

Multimodal Assessment of Biometric Changes in Injection Sites and Physiology and Behavior in Beef Calves Receiving Two Different Clostridial Immunizations Compared to Negative Controls

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ABSTRACT

Clostridial immunizations are commonly administered to cattle, and understanding the physiologic and behavioral effects of vaccination may influence preventative health program design. A randomized, controlled, blinded clinical trial was conducted using multimodal assessment of injection sites, physiologic, and behavioral changes in calves receiving one of two clostridial vaccinations and negative controls. Calf response after injection was monitored daily utilizing physiologic parameters (rectal temperature, pulse, respiratory rate, and

body weight), measurements of injection site reaction (lesion surface area, depth, and volume), skin surface temperature (thermography), and avoidance response to pressure at injection site (algometer). Calf behavior was remotely monitored using pedometers, accelerometers, and a remote triangulation system. Calves vaccinated with the clostridial products had larger injection site lesions and greater injection site surface temperature ratios compared to saline controls. The pressure required to stimulate a reaction differed among treatments and this effect was modified by treatment group with control calves requiring more pressure to stimulate avoidance response early in the trial. The percent of time calves spent stand-

ing was greater in one of the vaccine groups relative to controls as measured by the accelerometers. The location monitoring system revealed differences among treatment groups relative to time spent at the hay and grain feeding areas. This research illustrates that clostridial vaccination induced multiple changes in injection site, physiological, and behavioral variables as compared to negative controls. The biometric profile generated by the combination of multimodal assessment tools employed in this project demonstrates the advantages of objective assessment to describe effects of vaccine administration.

INTRODUCTION

Injectable immunizations are frequently administered to cattle, and vaccination against clostridial diseases is relatively common throughout the beef industry. A reported 78% of cattle receive a clostridial vaccine upon arrival to the feedlot.¹³ In cow-calf operations with over 200 breeding cows, 91% of the ranches vaccinated calves for clostridial diseases.¹² Previous research has documented the efficacy of commonly used clostridial vaccines ability to stimulate an antibody response.^{10,16} Clostridial vaccines are commonly associated with injection site reactions and cause concern for adverse events after injection. Interestingly, increased injection site reactions after clostridial vaccination have been associated with enhanced serum clostridial titers,¹¹ yet these inflammatory responses can adversely impact calf physiology and behavior. Although injection site reaction after clostridial vaccination has been described, there is limited literature describing changes in behavior and physiology of calves.

Specific types of clostridial vaccines have been shown to influence the inflammatory response, feeding performance and behavior of calves.^{2,8} Other research illustrates few differences in feeding performance or behavior based only on varying injection site location.¹ The aforementioned research evaluated longer term post-clostridial vaccination reactions. Little research exists providing detailed information on the acute

phase physiologic and behavioral changes that occur in calves after clostridial vaccination. An improved understanding of acute changes is important because this time frame occurs concurrently with the high risk period for other diseases, such as bovine respiratory disease, when vaccinations are administered upon arrival to a stocker or feedlot operation.

The objective of this research was to evaluate potential differences in short-term (13-day) injection site reactions, physiologic and behavioral changes between two commonly used 7-way clostridial vaccines and a negative control. We hypothesized that calves administered the clostridial vaccines would have larger injection site reactions, greater physiologic changes, and behavioral modifications relative to saline control calves. This research is unique in vaccine reaction evaluation as it institutes a series of biometric measurements using multimodal tools to simultaneously monitor the injection site reaction and the potential influence on calf well-being.

MATERIALS AND METHODS

All methods and procedures in this experimental protocol were approved by the Kansas State University Animal Care and Use Committee. (Protocol #3064).

Animals

Black angus-cross heifer calves (n=13) were purchased through a local auction and housed in one 12 m x 25 m dry lot pen (Large Animal Research Center, KSU, Manhattan, Kansas). All calves were fed a commercial ration (Calf Grower B-68, Farmers CO-OP Assn., Manhattan, KS) at a rate of 1.4 kg/head/day and free choice brome and alfalfa hay throughout the trial period.

