Weaning management of newly received beef calves with or without continuous exposure to a persistently infected bovine viral diarrhea virus pen mate: Effects on rectal temperature and serum proinflammatory cytokine and haptoglobin concentrations

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ABSTRACT: Exposure to animals persistently infected (PI) with bovine viral diarrhea virus (BVDV) results in immunomodulation in cohorts. It is hypothesized that the extent of modulation differs for low-risk, preconditioned (PC) vs. high-risk, auction market (AM) beef cattle. Our objective was to compare immune responses of PC or AM calves in the presence (PI) or absence (CON) of a PI-BVDV pen mate. Crossbred PC steers (n = 27) from a single ranch origin were weaned, dewormed, vaccinated against respiratory and clostridial pathogens, tested for PI-BVDV, and kept on the ranch for 61 d. Subsequently, PC steers were transported to a receiving unit (RU), weighed, stratified by d −1 BW, and assigned randomly to treatment (PCPI or PCCON) with no additional processing. Simultaneously, crossbred AM calves (n = 27) were assembled from regional auction markets and transported to the RU. The AM calves were weighed, stratified by gender and d −1 BW, processed under the same regimen used for PC steers at their origin ranch, except bull calves were castrated, then assigned randomly to treatment (AMPI or AMCON). Treatment pens were arranged spatially so that PI did not have fence line contact with CON. Blood samples were collected on d 0, 1, 3, 7, and 14 to determine serum concentrations of haptoglobin (Hp), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), IL-4, and IL-6. Rectal temperature (RT) was recorded concurrent with blood sampling. In AM calves, RT and Hp increased (management effect; P < 0.001) sharply on d 1; however, exposure to a PI-BVDV pen mate did not affect either variable (P ≥ 0.79) during the 14-d evaluation period. Serum concentrations of TNF-α tended to increase (P = 0.09) for the PI cohort. A treatment × day interaction (P ≤ 0.05) was observed for IFN-γ on d 7 and 14 and IL-6 on d 14; these indices were greatest for AMPI. Results indicate weaning management and PI-BVDV exposure alter the immune status of newly received beef cattle. These main effects may be additive because proinflammatory cytokine concentrations were greatest for AMPI. Therefore, results further indicate that potential health or growth consequences in cohorts exposed to a PI-BVDV pen mate are impacted by previous management and health history.

Key words: bovine viral diarrhea virus, cytokine, haptoglobin, immunomodulation


INTRODUCTION

Physiological alteration in response to specific stress events, such as weaning (Hickey et al., 2003; Carroll et al., 2009), transportation (Blecha et al., 1984; Stanger et al., 2005; Buckham Sporer et al., 2008), castration (Chase et al., 1995; Fisher et al., 1997), and commingling (Gupta et al., 2005; Step et al., 2008), have been observed in beef cattle. Typically, high-risk, newly received beef cattle acquired from the auction
Table 1. Timeline of management and processing events for different treatment groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Weaned</th>
<th>Initial respiratory vaccination</th>
<th>Second respiratory vaccination</th>
<th>Arrival to RU</th>
<th>Treatment group assignment</th>
<th>Castrated</th>
<th>Blood samples collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>Unknown</td>
<td>d −62</td>
<td>d 14</td>
<td>d −2</td>
<td>d 0</td>
<td>Unknown or d 0</td>
<td>d 0, 1, 3, 7, 14</td>
</tr>
<tr>
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<td>d −62</td>
<td>d −62</td>
<td>d −48</td>
<td>d −1</td>
<td>d 0</td>
<td>Neonatal</td>
<td>d 0, 1, 3, 7, 14</td>
</tr>
</tbody>
</table>

1 AM = auction market; PC = preconditioned.
2 RU = receiving unit, University of Arkansas Agricultural Experiment Station located near Savoy.

market system encounter multiple stressors simultaneously. The magnitude of stress and morbidity may be mitigated for cattle preconditioned on their origin ranch before marketing occurs (Duff and Galyean, 2007; Taylor et al., 2010). Loerch and Fluharty (1999) suggested that the most significant impact of physiological stress is the negative effect it has on feed intake and immunocompetency, and these predisposing factors may ultimately contribute to bovine respiratory disease (BRD). Stress-induced immune dysfunction allows viruses to evade host immune defense mechanisms (Sheridan et al., 1994); thus, viral pathogenesis is likely enhanced for animals also experiencing physiological stress.

