase during times of dehydration.[15] The CHRINT and CHRSUB groups were restricted from water on d -6 and -4 and as a result, on d -3 RBC increased to $10.2 × 10^6$/ μL and $10.6 × 10^6$/ μL, respectively. From d 0 to 3, RBC gradually increased overall after the approximately 14-hour transport from SWREC to WTAMU.

Rectal Temperature

There was a treatment × d interaction ($P < 0.01$) observed for RT. Figure 3 illustrates that treatment differences
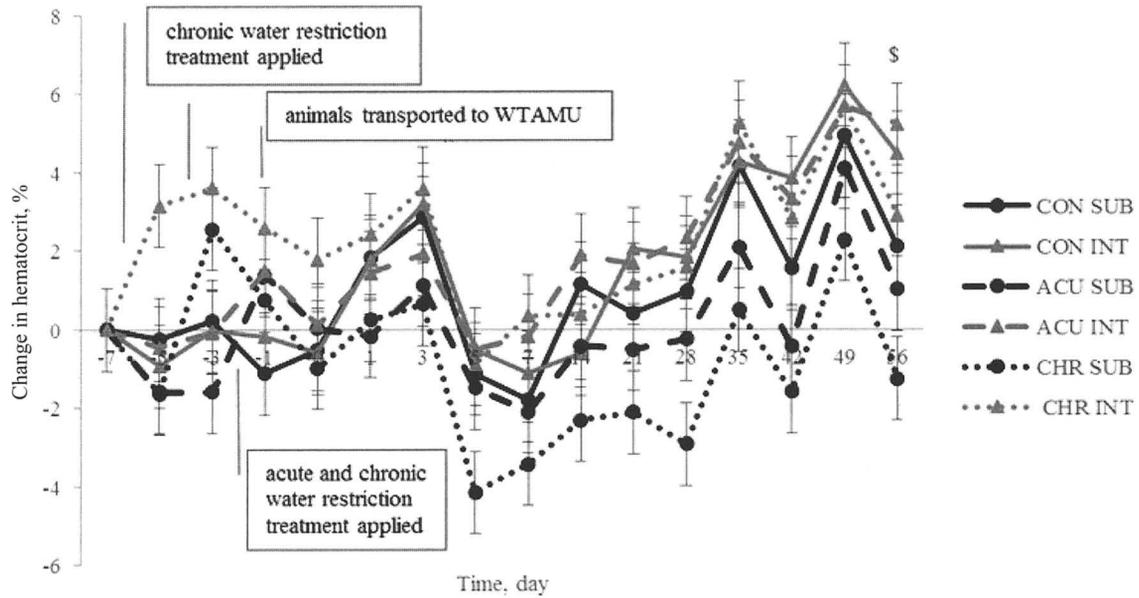
Figure 1. Effect of control (CON), acute (ACU) and chronic (CHR) water restriction with subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V]*) or intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V†) vaccination on change in percent hematocrit (treatment × day; $P < 0.01$). $ CHR SUB differs from CON INT and ACU INT, $P < 0.05$. Error bars represent the pooled standard error of the mean.
*Express® 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
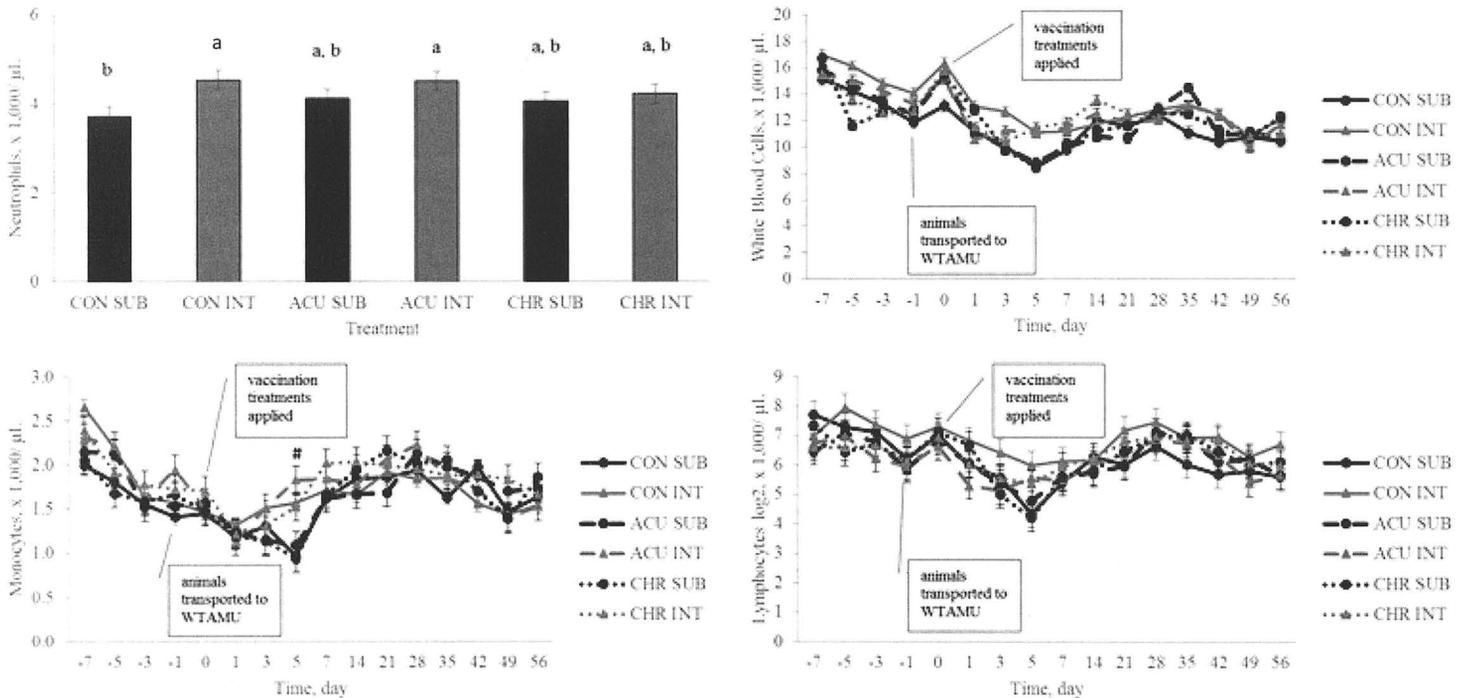†Inforce™ 3, Zoetis, Parsippany, NJ