Preventive Health Programs

Upon arrival (9 days prior to clostridial vaccination, study day -9), all calves were individually weighed and given a unique identifying tag in the right ear. Each calf received metaphylaxis using ceftiofur CFA (Excede,TM Pfizer Animal Health, New York, NY; dosed at 6.6 mg/kg and based on aver-

age weight of 227 kg BW) administered subcutaneously in the left ear base as per label directions. An injectable modified live viral vaccine (including parainfluenza 3 virus, bovine respiratory syncytial virus, infectious bovine rhinotracheitis virus and bovine viral diarrhea virus types 1 and 2 (Express[®] 5, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) was administered to all calves subcutaneously over the left rib cage. This injection site ensured that the neck was free of confounding injection sites. All calves were monitored daily for signs of clinical illness after arrival until study conclusion.

Clostridial Vaccine Allocation and Administration

All calves were randomly assigned using commercial software (Microsoft Office Excel[®] 2007, Microsoft, Redmond WA) to one of three treatment groups: V7 (Vision[®] 7 with Spur; Merck Animal Health, Summit, NJ; serial # 09580912C, exp. 05/01/2013, n = 5), C7 (Caliber[®] 7; Boehringer Ingelheim Vetmedica, Inc; St. Joseph, MO; serial # 333-058, exp. 10/12/2012; n = 5), or CN (negative control; sterile saline only; n = 3). Both clostridial vaccinations were bacterintoxoids containing the following antigens: Clostridium chauvoei, Clostridium septicum, Clostridium novyi, Clostridium sordellii, and Clostridium perfringens Types C and D. All products were handled and administered in accordance with label directions. The same veterinarian [AD] performed all daily observations and was blinded to the treatment allocation of each calf.

On study day 0 (9 days after arrival), all vaccines were administered subcutaneously (using a 1.5 inch, 18-gauge needle) in the center of a previously clipped (No. 40 clipper blade) 20 cm x 20 cm area on the right side of the neck. The center of the clipped area was determined using a pliable square with crosshairs. Alcohol was applied to the skin prior to vaccination. All vaccines were subcutaneously administered by the same veterinarian [DEA] using the “skin tenting” method. Confirmation of subcutaneous placement of the needle was ensured prior

to injection. All control group calves were treated in similar manner except that instead of a clostridial product, 2 ml of 0.9 % saline was injected subcutaneously.

Pre- and Post-Vaccination Measurements

Daily measurements of the calves were done in a completely enclosed building. Each calf was restrained and weighed in a weigh scale chute (For-Most Livestock Equipment, Harwarden, IA) daily at approximately the same time of day. The following measurements were taken on each calf on each day of the 13-day trial period:

- vital parameters
- lesion surface area dimensions
- lesion depth
- skin surface thermal readings, and
- response to pressure applied to the lesion.

Vital parameters measured daily included body weight, rectal temperature, heart rate (beats per minute), and respiratory rate (breaths per minute). The dimensions of the surface area of the injection site reaction were measured using digital calipers (Metr. 150, Whitworth Instruments) to estimate the surface area of the injection site reaction. A total of 6 directional diameters were measured using a clock-face template (eg, 12-6 o'clock position, 1-7, 2-8, 3-9, 4-10, and 5-11). Total lesion surface area was then estimated using calculations for the geometric area of a circle by using the mean of the 6 diameters. If no reaction could be detected visually or by palpation, the lesion size was recorded as 0.

The point of maximum depth for each detectable lesion was determined using ultrasonography (5.0 MHz linear transducer; Aloka Model SSD-900V, Hitachi Aloka Medical, Ltd., Tokyo, Japan). Depth was measured at the point of maximum reaction within each lesion. The lesion depth measurement was determined by measuring the distance, in mm, from the skin surface to the interface of the subcutaneous skin and the fascial covering of the underlying muscle. This number was used in combination with

total lesion surface area measurements to determine the geometric volume of an ellipse to estimate the total volume of the size of each lesion.

