Successful treatment of suckling Red Angus calves for bovine respiratory disease is not associated with increased mean pulmonary arterial pressures at weaning

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ABSTRACT: The purposes of this study were to determine if the successful treatment of bovine respiratory disease (**BRD**) in suckling calves was associated with a long-term increase in mean pulmonary arterial pressure (**mPAP**) and, to screen for associations between blood leukogram variables and mPAP. A cohort of Red Angus calves (n = 74) were followed from birth to weaning at an altitude of 975 m. Calves were weaned at 172 ± 14 d when their mPAP was measured and whole blood collected. Thirty calves that had been treated for BRD (34 to 45 d prior) and 30 calves that had not required treatment for BRD were sampled. Treatment for BRD had no effect on mPAP (P = 0.37). Mean mPAP was 48 ± 8 mm Hg (± SD) with a minimum of 34 mm Hg and a maximum at 69 mm Hg. Weaning weight and sex tended to be associated with mPAP, but they explained just 5% of the variation in mPAP (P = 0.08; Adj. r^2 = 0.05). Fibrinogen (P = 0.008) and absolute lymphocyte count (P = 0.06) were negatively associated with mPAP, whereas absolute monocyte count was positively associated with mPAP (P = 0.01). The findings of this study suggest that pre-weaning treatment for BRD does not increase a calves' post-weaning risk of congestive right heart failure. Further, components of the immune and acute phase response system may play a role in the development and progression of pulmonary hypertension.

Key words: calf, heart failure, inflammation, leukocytes, pulmonary arterial pressure, respiratory disease

INTRODUCTION

Cattle are highly susceptible to congestive right heart failure secondary to pulmonary hypertension or cor pulmonale. Pulmonary hypertension is synonymous with an increase in mean pulmonary arterial pressure (**mPAP**), a product of steady-state vascular resistance. The most commonly recognized cause of pulmonary hypertension in cattle is hypoxia-induced pulmonary arterial vasoconstriction and remodeling associated with high-altitude exposure. Respiratory disease, however, may also lead to increased mPAP by predisposing affected individuals to alveolar hypoxia (Holt and Callan, 2007) and, in severe cases, reducing pulmonary vascular capacity through fibrosis and obliteration of pulmonary vessels. Feedlot cattle treated for bovine respiratory disease (**BRD**) were three times more likely to die of congestive right heart failure than cattle that did not require treatment (Neary et al., 2016a). It has also been determined that cattle with the greatest mPAP as suckling calves typically have the greatest mPAP through the confined feeding phase (Neary et al., 2015); consequently, interventions that reduce mPAP in the cow–calf phase may have beneficial carryover health effects as cattle enter the next phase of production.

The purposes of this study were to determine if healthy calves that were successfully treated for

BRD during the suckling phase had significantly greater mPAP than healthy herdmates that did not require treatment for BRD and, to screen for associations between the blood leukogram and mPAP. It was hypothesized that calves treated for BRD would have greater mPAP than calves that did not require treatment.

MATERIALS AND METHODS

Study Site and Husbandry

Seventy-four purebred Red Angus calves were born in a pasture setting from January 9, 2017 to February 22, 2017 at the Texas Tech Beef Center in New Deal, TX. The calves were raised, and the study conducted, at an altitude of 975 m. These calves were a result of a fresh in vitro and conventional embryo transfer program that was transferred into commercial black-hided dams. All recipients were second-calf heifers. Calving took place on a dormant pasture containing WW-B. Dahl Old World Bluestem (*Bothriochloa bladhii*) and Bermuda (*Cynodon dactylon*) grasses. Cows were monitored every 3 h for signs of calving difficulty and assistance provided as necessary. Once all cows had calved, the cow–calf pairs were moved to corn stalks until March 30, 2017. Cows were transported to a dry-lot setting for 7 d, so calves could be processed and resorted prior to being placed on nonirrigated wheat pasture. Cows were rotated to multiple wheat pastures as forage availability dictated. In general, conditions were fairly dry during the pre-weaning, foraging phase.