Bovine viral diarrhea virus (BVDV) may be an important respiratory pathogen involved in the pathogenesis of BRD either directly via acute clinical disease or through indirect effects of immunosuppression (Welsh et al., 1995). Calves born persistently infected (PI) with BVDV are a key source of BVDV transmission (Fulton et al., 2005), and exposure to PI-BVDV animals results in immunomodulation of cohorts (Buriaca-Robles et al., 2010). It is hypothesized that the immune response is altered by PI-BVDV exposure; however, the extent of alteration may be impacted by previous management and health history of cohorts. Our objective was to determine rectal temperature and serum proinflammatory cytokine and haptoglobin concentrations in high-risk, commingled, auction market vs. low-risk, single-source, preconditioned calves with or without 14-d continuous exposure to a PI-BVDV pen mate.

**MATERIALS AND METHODS**

Animal methods and experimental procedures were approved by the University of Arkansas Animal Care and Use Committee.

**Cattle**

*Experimental Cattle.* Two different cattle management systems were used for the 14-d evaluation period: 1) a low-risk, single-source, preconditioned (PC) group of British × Continental crossbred steer calves (n = 27; initial BW = 282 ± 1.6 kg) from a single ranch located in Izard County, AR, and 2) a high-risk, commingled, auction market (AM) group of crossbred bull (n = 15) and steer (n = 12) calves (initial BW = 268 ± 2.3 kg) acquired from an Arkansas auction market (Table 1). The PC steers arrived at the University of Arkansas Agricultural Experiment Station located near Savoy (receiving unit; RU) on December 6, 2009, and were considered to be low-risk for developing BRD because they had been previously vaccinated against BRD pathogens, were weaned on their origin ranch for 61 d, and were maintained as a single source without commingling. The AM calves were assembled by an order buyer from a public auction market located in north central AR and arrived at the RU on December 5, 2009. The order buyer was instructed to purchase AM cattle of similar BW and phenotype as the PC calves. The AM cattle were considered to be high risk for developing BRD because they did not have known health or vaccination history and were commingled extensively, resulting in greater probability of increased stress and exposure to BRD pathogens.

The main effects of management (AM or PC) and exposure (not exposed to a PI-BVDV pen mate = CON or exposed = PI) were tested in a 2 × 2 factorial arrangement resulting in 4 treatments (AMCON, AMPI, PCCON, and PCPI). For PC treatments, 62 d before trial initiation, steers (castration occurred at birth) were weaned on their origin ranch and confirmed negative for PI-BVDV via ear-notch skin samples tested for the presence of BVDV using the antigen-capture ELISA (ACE) method (Idexx Laboratories Inc., Westbrook, ME) at a commercial laboratory (Cattle Stats, LLC, Oklahoma City, OK). Also on the day of weaning, PC calves were administered 1) a pentavalent modified-live virus (MLV) respiratory vaccine containing infectious bovine rhinotracheitis virus, BVDV types 1a and 2a, parainfluenza-3 virus, and bovine respiratory syncytial virus isolates [Express 5, Boehringer Ingelheim Vetmedica Inc. (BIVI), St. Joseph, MO], 2) *Manheimia haemolytica-Pasteurella multocida* bacterin-toxoid (Pulmo-guard PHM-1, BIVI), and 3) pour-on anthelmintic (Cydectin, BIVI). Fourteen days later (d −48), PC calves were administered a clostridial bacterin-toxoid (Alpha 7, BIVI) and revaccinated with pentavalent MLV respiratory vaccine. Preconditioned calves were housed in an isolated drylot pen, were fed hay along with a supplement, and remained on their origin ranch during the preconditioning phase until d −1,
when they were transported 322 km (approximately 4 h) to the RU. Upon arrival at the RU (d −1), PC calves were held in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d −1, PC calves were weighed and returned to their isolated holding pen. The next day (d 0), PC calves were bled, stratified by d −1 BW, then assigned randomly to treatment (PC-CON or PC-PI).