Skin surface temperature was recorded using high definition thermal imaging (ThermaCAM S65, ThermaCAM Researcher Pro 2.8 SR-1, FLIR Systems, Inc, Wilsonville, OR) of the injection site and surrounding clipped area. All images were obtained at a predetermined distance of 1-meter separation from the skin to the camera lens. The skin surface temperatures were assessed in a defined zone around the injection site on each day of study for each calf. Analysis was performed by drawing a region of interest around the lesion site and recording the mean temperature for that area. Using the same thermal image, another temperature recording was made of a pre-selected control site near to the cranial dorsal margin of the shaved area (~15-cm distant from the lesion site). Skin surface temperature was standardized to the control area so as to minimize any effect of variations of the imaging environment. The ratios of the mean temperature in the lesion region of interest to control region of interest (TL: TCAvg) and the maximum temperatures in the same areas (TL: TCMax) were determined and used for statistical analysis.

Adverse response to pressure applied to the lesion site was measured using algometry (Algometer Model FDIX, USB/RS232, 1cm² flat rubber tip, Wagner Instruments, Greenwich, CT). Adverse response to pressure applied to the injection site using algometry allows pressure to be applied over a controlled surface area and the magnitude of force applied over that area is recorded. This allowed semi-quantitative assessment of the intensity of discomfort over the center of each lesion. The algometer was aligned perpendicular to the injection site (as determined in the center of the shaved area) and pressure applied until calf reaction to the pressure was noted. Calf reaction was gauged as moving away from the algometer, movement in the head and neck region or

weight shifting away from the pressure.

Behavioral Monitoring

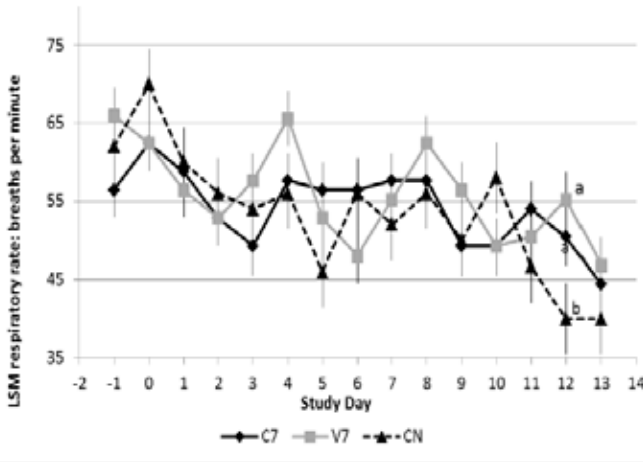
Following a 1 week acclimation period (study day -2), all calves were individually weighed and each calf was equipped with behavioral assessment technology (location triangulation ear tags, accelerometers, pedometers). This individual calf data provided detailed and quantitative analysis of calf behavior and activity. Location triangulation tags (Ubisense Series 7000 Compact Tag, Ubisense, Denver, CO) were placed in the dorsal aspect of the left ear and attached using a commercial ear tag button. These tags include a dual-radio architecture that transmits radio pulses that are then used to determine the precise 3-dimensional location of each calf within the pen as previously described.¹⁴ Locational patterns of the time calves spent at the grain bunks, hay bunks, water trough, and shelter area were recorded and aggregated over each 24 hour period to identify behavioral changes.

Accelerometers (GPI Programmable Accelerometer, Sensr, Elkader, Iowa) and pedometers (NL-800 Activity Monitor, New Lifestyles, Lee's Summit, Missouri) were placed on the lateral aspect of the right metatarsus within a padded neoprene pouch and secured with adhesive tape. The pedometers record the number of steps taken aggregated per 24-hour period for each calf. The accelerometers record relative changes in force (g) in three axes (x,y,z) and these data can be used to calculate the percentage of time spent standing, lying, or walking aggregated per 24-hour period using a previously defined method.⁷ The periods of time during which calves were being processed (eg, daily measurements of injection sites) were removed from each behavioral dataset prior to analysis to allow data to represent only the time calves spent in the pen.

Health Monitoring

All calves were visually assessed twice daily (morning and evening) for the entire trial by the same veterinarian blinded to injection group assignment. During these periods, each calf was assigned a clinical

Figure 1. Model estimated least square mean (and standard error) respiratory rate by treatment group and study day. Treatment groups were administered either C7, (Caliber® 7, Boehringer Ingelheim Vetmedica, St. Joseph, MO, n=5), V7 (Vision® 7 with Spur, Merck Animal Health, n = 5), or CN (negative control, sterile saline, n=3). Study dates relative to time of clostridial (or saline for control group) administration on study day 0. Unique superscripts denote statistical ($P < 0.05$) differences between treatment groups within study day.



illness score (CIS). The CIS system was as follows:

- 1 = normal behavior
- 2 = mild apparent depression and/or cough
- 3 = moderate apparent depression, and/or labored breathing and/or cough
- 4 = severe illness, moribund with little response to human approach.