On June 5, 2017, when the calves were 127 ± 14 (± SD) d of age, the cow–calf pairs were moved permanently into a dry-lot setting (27.4 m x 36.5 m with a 3.6 m concrete apron adjacent to feed bunks). In the dry-lot, cows were fed a total mixed ration consisting of corn gluten feed, cotton burrs, cracked corn, and a custom mineral supplement that met their lactating nutritional requirements (NRC, 2016). Each pen had shade and a creep area where calves could have access to free choice Bermuda grass hay along with a molasses-based lick tub at 12% crude protein (Purina Stress Tub; Gray Summit, MO). The cattle remained in the dry-lot setting until July 24, 2017, when PAP was measured immediately prior to weaning. All procedures were approved by the Texas Tech University Animal Care and Use Committee (Protocol 17016-01) prior to commencing the study.

Health Management

Prior to calving, cows were vaccinated for the following pathogens: infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD types 1 and 2), parainfluenza-3 virus (PI3), bovine respiratory syncytial (BRSV), and *Leptospira canicola* bacteria (Bovi-Shield Gold FP5 VL5 HB; Zoetis, Florham Park, NJ), rotavirus, coronavirus, *Clostridium* type C, *Escherichia coli* Bacterin (Scour Guard 4KC; Zoetis, Florham Park, NJ), and *Clostridium chauvoei-septicum-novyi-sordellii-perfringens* types C and D bacterin-toxoid (Ultrabac 7; Zoetis, Florham Park, NJ). The cows also received doramectin (Dectomax Injectable; Zoetis, Florham Park, NJ) for internal parasites and albendazole (Valbazen Oral Drench; Zoetis, Florham Park, NJ) for external parasites.

Within 24 h of birth, calves received a unique numbered ear tag, were weighed using hand-held digital scale (Cabela's 330-lb. Digital Scale, Cabela's, Sidney, NE), vaccinated against BRSV, IBR and PI3 (Inforce 3; Zoetis, Florham Park, NJ), and rotavirus and coronavirus (Calf-Guard; Zoetis, Florham Park, NJ). All calves were observed nursing within 24 h of birth.

All calves received a second series of vaccinations on March 30, 2017, when the cow–calf pairs were brought into a dry-lot temporarily before moving to wheat pasture. Calves were revaccinated against BRSV, IBR, and PI3 (Inforce 3; Zoetis, Florham Park, NJ), and given new vaccinations that included *Mannheimia haemolytica*, BVD types 1 and 2 (One Shot BVD; Zoetis, Florham Park, NJ), *Clostridium chauvoei-septicum-novyi-sordellii-perfringens* types c and d bacterin-toxoid (Ultrabac 7; Zoetis, Florham Park, NJ), D-alpha-tocopherol with vitamins A and D (Vitamin E-AD-300; VetOne, Boise, ID), zinc, manganese, selenium, and copper (Multimin 90; Multimin USA, Fort Collins, CO). Calves were also injected with the anthelmintic doramectin (Dectomax; Zoetis, Florham Park, NJ).

Calves were checked twice daily (morning and evening) for clinical signs of illness. Calves showing signs of illness were caught and treated, as necessary, by the manager of the Texas Tech University Beef Center. The manager had extensive experience and training in identifying ill cattle within a feedlot setting. Visual signs of respiratory disease included lethargic movement, drooping ears, cough, labored breathing, nasal discharge, and diminished appetite. If the calf showed signs consistent with respiratory disease and had a rectal temperature

greater than 39.4 °C (Handheld Thermistor Rectal Thermometer, Cooper-Atkins, Middlefield, CT), the handler treated the calf with tulathromycin (2.5 mg/kg; Draxxin; Zoetis, Florham Park, NJ). If no improvement in clinical signs occurred within 7 d, calves were retreated for respiratory disease with ceftiofur crystalline free acid (6.6 mg ceftiofur equivalents/kg; Excede; Zoetis, Florham Park, NJ). No calves were treated more than twice.