To coincide with PC calves, AM calves were assembled from an auction market located in north central AR and transported 311 km (approximately 4 h) to the RU. Upon arrival at the RU (d −2), AM calves were maintained in an isolated holding pen with ad libitum access to hay and water until treatment allocation on d 0. On d −1, AM calves were weighed, identified with a unique ear identification tag, ear notched to test for PI-BVDV status (all experimental calves tested negative) at a commercial laboratory as previously described, and returned to their isolated holding pen. On d 0, AM cattle received a pentavalent MLV respiratory vaccine containing infectious bovine rhinotracheitis virus, BVDV types 1a and 2a, parainfluenza-3 virus, and bovine respiratory syncytial virus isolates (Express 5, BIVI), Mannheimia haemolytica-Pastuerella multocida bacterin-toxoid (Pulmo-guard PHM-1, BIVI), and pour-on anthelmintic (Cydectin, BIVI); therefore, the first known processing for AM occurred on d 0 rather than 62 d previously for PC. Additionally, for AM on d 0, blood samples were collected from calves, bulls were castrated surgically, stratified by gender and d −1 BW, then assigned randomly to treatment (AM-CON or AM-PI). Cattle were then moved to their designated pens and provided 0.91 kg/d (as-fed basis) of a receiving supplement (15.1% CP, DM basis) and ad libitum access to bermudagrass hay (13.1% CP, 52.3% NDF, 40.6% ADF, DM basis) and water. The supplement offered was stepwise increased to a maximum of 2.73 kg/d as each pen completely consumed the supplement offered for 2 consecutive d.

Persistently Infected Cattle. Calves that had been previously ear notched, tested at a commercial laboratory (Cattle Stats), and identified as PI-BVDV according to ACE were acquired from a stocker cattle operation in Washington County, OK, to be used as a PI-BVDV exposure source. Upon arrival to the RU, each PI-BVDV calf was ear notched a second time, and samples were shipped via overnight parcel service to the ARS-USDA National Animal Disease Center (NADC) located in Ames, IA, for rapid affirmation of positive PI-BVDV status using the same ACE procedure. Six PI-BVDV calves were each assigned randomly to the 6 PI-designated treatment pens by drawing pen assignment number. Additionally, 6 mL of anticoagulated blood was collected from the right jugular vein into evacuated tubes (Vacutainer, 6 mL tube containing 10.8 mg K2 EDTA, Ref 367863) from each PI-BVDV animal and shipped on ice via overnight parcel service to NADC for subsequent differentiation of BVDV subgenotype strain using virus isolation of buffy coat cells and phylogenetic analysis previously described by Ridpath et al. (2011). One PI-BVDV animal was identified as subgenotype 1a, and the 5 remaining were subgenotype 1b. It is important to note that BVDV 1b, the subgenotype strain in which 5 of 6 PI-BVDV calves used in this experiment were identified, is the predominant BVDV subgenotype strain isolated from cattle in the United States (Fulton et al., 2002; Ridpath et al., 2010).

Pen Assignment and Arrangement

To avoid unwanted PI-BVDV fence line or water source contact with CON, drylot receiving pens were arranged spatially before treatment allocation. The spatial pen arrangement used in the current study may have contributed to confounding effects of location within the RU facility; however, we determined the potential confounding effects of fence line and water source contact between PI and CON to be more significant. Pens measured 3.7 × 29 m and contained 3 m of feed bunk and a fence line water source shared with an adjacent pen of the same treatment. Furthermore, the treatment pens were configured so that unlike treatment pens were separated by 1 or 2 unoccupied pens and did not share the same water source. There were 3 pens/treatment. Within management group (AM or PC), calves were stratified by gender (AM only) and d −1 BW, then assigned randomly to 1 of 6 pens. One PI-BVDV calf was assigned to each of the PI-designated treatment pens (3 pens within each management group). Each pen contained 5 animals, resulting in 4 and 5 experimental animals/pen for PI and CON treatment pens, respectively. During all blood sampling procedures, CON treatments were evaluated first, followed by PI treatments, to avoid unwanted CON contact with PI-BVDV animals or experimental cattle in the PI treatments and to reduce potential exposure to fomites contaminated with body fluids or fecal material containing BVDV (Stevens et al., 2011). Because of spatial treatment arrangement and the necessity to evaluate CON followed by PI, morbidity investigators were not blinded to experimental treatment.