Protocol dictated that any calf having a CIS greater than 1 would be taken to the working facility for physical examination and treatment administration if the rectal temperature was found to be greater than or equal to 40°C.

Necropsy

Vaccinated calves (n=10) were humanely euthanized 14 days after vaccine administration and necropsy examination of injection sites, thorax, and abdomen performed. Euthanasia was conducted according to AVMA guidelines using a penetrating captive bolt

(Koch Magnum .25™ Stunner, Koch Supplies Inc., Kansas City, MO). Lesions were then dissected from the neck, cross-sectioned, and samples submitted for histopathology.

Statistical Analysis

Data were imported to and analyzed with a commercial software program (JMP® 9.0.2, SAS Institute, Cary, NC). All continuous outcomes were assessed using generalized linear models to evaluate potential associations between the outcome of interest and treatment group (C7, V7, CN), study day (-1 through 13), and the potential interaction between treatment and study day. When the interaction between study day and treatment group was not significantly ($P < 0.05$) associated with a specific outcome, the interaction was removed from the model and only

main effects were reported. All statistical models included calf identification number as a random effect to account for the lack of independence due to repeated measures on individual calves. 3 A P-value of ≤ 0.05 was considered significant for all models and least square means are reported when the effect was significant.

RESULTS

Study subjects

All calves were healthy (CIS = 1) from arrival to study day 0 (vaccine administration) as determined by twice daily observation and baseline physiologic measurements done on study days -1 and 0. No calves received a CIS greater than 1 or required any treatment at any point during the study period. Data were available on all measured parameters on all animals throughout the trial period and no data were excluded from the analysis.

Physiologic Parameters

There were no significant interactions between study day and treatment group when body weight, temperature, and heart rate were evaluated. Of the basic physiologic parameters, only respiratory rate (RR) showed a significant interaction between treatment and study day (Figure 1). Body weight was not significantly ($P = 0.84$) associated with treatment group; however, body weight did increase over study days. Rectal temperature also varied by study day, but was not impacted by treatment group ($p = 0.17$). Model estimated least square mean heart rate was greater ($P < 0.01$) in both groups receiving clostridial vaccinations (C7, 114.5 ± 3.2 ; V7 107.8 ± 3.2) compared to the CN group (97.5 ± 3.7). Calculated average daily gain (ADG) was not significantly different between any of the treatment groups and calculated values were: 1.3 kg/head/day for V7, 1.5 kg/head/day for C7, and 1.2 kg/head/day for CN calves.

Lesion Size

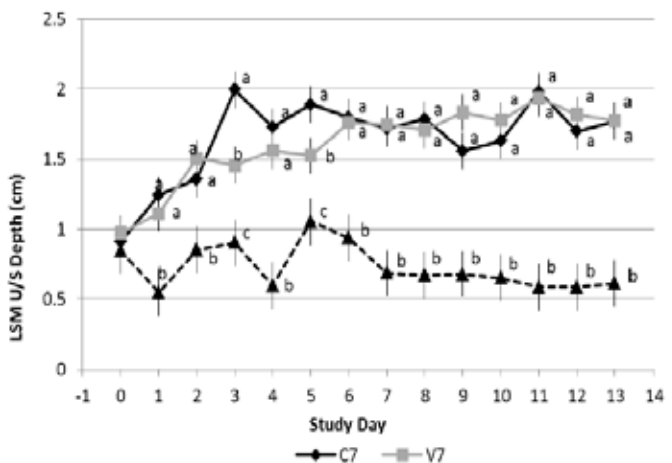
Evaluation of injection site lesion surface area and total ellipse volume revealed no significant interactions between treatment and study day; however, both main effects were significant. Model estimated lesion surface area ($\text{mm}^2 \pm \text{SE}$) was significantly greater for both group C7 (5464.9 ± 714.3) and V7 (4847 ± 714.3) calves compared with CN calves (CN calves received a 0 measurement at each reading), but C7 surface area did not differ from V7. The estimated total injection site volume (ellipse volume, $\text{mm}^2 \pm \text{SE}$) was significantly greater for both group C7 (37548.5 ± 5656.7) and V7 (32333.3 ± 5656.7) calves as compared with CN calves, yet no differences