Pulmonary Arterial and Right Atrial Pressure Measurement

On July 24, 2017, the day of weaning, pulmonary arterial pressures (PAP) and right atrial pressures (RAP) were obtained from 60 calves: 30 that had been treated for respiratory disease and 30 calves that had not required any treatment for respiratory disease. Blood samples were collected during the procedure. The first treated and untreated calves to be processed in the chute were sampled. All calves, including those not sampled, were clinically healthy. Calves were restrained in a hydraulic chute and individually weighed on an electronic scale certified by the Texas Department of Agriculture (accuracy ± 0.454 kg). Calves were housed in a pen without food or water for 3 h to get an accurate 4% shrink in gross weight.

Once adequately restrained within the chute, a hydraulic neck bar was used to expose the right side of the calf's neck. The neck was then liberally sprayed with chlorhexidine solution before a 12-G, 8.9-cm hypodermic needle was inserted into the jugular vein. Blood was collected in 10 mL glass tubes containing the anticoagulant EDTA. The tubes were inverted five times before storage in an insulated container cooled with ice packs. Flexible, saline-filled polyethylene catheter tubing (1.3 m length and external and internal diameters of 17 and 12 mm, respectively) was then fed through the needle and into the jugular vein. A pressure transducer (TranStar DPT, Smiths Medical ASD, Inc., Dublin, OH) connected the catheter and oscilloscope (BM5Vet, Bionet America, Inc. Tustin, CA). The change in the pressure waveform that occurred as the catheter tip was advanced through the right atrium, right ventricle, and finally into the pulmonary artery was monitored on the oscilloscope. The jugular vein, right atrium, right ventricle, and pulmonary artery have distinct pressure waveforms. Blood samples were shipped overnight in an insulated container lined with icepacks and a complete blood count performed the next day (West Texas A & M Diagnostic Laboratory, Amarillo,

TX). Plasma protein was assessed by refractometry. Plasma fibrinogen was estimated by using an established rapid heat precipitation micromethod (Millar et al., 1971). The calves remained in dry-lot setting for 1-mo post-procedure.

Statistical Analyses

Statistical analyses were performed using a commercially available software (Stata version 12.1, College Station, TX). Summary statistics are presented as mean ± SE unless otherwise specified. Between group differences were evaluated using Student's t-test with equal variances or Kruskal–Wallis equality of populations rank test, depending on the distribution of the data assessed graphically. Two backwards step-wise linear regression models that screened phenotypic and blood leukogram variables for association with the outcome variable mPAP were performed. Variables were screened for a univariable association with mPAP. Those that had a probability of a type 1 error of 0.20 or less were included in the full regression model. The phenotypic variables included treatment for respiratory disease, weaning weight (calculated with a 4% shrink), sex (bull or heifer), and age. Multiple BRD treatments were not accounted for in the analyses due to the small number of calves that this involved. The blood leukogram variables included concentrations of leukocytes (neutrophils, lymphocytes, monocytes), erythrocytes, hemoglobin, platelets, plasma protein, and fibrinogen, and the ratio of neutrophils-to-lymphocytes. The marginal means were calculated and plotted with the observed values against mPAP. Evaluations of model fit included graphical and statistical assessments of residual error normality (Shapiro–Wilk test), heteroskedasticity (Breusch–Pagan/Cook–Weisberg test), and linearity.

RESULTS

Descriptive

Calves weighed 33.3 ± 4.5 kg (± SD) at birth. Birth weight was not associated with future BRD treatment status ($P = 0.27$). Three cows required calving assistance; the calves were pulled without mechanical assistance. The mean age of the calves tested was 172 ± 14 d (± SD). The mean body mass of calves treated for BRD (177 ± 4.5 kg) tended to be less than calves that had not been treated (189.3 ± 4.9 kg, $P = 0.09$). Average daily

gain from birth to weaning was 0.88 ± 0.13 kg (± SD) and was not affected by BRD treatment status ($P = 0.26$). Calves were treated for BRD from 45 to 34 d prior to weaning. The majority (75%) of calves were treated between 40 and 45 d prior to weaning. Three of the calves sampled were treated twice for BRD with a 1-wk interval between treatments. Mean mPAP was 48 ± 8 mm Hg (± SD) with a minimum of 34 mm Hg and a maximum at 69 mm Hg. Mean mRAP was 28 ± 6 mm Hg (± SD) with a minimum of 13 mm Hg and a maximum at 39 mm Hg. An increase in mPAP by 1 mm Hg was associated with a 0.5 ± 0.1 mm Hg increase in mean right atrial pressure ($P < 0.001$; Adj. $r^2 = 0.40$).