Proinflammatory Cytokine and Haptoglobin Analyses

Blood samples were collected from experimental animals at approximately 0800 h on d 0, 1, 3, 5 (d 5 samples were excluded from analysis), 7, and 14 to determine serum concentrations of IL-4, IL-6, interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and haptoglobin (Hp). Blood was collected via jugular venipunc-
tured from the right vein of each animal into plain evacuated tubes (Vacutainer SST, 10-mL tube, Ref 367985; BD Inc., Franklin Lakes, NJ). Within 4 h of collection, blood was centrifuged at 2100 × g for 20 min at 20°C, and serum was decanted into duplicate aliquots and stored frozen at −20°C until subsequent analysis. Subsequently, 4 (PI) or 5 (CON) serum sample aliquots from each treatment pen from d 0, 1, 3, 7, and 14 were transported on ice to NADC for determination of serum concentrations of IL-4, IL-6, IFN-γ, and TNF-α using a custom-developed, bovine-specific multiplex ELISA assay (SearchLight, Aushon Biosystems Inc., Billerica, MA) with intra- and interassay CV of 10% and 15%, respectively. A second aliquot from the same animals and sampling times for the proinflammatory cytokine analysis were used to determine serum Hp concentrations using a commercial, bovine-specific Hp ELISA kit (Immunology Consultants Laboratory Inc., Newberg, OR). For both Hp and cytokine analyses, serum samples were diluted within appropriate range of the known standards provided in each assay kit.

Rectal Temperature Evaluation and Treatment of BRD

Rectal temperature (RT) was recorded concurrent with blood sampling time points via a digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA; readability = ±0.1°C). During the first week, for initial diagnosis of BRD, calves were considered morbid and treated with an antibiotic based solely on RT observed on predetermined sampling days. If RT was ≥40°C, calves were administered antibiotic therapy subcutaneously (sc) with enrofloxacin (Baytril, Bayer Animal Health, Shawnee Mission, KS) at a dosage rate of 10 mg/kg of BW and were immediately returned to their home pen. A 48-h posttreatment interval (PTI) was implemented after administration of enrofloxacin, and RT was evaluated on expiration of the initial antibiotic PTI. Therefore, visual signs of BRD were not used to determine morbidity for the current study during the first week, and calves were only removed from their home pen to evaluate RT on predetermined sampling days or if the antibiotic PTI expired on a day when sampling was not scheduled. If the second RT was ≥40°C, a second antibiotic treatment with florfenicol (Nuflox, Schering-Plough Animal Health, Summit, NJ) was administered sc at a dosage rate of 40 mg/kg of BW. A 48-h PTI was also implemented for cattle administered florfenicol, and RT was evaluated on expiration of the second antibiotic PTI. If the temperature was ≥40°C, a third and final antibiotic treatment with ceftiofur HCl (Excenel RTU, Pfizer Animal Health, New York, NY) was administered sc at a dosage rate of 2.2 mg/kg of BW and repeated for 2 consecutive d after the initial injection of ceftiofur HCl. Treatment data were recorded for individual animal including treatment date, RT, and the amount (mL) of each antibiotic administered. From d 8 to 14, cattle were observed daily for clinical signs of BRD (depression, nasal discharge, ocular discharge, cough, gaunt appearance, inappetence) by 2 experiment station personnel with a combined 35 yr of experience evaluating cattle with BRD. If ≥2 visual signs existed, calves were brought to the restraining chute and weighed, and RT was recorded via a digital thermometer.

Statistical Analyses

Data were analyzed as a completely randomized design using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model included treatment, day, and treatment × day. If a treatment × day interaction was evident (P ≤ 0.10), differences of least squares means within a day were reported if the resulting t test was statistically significant (P ≤ 0.05). Kenward-Roger was specified as the degrees of freedom selection method. Treatment, day, replicate, pen, and animal were used in the class statement. Replicate was the random variable, and animal was specified as the subject. The repeated statement was day, and the spatial power law structure [SP(POW)] in SAS was used as the covariance model to account for unequally spaced sampling times. To evaluate the 2 × 2 factorial arrangement of treatments, single-degree-of-freedom orthogonal contrasts evaluating the main effects of management (PC or AM), exposure (PI or CON), and their interaction were used. If the interaction was significant (P ≤ 0.10), treatment means were separated with a t test using the PDIFF option in SAS. For the main effects of management and exposure, a P-value ≤ 0.05 was considered statistically significant. Proinflammatory cytokine and haptoglobin concentrations were log transformed to improve the normality of the data. The Pearson correlation coefficient between RT and log-transformed Hp concentration was generated using the CORR procedure of SAS.