were found between C7 and V7. A significant interaction was identified between treatment group and study day when ultrasonographic measurements of injection site depth were assessed (Figure 2). Injection site tissue depth was significantly less in CN calves compared to V7 and C7 groups at all time points except day 0. Injection site lesion depth did not differ between V7 and C7 calves except on days 3 and 5 when C7 had larger injection depth compared to V7.

Skin Surface Temperature

Summary of infrared thermography data (ratios between the lesion site and a control area) for maximum and average temperatures in each region were determined (Table 2). The maximum temperature ratio differed by study day, but was not significantly associated with treatment group. The ratio between average temperatures of injection site and control location was greater in the V7 group (1.015 ± 0.004) compared to calves in the C7 group (1.005 ± 0.004). Both treat-

Figure 2. Model adjusted least square mean injection site reaction depth (cm) by treatment group and study day. Treatment groups were administered either C7, (Caliber® 7, Boehringer Ingelheim Vetmedica, St. Joseph, MO, n=5), V7 (Vision® 7 with Spur, Merck Animal Health, n = 5), or CN (negative control, sterile saline, n=3). Study dates relative to time of clostridial (or saline for control group) administration on study day 0. Unique superscripts denote statistical ($P < 0.05$) differences between treatment groups within study day.



ment groups had significantly higher temperature ratios compared to the CN group (0.998 ± 0.004).

Injection Site Response to Pressure

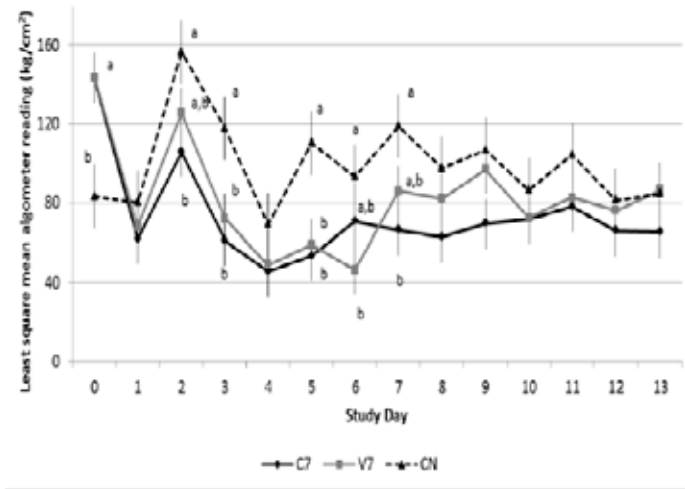
Adverse response to pressure applied to the injection site was assessed using algometry. A significant interaction was present in the amount of pressure required to stimulate an aversion reaction from the algometer (Figure 3); with CN calves requiring more pressure to stimulate a reaction in the early portion of the trial relative to the C7 and V7 calves.

Behavioral Monitoring

The mean number of steps taken per day, as measured by pedometers, was not significantly associated with treatment group, but did show an association with study day. Calves took significantly more steps prior to vaccination with the clostridial product or saline than at any point following day 0, but this effect did not vary by treatment group. Accelerometer data revealed a significant interaction of treatment group by study day with regard to time spent walking (Figure 4). The calves in the C7 group spent less time walking during the first 4 days of the trial compared to V7 or CN calves. However, after day 8, V7 calves spent more time walking than C7 or CN calves. The C7 calves spent more time standing ($49\% \pm 5$) and compared to CN ($25\% \pm 5$) calves, but V7 calves ($39\% \pm 5$) were not significantly different from either group.