Effect of BRD on mPAP

Treatment for BRD had no effect on mPAP ($P = 0.37$). Only weaning weight and sex tended to be associated with mPAP, but they explained just 5% of the variation in mPAP ($P = 0.08$; Adj. $r^2 = 0.05$). For every 100 kg increase of weaning weight, mPAP increased by 6 ± 4 mm Hg ($P = 0.08$) when controlling for sex. Males tended to have a mPAP 3 ± 2 mm Hg less than females ($P = 0.13$) when controlling for weaning weight.

Effect of BRD on Blood Leukogram

Only absolute neutrophil count tended to differ according to whether calves had been treated for BRD ($P = 0.09$). Untreated calves had a median of 3.4×10^3 neutrophils/µL of blood and treated calves had a median of 4.2×10^3 neutrophils/µL of blood. A descriptive leukogram summary is provided in Table 1.

Association Between Leukogram and mPAP

Fibrinogen, absolute monocyte count, and absolute lymphocyte count were associated with mPAP ($P < 0.001$; Adj. $r^2 = 0.30$). Fibrinogen ($P = 0.008$) and absolute lymphocyte count ($P = 0.06$) were negatively associated with mPAP, whereas absolute monocyte count was positively associated with mPAP ($P = 0.01$; Figure 1).

DISCUSSION

The findings of this study indicate that the successful treatment of BRD in suckling Red Angus calves does not lead to an increase in mPAP when measured at weaning. Importantly, this suggests that pre-weaning treatment for BRD does not increase a calves' post-weaning risk of congestive right heart failure. Our study did indicate, however,
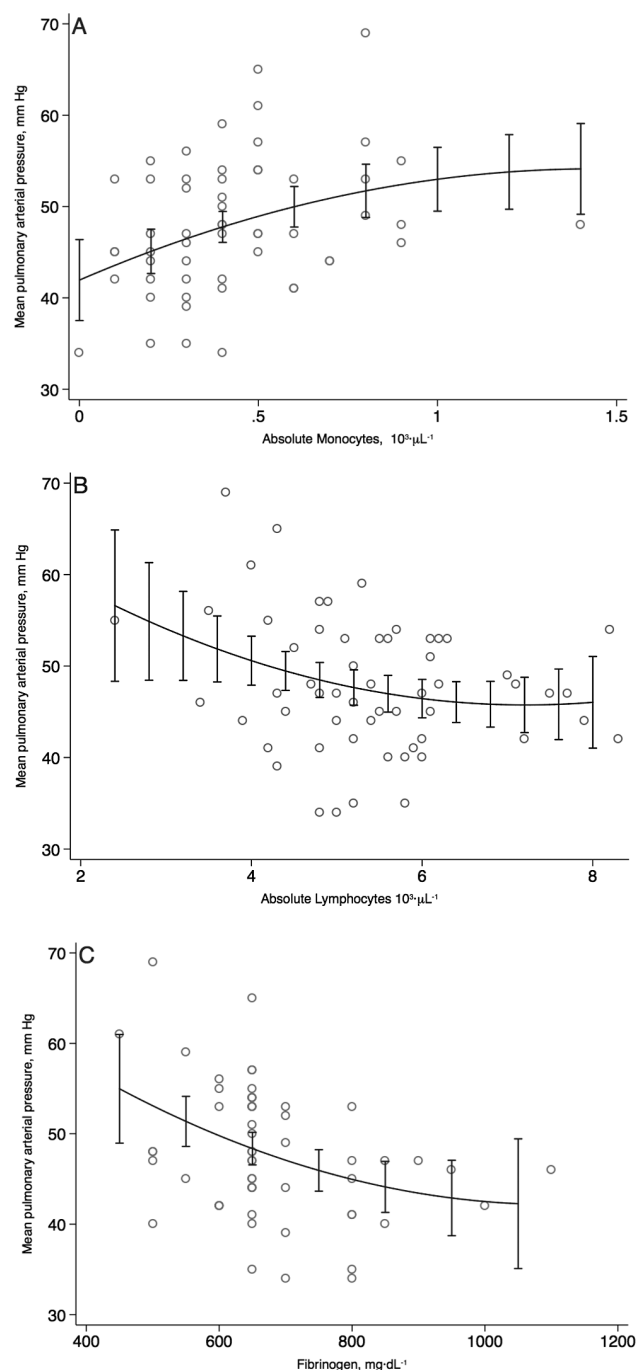