RESULTS AND DISCUSSION

Rectal Temperature

Mean RT for AM calves increased sharply (treatment × day interaction; P < 0.001) on d 1 and remained elevated until returning to baseline (d 0) levels on d 14, such that a main effect of management (P < 0.001) was evident (Fig. 1). Febrile responses have been consistently reported in experimental models of viral (Chirase et al., 1991; Muller-Doblies et al., 2004), endotoxin (Reuter et al., 2008), and corticotrophin-release hormone (Cooke and Bohnert, 2011) challenge. Furthermore, RT ≥ 40°C
is a standard objective index used to determine clinical BRD morbidity of cattle both in the production setting (Edwards, 2010) and in the current study. Peak RT was observed on d 1, averaging 40.3°C and 39.1°C for AM and PC, respectively. The elevated RT observed for AM resulted in markedly greater (management effect; \( P < 0.001 \)) morbidity for AM (86%) vs. PC (4%) during the 14-d evaluation period. The majority of the animals diagnosed with BRD received the first antibiotic on d 1 (21 of 24 morbid cattle). The AM calves also were treated with a second antibiotic more often (33% vs. 4% for AM and PC, respectively; \( P = 0.01 \)), and 2 AM calves received a third antibiotic during the 14-d study (management effect; \( P = 0.20 \)). Consequently, antibiotic treatment costs were $23.25 vs. $1.53/animal for AM and PC, respectively; \( P \leq 0.05 \).

The greater RT observed for AM may be in response to physiological stress, pathogenic infection, or MLV respiratory vaccination; however, differentiation of these factors on febrile response was not determined in the current study. Nevertheless, the presence of a PI-BVDV pen mate did not affect (\( P = 0.95 \)) RT. Others reported RT being increased subsequent to PI-BVDV challenge (Burchiga Robles et al., 2010) or oral inoculation of BVDV (Muller-Doblies et al., 2004); however, the sampling interval in those studies was more frequently concentrated, and differences in RT that may have occurred between sampling intervals in the current study are unknown. Furthermore, the potential of a febrile response to the MLV respiratory vaccine administered on d 0 to AM but not PC may have impacted our RT results (Roth and Kaeberle, 1983).

**Serum Proinflammatory Cytokines**

There were no main effects of management (\( P \geq 0.15 \)) observed for serum proinflammatory cytokine concentrations. There were also no effects of management, exposure, or their interaction on serum IL-4 concentrations (data not shown; \( P \geq 0.46 \)). Serum TNF-\( \alpha \) concentration tended (\( P = 0.09 \)) to be greater in calves exposed to a PI-BVDV pen mate (Fig. 2). A day effect (\( P \leq 0.001 \)) was observed for all 4 of the proinflammatory cytokines evaluated. Most proinflammatory cytokine concentrations increased transiently with time; however, an exception was noted for TNF-\( \alpha \). For CON, TNF-\( \alpha \) concentrations were decreased throughout the 14-d evaluation period. Although the treatment \( \times \) day interaction was not significant (\( P = 0.21 \)), by d 14 TNF-\( \alpha \) increased sharply for AMPI. The numerical difference in TNF-\( \alpha \) suggests an additive effect of management, and PI exposure was evident for serum TNF-\( \alpha \) concentration and is consistent with our hypothesis of immunomodulation resulting from PI-BVDV exposure being greatest in AM. Activated macrophages in response to gram-negative bacteria (i.e., *M. haemolytica*) or other infectious microbes are the primary cellular source of TNF-\( \alpha \), and some of the biologic effects of TNF-\( \alpha \) include activation of neutrophils and endothelial cells, fever, and induction of acute-phase proteins by hepatocytes (Abbas et al., 2007). Furthermore, BW loss from anorexic or catabolic effects of increased TNF-\( \alpha \) resulting from exposure to a PI-BVDV pen mate may occur for highly stressed AM calves (Johnson, 1997).