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]*) and intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V†) vaccination on neutrophil count (treatment effect; $P = 0.06$), total white blood cell count (treatment × day; $P = 0.05$), monocyte count (treatment × day; $P = 0.01$), and lymphocyte count (treatment × day; $P = 0.01$). Treatments with unlike letters tend to differ, $P \leq 0.09$. # ACUINT tended to differ from CHRSUB, $P = 0.08$. Error bars represent the pooled standard error of the mean.
*Express® 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
†Inforce™ 3, Zoetis, Parsippany, NJ

did not exist within d ($P \geq 0.63$), yet there were clear temporal patterns evident (day effect; $P < 0.01$). Overall, the RT decreased after transport on d -1 to 3 and increased beginning on d 5. This RT pattern may be in response to viral replication from the MLV vaccines administered to all calves on d 0. In the event of invading pathogens, RT increases as part of the innate immune response.[7] In a previous study[6] that compared the effects of stress via repeated dexamethasone administration and vaccination on rectal temperature, it was reported that following vaccination, the CON animals that were not administered dexamethasone had greater RT compared to animals that were administered dexamethasone before vaccination. However, as previously indicated no treatment effect on RT was resolved in the current study. In a previous study, RT decreased following 48 h deprivation of feed and water in steers;[14] in the current study CHRSUB and CHRINT had numerically reduced RT on d -3 compared to the other treatments that had not been restricted from water access at this time, but the difference was not statistically significant ($P \geq 0.63$).