Figure 1. Plot of mPAP against (A) monocyte, (B) lymphocyte, and (C) fibrinogen concentrations showing the observed values and a line of best fit with 95% confidence intervals. Fibrinogen ($P = 0.008$) and absolute lymphocyte count ($P = 0.06$) were negatively associated with mPAP. Absolute monocyte count was positively associated with mPAP ($P = 0.01$).

that components of the immune and acute phase response system may play a role in the development of pulmonary hypertension. Furthermore, the mPAP levels reported in this study were considerably greater than mPAP reported in other mammalian species and even similarly aged calves located at a higher altitude. These findings are much more than a physiological curiosity; pulmonary hypertension is deleterious to the health and survival of cattle (Neary et al., 2016b).

Table 1. Blood leukogram of Red Angus calves at the time of weaning ($n = 60$)

Variable	Mean ± SD or (Min, Q_{25}, Median, Q_{75}, Max)[1]
Neutrophils, $10^3 \cdot \mu L^{-1}$	(1.7, 3, 4, 5.1, 10)
Lymphocytes, $10^3 \cdot \mu L^{-1}$	5.4 ± 1.3
Monocytes, $10^3 \cdot \mu L^{-1}$	(0, 0.3, 0.4, 0.7, 2)
Erythrocytes, $10^6 \cdot \mu L^{-1}$	9.8 ± 1.1
Hemoglobin, $g \cdot dL^{-1}$	12.1 ± 1.0
Hematocrit, %	23 ± 13
Platelets, $10^2 \cdot \mu L^{-1}$	647 ± 167
Plasma protein, $g \cdot dL^{-1}$	7.4 ± 0.3
Fibrinogen, $mg \cdot dL^{-1}$	687 ± 137

[1]Minimum (Min), 25th percentile (Q_{25}), 75th percentile (Q_{75}), Maximum (Max).

Cattle have a pronounced hypoxia-induced pulmonary pressor response (Tucker et al., 1975), which means that an animal's mPAP increases in association with altitude. We would, therefore, anticipate that the mPAP of calves at the moderate altitude of 975 m to be lower than the mPAP of similarly aged calves located at a higher altitude. The values recorded in our study, however, are greater than those observed in 6-mo-old male Black Angus calves at an altitude of 2,170 m (42 mm Hg; Neary et al., 2015) and only slightly less than 6-mo-old crossbred Angus heifers (51 mm Hg) and steers (54 mm Hg) at an altitude of 2,730 m (Neary et al., 2013).

Both environmental and genetic factors are likely etiological factors for the high mPAP observed in our study. Perhaps, the greatest environmental difference is that the calves in our study were managed in a confinement or dry-lot system rather than a traditional pasture-based system. Research conducted at the same facility on the previous year's calf crop reported that confinement was deleterious to calf health (Burson, 2017). Although the calves in our study appeared healthy when mPAP was measured, the possibility that subclinical disease or long-term sequela of a prior infection contributed to an increase in mPAP cannot be ruled out.

Genetics is also a key determinant of mPAP. Studies of Angus cattle suggest that mPAP is moderately heritable (Shirley et al., 2008; Crawford et al., 2016). A recent evaluation of mPAP obtained from over 2,400 bulls at an altitude of 2,255 m reported that Red Angus bulls had the second greatest mPAP among the 10 different beef breeds and breed-crosses studied (Crawford et al., 2017). This may be partly attributable to the relatively greater frequency of the hypoxia-inducible transcription factor 2A (HIF2A) isoform in the Red Angus breed (Heaton et al., 2016), which has been associated with increased risk for pulmonary hypertension in Angus cattle (Newman et al., 2015). Red Angus and Angus cattle had the second and fifth greatest frequencies of the HIF2A isoform associated with pulmonary hypertension among the 46 breeds evaluated (Heaton et al., 2016).