A treatment \( \times \) day interaction (\( P \leq 0.05 \)) was observed for both IFN-\( \gamma \) and IL-6 (Figs. 3 and 4, respectively). Peak IFN-\( \gamma \) concentration during the 14-d sampling period occurred on d 7 (day effect; \( P = 0.001 \)), and serum IFN-\( \gamma \) on d 7 was 3.3-fold greater for AMPI than...
PCCON (treatment × day interaction; \( P = 0.05 \)). By d 14, overall IFN-γ concentrations had decreased; nevertheless, AMPI was greatest, PCPI was intermediate, and AMCON and PCCON were least. Interferon-γ is produced by activated natural killer or cytotoxic T cells in response to stressed or virus-infected cells (Abbas et al., 2007). The increased concentrations of serum IFN-γ observed for PI-exposed cohorts is likely in response to enhanced BVDV infection attributable to the presence of a PI-BVDV pen mate, and differences in management and vaccination status for AM may have further contributed to this occurrence. Peak concentrations of IL-6 were observed on d 1 and returned to near baseline by d 3 (day effect; \( P < 0.001 \)); nevertheless, a treatment × day interaction (\( P = 0.006 \)) was evident. On d 1, IL-6 concentrations were greatest for AMCON, intermediate for AMPI and PCPI, and least in PCCON. On d 14, AMPI had greater serum concentrations of IL-6 compared with AMCON. Among other actions, IL-6 prompts hepatic production of acute-phase proteins, and an association among psychological stress and increased IL-6 production was observed in humans after experimental influenza challenge (Cohen et al., 1999).

Similar to our observations of increased proinflammatory cytokine profiles for AMPI, Hodgson et al. (2012) reported that IFN-γ and LPS-induced TNF-α production were greater in calves subjected to weaning and maternal separation (stress) compared with calves preadapted to the weaning stress after experimental challenge with bovine herpesvirus-1. The authors also observed that weaning stress increased mortality after \( M. \) haemolytica challenge; 90% of the wean-stressed calves died vs. 50% of calves preadapted to the stressor. Burciaga-Robles et al. (2010) observed increases in TNF-α, IFN-γ, and IL-6 when steers were exposed for 72 h to 2 PI-BVDV calves. Carroll et al. (2009) observed that both TNF-α and IL-6 concentrations increased after endotoxin challenge in calves, and the magnitude of increase was greater for calves weaned at 250 d of age vs. calves weaned early at 80 d of age and shipped simultaneously to a receiving facility. Proinflammatory cytokine concentrations were greatest for AMPI during some of the sampling times, suggesting that physiological stress and PI-BVDV exposure may be synergistic in stimulating an inflammatory response. Increased inflammation associated with these factors may consequently affect health and growth performance (Spurlock, 1997). Although differences in growth between CON and PI were not evident during the 14-d evaluation period, observations from a companion study (Richeson et al., 2012) suggest that differences in growth performance may not be detected until d 28 from onset of PI-BVDV exposure. One explanation for reduced growth performance being deferred is that peak TNF-α concentration did not occur until d 14 in this study, and the response pattern of proinflammatory cytokines in a PI-BVDV-exposed cohort may be a function of BVDV replication in the host.

Serum proinflammatory cytokine concentrations were not different between PCCON and PCPI in the current study. This may explain why previous field studies using experimental animals with relatively low clinical BRD observations do not report negative effects in health or performance among PI-exposed and nonexposed pens (O’Connor et al., 2005; Booker et al., 2008; Elam et al., 2008; Stevens et al., 2009; Richeson et al., 2012). The increased inflammatory response and im-

**Figure 3.** Effects of weaning management and persistently infected bovine viral diarrhea virus (PI-BVDV) exposure on serum interferon-γ concentration of newly received beef calves. Effect of day (\( P < 0.001 \)) and treatment × day (\( P = 0.05 \)). a–cMeans within a day without a common superscript differ (\( P \leq 0.05 \)). Statistical analysis was performed on log-transformed data, and geometric means are reported; SEM for log-transformed data = 0.2844. AMCON = auction market, control; AMPI = auction market, exposed to a PI-BVDV pen mate; PCCON = preconditioned, control; PCPI = preconditioned, exposed to a PI-BVDV pen mate.