Performance

Change in body weight differed between water restriction treatments from d -7 to d -1 ($P < 0.01$; Table 2). The CONSUB and CONINT gained an average of 9.4 and 14.9 lb (4.3 and 6.8 kg), respectively, during the 6-d period while the water restricted treatment groups lost body weight during the same time period. This decrease in body weight may be attributable to water loss due to dehydration rather than weight loss from tissue catabolism. Feed and water deprivation of >24 hours has been observed to decrease BW,[22] increase percentage shrink, and decrease ADG for 28 d following water restriction.[9] Conversely, percent 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 subsequently weighed on d 0. The increase in body weight observed for water restricted treatments 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 compensation for 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 numerically increased for CONSUB and CONINT compared to the water restricted treatments, but this difference was not statistically resolvable ($P = 0.20$). Nevertheless, this observation is likely due to more diminished gut fill for CONSUB and CONINT during transport and rapid replenishment immediately following transport.
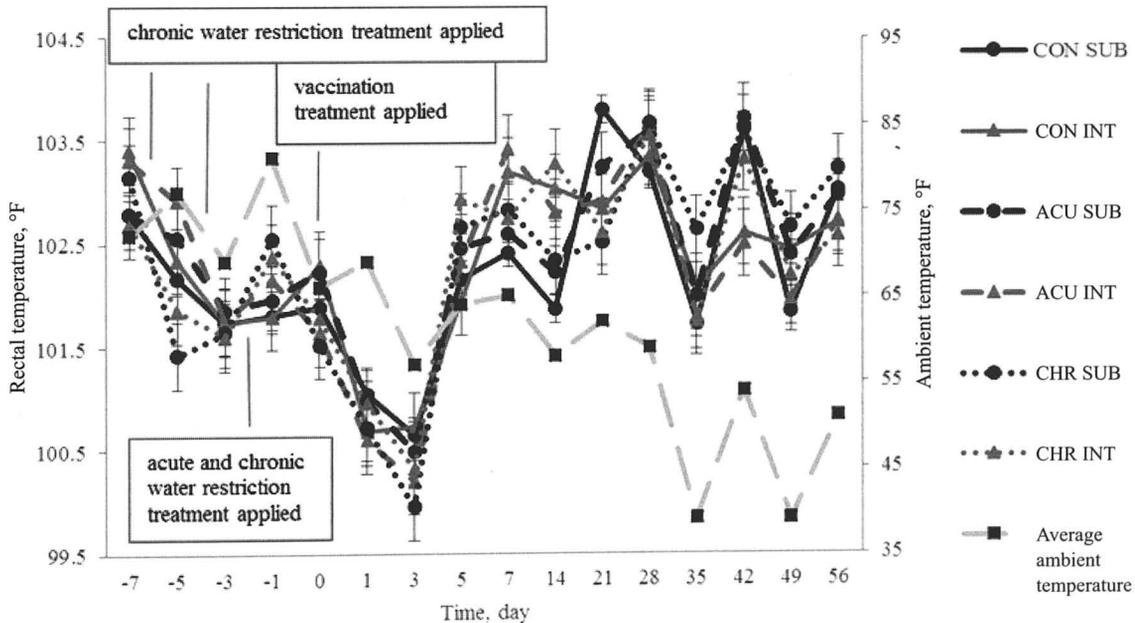

Figure 3. Effect of control (CON), acute (ACU) and chronic (CHR) water restriction treatment with subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V]*) or intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V†) on rectal temperature (treatment × day, $P < 0.01$). Error bars represent the pooled standard error of the mean. Average ambient temperature for d -7 to -1 are according to NOAA data reported from the Texarkana Regional Airport, Texarkana, AR; d 0 to 56 are according to NOAA data reported from the Rick Husband Amarillo International Airport, Amarillo, TX. Note: rectal temperature is indicated in the left y-axis scale and ambient temperature is indicated in the right y-axis scale.

*Express® 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
†Inforce™ 3, Zoetis, Parsippany, NJ

Table 2. Effect of control (CON), acute (ACU) and chronic (CHR) water restriction with subcutaneous (SUB) or intranasal (INT) vaccination on percent shrink and performance.