Data acquired through location triangulation tags revealed that time spent at the hay was greater for calves in the C7 group ($16\% \pm 1.9$) and CN ($13\% \pm 2.0$) compared with that of V7 ($10\% \pm 1.9$) calves. Alternatively, time at the grain bunk was greater for

Figure 3. Model adjusted least square mean algometer pressure (kg/cm²) at reaction point by treatment group and study day. Treatment groups were administered either C7, (Caliber[®] 7, Boehringer Ingelheim Vetmedica, St. Joseph, MO, n=5), V7 (Vision[®] 7 with Spur, Merck Animal Health, n = 5), or CN (negative control, sterile saline, n=3). Study dates relative to time of clostridial (or saline for control group) administration on study day 0. Unique superscripts denote statistical ($P < 0.05$) differences between treatment groups within study day.



V7 ($2\% \pm 0.4$) and CN ($2\% \pm 0.4$) compared to the C7 group ($1\% \pm 0.4$). No significant associations with treatment group were found for the time calves spent at the water trough. Calves in the CN group spent more ($5\% \pm 1.8$) time in the loafing shed compared to calves in either the C7 or V7 groups ($3\% \pm 1.7$, $3\% \pm 1.7$, respectively).

Necropsy and Histopathology Assessment

The gross necropsy evaluation of all calves revealed no abnormalities in any organ system. Injection site lesions were characterized by multiple nodules surrounded by ecchymoses, edema, and scar tissue. Histopathology was performed on representative samples taken from the center of each lesion. All vaccine injection site lesions showed similar multifocal to coalescing pyogranulomatous cellulitis.

DISCUSSION

The results of this study demonstrate the utility of using multimodal tools to characterize biometric changes in calves follow-

ing an injection. In this case, clostridial vaccination was shown to induce changes not only at the injection site but also physiological variables and behavioral patterns of vaccinated calves compared to negative controls. The local inflammatory reaction in clostridial vaccinated calves was reflected by the larger surface area of lesions, greater injection site temperature ratios, and less pressure was required to stimulate an aversion reaction compared to negative controls. Changes in behavior patterns were manifested differently based on which clostridial vaccine they received. Cattle in the C7 group spent more time standing relative to CN calves, but V7 calves did not differ in standing frequency from the other treatment groups. Cattle behavior as monitored by the location monitoring system revealed differences among treatment groups relative to time spent at the hay and grain feeding areas. This research illustrates that the routine practice of injecting calves with a clostridial vaccine induces a local injection site reaction and modifies the activities and behaviors in the animals. The findings

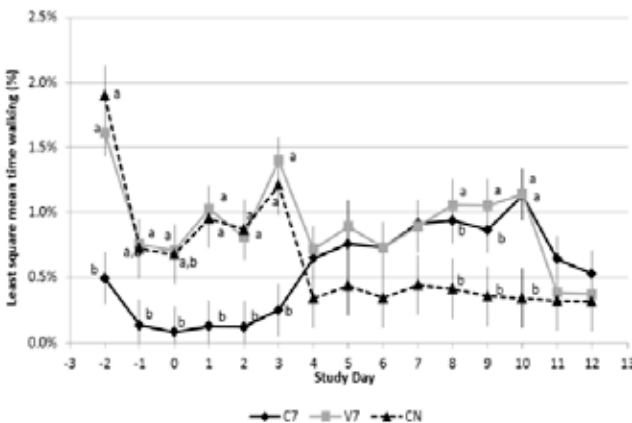
also display the ability of applying multiple Biometric measures to gauge the animal level reaction to the application of a Biological product. Understanding the animal response to common vaccination is critical when considering how to most effectively create and employ a preventative immunization program.

Cattle were apparently healthy throughout the entire trial period. However, injection site, physiologic, and behavioral changes occurred following injection. Similar to previous reported work,^{11,16} cattle vaccinated with clostridial vaccines developed local inflammatory reactions. Both vaccines were administered as labeled using a 2 ml subcutaneous dose, and the size of the reaction for both products was larger than the CN injection site. Injection site lesion depth has not previously been reported in cattle following clostridial vaccination as measured by ultrasound. Ultrasonography data revealed that injection site depth increased in the V7 and C7 group calves until day 3 to 4 of the study and then remained relatively constant throughout the remaining

study period (Figure 2). HD thermography was useful to determine the magnitude of inflammation as reflected by surface temperature. Both groups that were vaccinated with clostridial vaccines (V7 and C7) had greater maximum skin surface temperature ratios compared to CN calves, and the V7 calves also had a higher maximum skin surface temperature ratio compared to C7 calves. This increased ratio is likely in response to vasodilation, increased blood flow, and inflammation at the local injection site.