The HIF-alpha proteins, encoded by the endothelial Per-ARNT-Sim (PAS) domain-containing protein 1 gene (*EPAS1*), are highly conserved regulators of the mammalian hypoxic response. Under normoxic conditions, HIF proteins are degraded, but under hypoxic conditions HIF proteins are protected from degradation and have numerous downstream effects. Missense mutations of EPAS1 affecting the oxygen-dependent degradation domain cause a gain-of-function. There has been speculation that HIF proteins may be responsible for hematopoietic stem cell activation, from which monocytes are derived (Florentin and Dutta, 2017). If so, the positive association between blood monocyte concentration and mPAP may be attributable to *EPAS1* variants within the calves tested.

Monocytes and macrophages are the main effector cells of lung inflammation, a hallmark of pulmonary hypertension (Frid et al., 2006). In a mouse model, chronic hypoxia led to monocytosis followed by the recruitment of monocytes into the lungs (Amsellem et al., 2017). Here, the macrophages predominantly acquired the M2 phenotype, which is conducive to the growth of pulmonary arterial smooth muscle cells (Amsellem et al., 2017). Fibrocytes – mononuclear cells of a monocyte/macrophage lineage – were reported to accumulate in the pulmonary adventitia of neonatal Holstein calves following 2 wk of hypoxia that led to the development of pulmonary hypertension (Frid et al., 2006). Whether the monocyte/macrophage infiltration and subsequent inflammation of the lung predispose cattle to other pulmonary diseases remains to be determined.

In agreement with our findings, a decline in blood lymphocyte concentration but stable neutrophil levels has been reported in human patients with pulmonary hypertension (Yıldız et al., 2013). In contrast to our study, however, most studies of human pulmonary arterial hypertension indicate that the neutrophil-to-lymphocyte ratio is a better predictor of disease severity than measurements of absolute neutrophils or lymphocytes alone (Özpelit et al., 2015; Harbaum et al., 2017). The reason for the negative association between lymphocyte concentration and mPAP is unclear, but it may be attributable to a dampening effect of

lymphocytes subtypes on pulmonary inflammation and remodeling (Taraseviciene-Stewart et al., 2007; Tamosiuniene et al., 2011).

The negative association between fibrinogen and mPAP found in our study suggests that abnormalities of coagulation may play a role in the development or progression of pulmonary hypertension. In one study, patients with primary pulmonary hypertension had impaired fibrinolytic activity (Welsh et al., 1996). In another study of human subjects exposed to high altitude, plasma fibrinogen and fibrinolytic activity increased regardless of whether or not an individual developed pulmonary hypertension; however, individuals with pulmonary hypertension had a significantly greater concentration of other pro-coagulant variables such as platelet adhesiveness, and clotting factors V and VIII (Singh and Chohan, 1972). This suggests that an increase in fibrinogen per se does not predispose to pulmonary thrombi but is facilitated by the presence of other pro-coagulant factors that were not measured in our study. The lower fibrinogen in calves with the greatest mPAP may be attributable to activated coagulation pathways within the pulmonary vasculature and, consequently, the consumption of fibrinogen.

Finally, and in agreement with prior observations, mPAP was found to be positively associated with weaning weight (Shirley et al., 2008). The strength of association, however, was small and, consequently, of little relevance within the cohort studied.

In conclusion, the successful treatment of BRD in suckling Red Angus calves did not lead to a long-term increase in mPAP. Irrespective of BRD treatment history, however, mPAP were high. The potential effects of pulmonary hypertension, and associated pulmonary inflammation, on bovine lung health and disease susceptibility need to be more extensively evaluated.

Conflict of Interest *The content is solely the responsibility of the authors. The authors have no conflicts of interest to declare.*

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