Item	Treatment*						SEM†	P-value‡
	CON SUB	CON INT	ACU SUB	ACU INT	CHR SUB	CHR INT		
Initial BW, lb	387.5	397.1	383.7	383.1	405.6	411.1	18.3	0.81
D -7 to -1 BWC, lb	9.4§	14.9§	-24.2‖	-17.7‖	-19.9‖	-22.7‖	3.96	<0.01
D -1 to 0 shrink, %	8.3§	8.0§	-3.1‖	-1.6‖	-2.2‖	-3.0‖	0.96	<0.01
D 0 to 14 ADG, lb	3.6	3.5	2.9	2.9	2.8	2.7	0.32	0.20
D 0 to 56 ADG, lb	2.9	2.8	2.8	2.6	2.8	2.7	0.16	0.71
Final BW, lb	523.7	537.8	528.0	513.7	553.6	548.8	22.2	0.77

*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. 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]§; INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V‖.
†Pooled standard error of the mean.
‡Treatments with unlike letter superscripts differ, $P \leq 0.05$.
§Express® 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
‖Inforce™ 3, Zoetis, Parsippany, NJ

Haptoglobin

Haptoglobin is an acute phase protein primarily produced by hepatocytes in response to inflammation, and has been observed to increase in response to transport, feed and water deprivation,[9] and vaccination.[2] However, previous research[1] reported greater serum haptoglobin concentration in non-transported calves compared to calves transported for 3 h, indicating that Hp response to transport is variable. There was no treatment effect (P = 0.62) or treatment × d interaction (P = 0.64) detected for serum Hp in the present study. Figure 4 reveals overall increased Hp on d -7 (day effect; P < 0.01), likely due to unknown and unanticipated stressor experienced immediately prior to study initiation. This con-

founded the potential serum Hp response due to treatment, yet Hp had declined by d -1, and increased numerically (d -1 to 5; P = 0.22) after transportation and relocation to WTAMU.

BRSV-Specific Secretory IgA

Secretory IgA has been observed to be the most prominent antibody class at mucosal surfaces, including the naris,[3] and is important for inhibiting bacterial adhesion, reducing the inflammatory effects of other Ig, neutralizing bacterial toxins and viruses as well as enhancing innate immune defense mechanisms in other species.[5] Due to numerous pathogens gaining access into the host via epithelial cells,[13] secretory IgA is an important component in determining
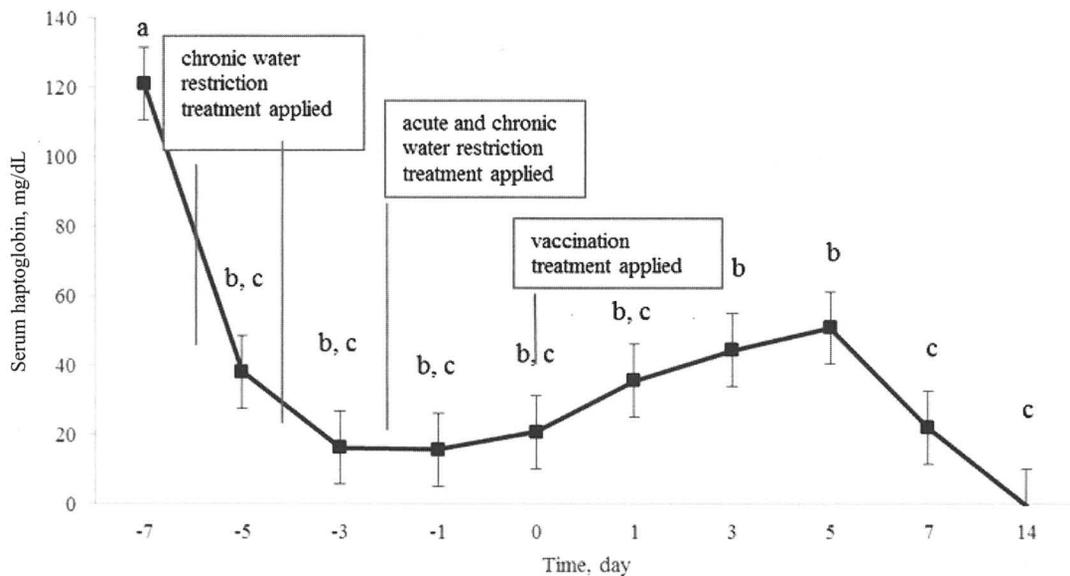