Algotmetry can be useful in serial assessment of pain or discomfort. This semi-quantitative test is affected

Figure 4. Model adjusted least square mean daily percent of time walking by treatment group and study day. Treatment groups were administered either C7, (Caliber® 7, Boehringer Ingelheim Vetmedica, St. Joseph, MO, n=5), V7 (Vision® 7 with Spur, Merck Animal Health, n = 5), or CN (negative control, sterile saline, n=3). Study dates relative to time of clostridial (or saline for control group) administration on study day 0. Unique superscripts denote statistical ($P < 0.05$) differences between treatment groups within study day.



by the individual calf's behavior. Therefore, a baseline must be determined for each calf. Previous research illustrates that algometers may be a sensitive instrument for measuring pain perception in a local area.^{4,5} Research in cattle has shown an increase in pressure required to stimulate a reaction when calves recently dehorned were treated with meloxicam compared to cattle not receiving an analgesic.⁴ Early in our study (2 and 3 days post-injection) the V7 and C7 calves required less pressure to stimulate a reaction relative to controls, but this difference in pain response appears to have abated toward the end of the trial period (Figure 3). The calves in this study may have become acclimated to the procedure and developed resilience to the test because of frequent manipulations. Pain associated with these local reactions may also have contributed to the increased heart rates observed in the clostridial vaccinated calves relative to CN calves.

Calves displayed different behavior patterns following the injections. The V7 and C7 calves spent more time walking compared to CN calves after study day 4. This coincides with the peak of local inflammation as judged by algometer and lesion depth measurements. The C7 calves spent more time standing after vaccination compared to CN calves and previous research has illustrated the relationship between standing behavior and painful procedures. In a project that evaluated short-term (24-hr) standing behavior following castration, calves displayed an increase in standing behavior after the painful procedure.¹⁵ However, in a longer period of time (3 d) following dehorning and castration, standing behavior was shown to decrease after the surgical procedures stimulating pain.⁶ Our findings did not display a treatment group by study day interaction and the overall effect indicates a difference from the control group which may still be related to the local inflammation associated with the injection site. Standing behavior may be representative of pain related to surgical procedures, but could be less associated with local inflammation due

to injection sites in the cervical region. The V7 calves did not differ in standing behavior from the other two groups, but this may be related to the relatively low sample size and level of variability in standing behavior among calves.

Time cattle spent in proximity to feeding locations within a housing area has been shown to be negatively associated with severity of lung consolidation in calves challenged with *Mycoplasma bovis*.¹⁴ Our results illustrated that the C7 group spent less time at the grain feeding area relative to the other two treatment groups and this may have been indicative of a reduction in appetite. This reduction of grain feeding may have been associated with increased inflammation in the calves as previous work has illustrated that calves receiving meloxicam following dehorning spent more time at the grain bunk in the immediate post-dehorning period relative to calves not receiving meloxicam.⁹ Both the C7 and CN calves spent more time at the hay feeding area compared to the V7 calves, and this behavior may also signal an interest in feeding.

Potential limitations of this work include the limited sample size for the clostridial vaccinates ($n=5$ per group) and the control calves ($n=3$). However, even with few animals per group, several differences were identified among the groups in the measured parameters. Continuous monitoring of calf activity (standing / lying) and pen behavioral patterns (percent of time spent at specific locations) illustrates the potential for multimodal assessment tools as a means of monitoring the health and well-being of cattle in addition to its sensitivity as a research tool for evaluation of responses to management changes. The importance of the magnitude of these differences is unknown, and further research should be performed to evaluate the production impact of these behavioral changes.

This research project utilized several technologies to allow objective characterization of calf injection site, physiological, and behavioral response to clostridial vaccina-

tion. Although sample size was relatively small, multiple parameters differed between the calves receiving a Clostridial vaccine and the control calves that received saline. The methods used to measure lesion size, surface temperature reaction, and behavioral changes show promise for future studies evaluating potentially subtle differences between reactions to biological products.

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