Figure 4. Effect of day on serum haptoglobin concentration (P < 0.01). Day means with a different letter superscript differ, $P \leq 0.05$. Error bars represent the pooled standard error of the mean.
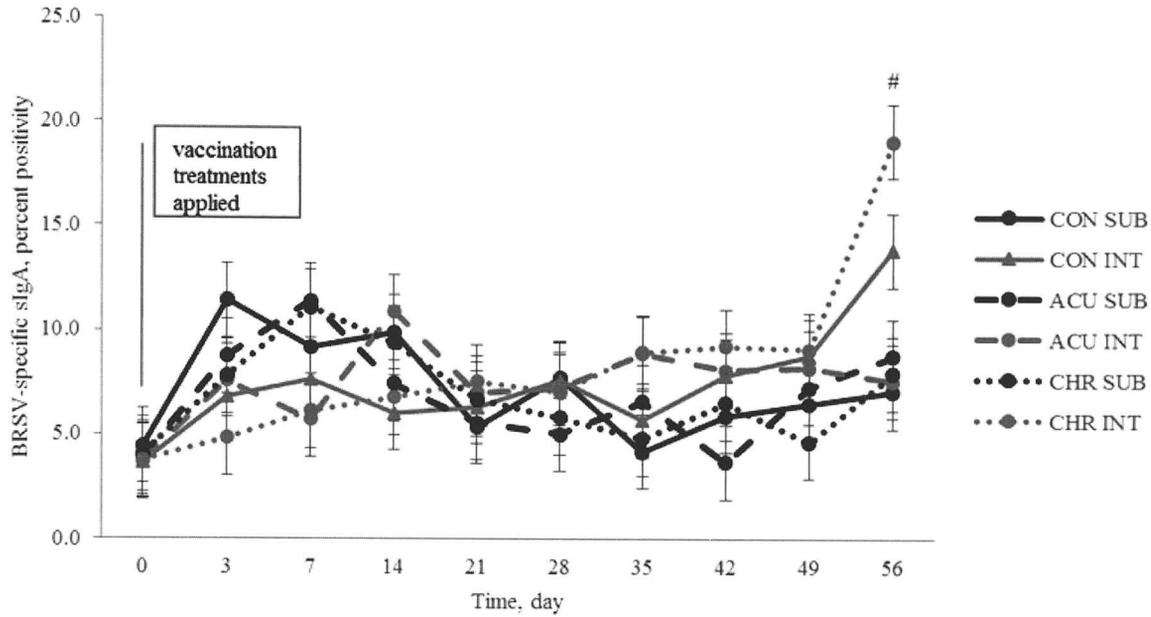
Figure 5. Effect of control (CON), acute (ACU) and chronic (CHR) water restriction with subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V]*) or intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V†) vaccination on BRSV-specific sIgA percent positivity index (treatment × day, $P < 0.01$). # CHR INT differs from CON SUB, ACU INT, ACU SUB, and CHR SUB, $P < 0.01$. Error bars represent the pooled standard error of the mean.

*Express® 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
†Inforce™ 3, Zoetis, Parsippany, NJ

vaccine efficacy because it is the primary antibody class present in nasal secretions.[5] Figure 5 illustrates the treatment × d effect ($P < 0.01$) on 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. The only difference observed in sIgA PPI in the current study was on d 56; CHRINT had greater sIgA PPI than CONSUB, ACUINT, ACUSUB, and CHRSUB ($P ≤ 0.01$) but did not differ from CONINT ($P = 0.99$). In another study in humans,[11] there was an increase in IgG following intramuscular administration of an influenza vaccine with antibodies in the serum detected prior to detection of IgG in mucosa, and the peak sIgA concentration was not observed until d 28. Our results suggest that intranasal vaccine treatments may have increased sIgA PPI slightly on d 56, but water restriction did not clearly impact sIgA PPI.

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, though systemic response tends to be less robust for the intranasal route.[10] This is also supported by studies[11,21] that reported greater serum antibody responses in systemic vs intranasal vaccinated humans and cattle, respectively. A more recent study[12] also 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 immunosuppressed animals. There was a treatment × d interaction ($P < 0.01$) observed for PI3V- and BVDV-specific serum antibody titer (Figure 6). On d 56, CONSUB had greater PI3V titer than CONINT, but no other treatment differences were detected. As expected, the CONSUB, ACUSUB, and CHRSUB treatments had greater BVDV titer than CONINT, ACUINT, and CHRINT from d 14 to 56 ($P ≤ 0.01$). The INT treatments 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. There was a treatment effect ($P < 0.01$) observed for BRSV-specific antibody titer. The CONINT and CHRINT treatments had increased BRSV titer compared to CONSUB, ACUSUB, and CHRSUB ($P ≤ 0.03$). The BRSV fraction contained in the SUB vaccine[a] used in the current study is labeled for revaccination 14 to 28 d after primary vaccination, but this did not occur for the current study. There were treatment differences noted for IBRV-specific antibody titer (treatment effect; $P < 0.01$); ACUSUB, ACUINT, and CHRINT were greatest ($P ≤ 0.03$), CONINT and CHRSUB were intermediate, and CONSUB was least but did not differ from CONINT or CHRSUB ($P ≥ 0.25$).
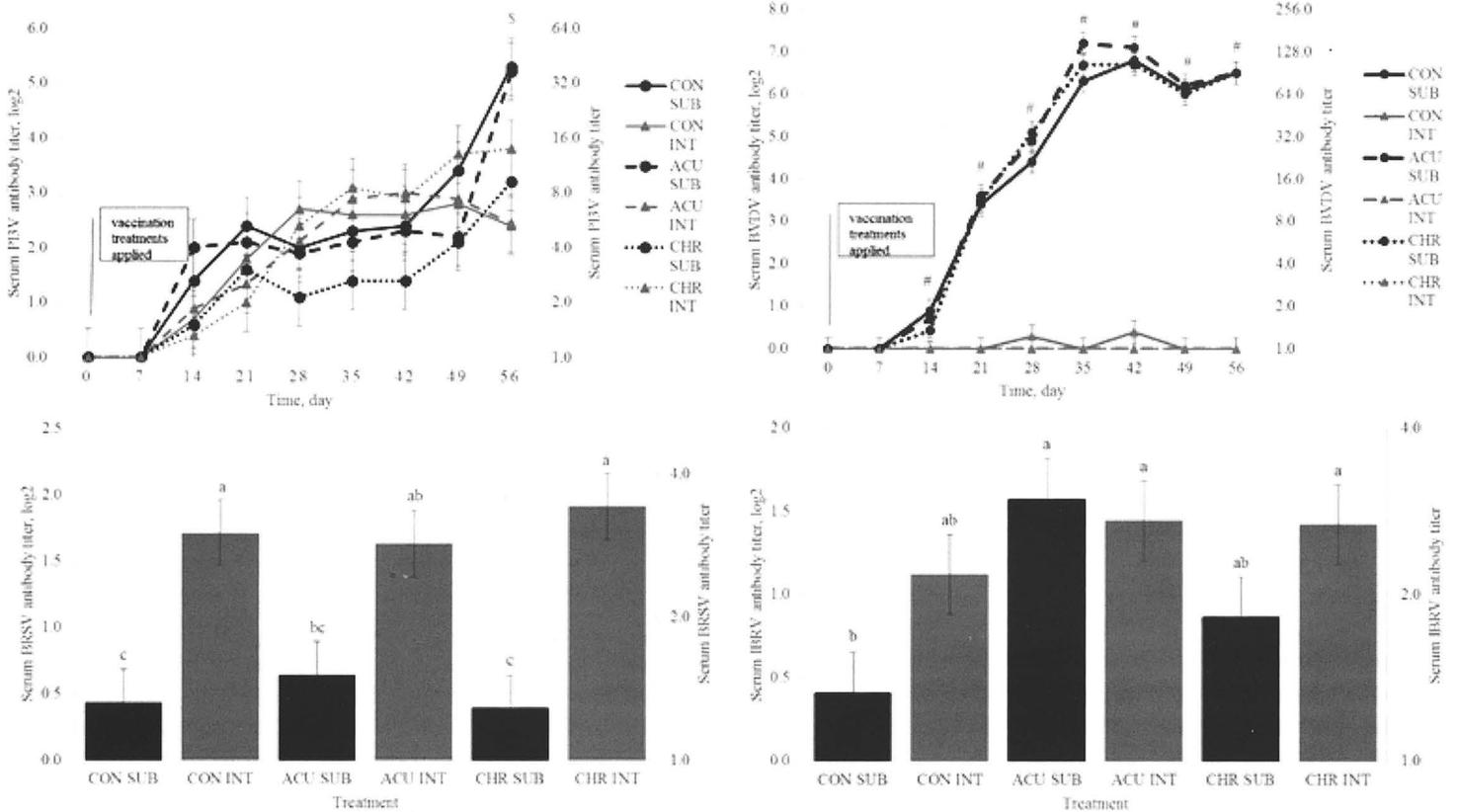
Figure 6. Effect of control (CON), acute (ACU) and chronic (CHR) water restriction with subcutaneous (SUB= subcutaneous vaccination on d 0 with bovine viral diarrhea virus [BVDV] type 1 and 2, infectious bovine rhinotracheitis virus [IBRV], bovine respiratory syncytial virus [BRSV], and parainfluenza-3 virus [PI3V]*) or intranasal (INT=intranasal vaccination on d 0 with IBRV, BRSV, and PI3V†) vaccination on serum PI3V-specific antibody titer concentration (treatment × day, $P < 0.01$); serum BVDV-specific antibody titer concentration (treatment × day, $P < 0.01$); serum BRSV-specific antibody titer concentration (treatment, $P < 0.01$); and IBRV-specific antibody titer concentration (treatment, $P < 0.01$). $ CON SUB differs from CON INT, $P < 0.05$. # CON SUB, ACU SUB, and CHR SUB differ from CON INT, ACU INT, and CHR INT, $P < 0.01$. Treatments with unlike letter superscripts differ, $P < 0.03$. Error bars represent the pooled standard error of the mean. Note: The $\log_2$-transformed values are indicated on the left y-axis and the non-transformed values are indicated on the right y-axis for each variable. Data were statistically analyzed using the $\log_2$-transformed values.
*Express® 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
†Inforce™ 3, Zoetis, Parsippany, NJ

Conclusions

There was no clear effect of water restriction on the antibody responses to either subcutaneously or intranasally administered vaccine antigens under conditions of the current study. Alterations observed in hematologic and performance variables indicates that water restriction during the marketing process impacts beef calves in a transient manner. There were slight differences in leukocyte profiles and antibody titers between SUB and INT treatments, but the overall antibody response in calves used in this study was very low. Further research is needed to understand the immunogenicity of different routes of respiratory vaccination administered to stressed, dehydrated beef calves.

Endnotes

[a]Express® 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO
[b]Inforce™ 3, Zoetis, Parsippany, NJ
[c]Vacutainer 10 mL tube, Ref #367985
[d]Immunology Consultants Laboratory, Newberg, OR
[e]Vacutainer, 7.2 mg K2-EDTA, 4 mL tube, Ref #367861
[f]Idexx, ProCyte DX Hematology Analyzer, Westbrook, ME
[g]EKF Diagnostics, HemataStat II, Cardiff, UK
[h]Puritan Medical Products #3306-PN
[i]Falcon 5 mL polystyrene round-bottom tube, Corning Inc., Corning, NY
[j]EMD MILLIPORE chemicals product #560000 10 ML

[k]Svanova, Svanovir BRSV-Ab, Boehringer Ingelheim Svanova, Uppsala, Sweden

[l]Bethyl Laboratories, Montgomery, TX

Acknowledgements

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