**Figure 4.** Effects of weaning management and persistently infected bovine viral diarrhea virus (PI-BVDV) exposure on serum IL-6 concentration of newly received beef calves. Effect of day (\( P < 0.001 \)) and treatment × day (\( P = 0.006 \)). a,bMeans within a day without a common superscript differ (\( P \leq 0.05 \)). Statistical analysis was performed on log-transformed data, and geometric means are reported; SEM for log-transformed data = 0.5363. AMCON = auction market, control; AMPI = auction market, exposed to a PI-BVDV pen mate; PCCON = preconditioned, control; PCPI = preconditioned, exposed to a PI-BVDV pen mate.
munomodulation observed for AMPI may suggest that highly stressed, commingled calves in which overall clinical BRD morbidity is high are more susceptible to PI-BVDV exposure and thus are more likely to experience negative health and performance consequences (Stevens et al., 2007; Hessman et al., 2009; Richeson et al., 2012). Typically, the majority of initial BRD diagnoses occur early in the receiving period for highly stressed calves (Buhman et al., 2000; Richeson et al., 2008). Therefore, the typical epidemic response may be such that subsequent health variables (i.e., relapse, chronically ill), rather than overall clinical BRD morbidity, are more likely to be impacted in highly stressed, commingled calves exposed to a PI-BVDV pen mate (Hessman et al., 2009; Richeson et al., 2012).

Serum Haptoglobin

A management effect ($P < 0.001$) was observed for serum Hp concentrations; AM calves had markedly greater Hp concentrations compared with PC (Table 2). Furthermore, a transient increase (day effect; $P < 0.001$) in Hp concentrations corresponded with other immune variables measured in the current study. The serum concentration of an acute-phase protein is altered either positively or negatively in response to conditions such as infection, trauma, surgery, or various other inflammatory conditions (Gabay and Kushner, 1999). In a study evaluating acute-phase protein changes in response to a natural BRD outbreak (Orro et al., 2011), serum Hp seemed to respond positively to secondary bacterial infection, rather than an initial infection with bovine respiratory syncytial virus. Serum Hp concentration and RT were positively correlated ($P < 0.001$; correlation coefficient $= 0.57$). Interestingly, IL-6 is important for inducing febrile body temperature and stimulation of the acute-phase protein (Hp) response (Petersen et al., 2004), and IL-6 was greatest for AM calves on d 1, which was the day of the highest RT for AM calves, whereas Hp peaked on d 2. Serum Hp concentrations (treatment × day, $P < 0.001$) responded similarly to febrile RT and morbidity observed for AM calves; therefore, these observations support the potential of serum Hp as a quantitative, objective biomarker for diagnosing BRD. This is similar to previous findings of positive Hp response to clinical BRD morbidity (Humblet et al., 2004; Burciaga-Robles et al., 2009) and pulmonary lesions evident at slaughter (Wittum et al., 1996), and it has also been reported that BRD diagnosis is more effective when both Hp and RT are included in the model (Svensson et al., 2007). Currently, lag time and cost of assay results severely limit the adoption of Hp or other biomarkers potentially useful for the diagnosis of BRD in the commercial production setting.

In conclusion, no differences were observed among PCCON and PCPI for any of the immune parameters measured in the current study; therefore, immunomodulation in PC calves exposed for 14 d to a PI-BVDV pen mate transmitting a low-virulent strain seems minimal. However, our results show continuous PI-BVDV exposure in AM calves increased some serum concentrations of proinflammatory cytokines, which may affect health or growth because of their associated anorexic and catabolic effects. Elucidating the contribution of physiological stress vs. viral and/or bacterial infection on the proinflammatory cytokine responses observed in the current study is difficult because both factors can influence production of the same cytokines; nevertheless, it

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</tr>
<tr>
<td>Avg</td>
<td>28.99</td>
<td>25.51</td>
<td>0.12</td>
<td>0.11</td>
<td>0.388</td>
<td>0.001</td>
<td>0.79</td>
<td>0.96</td>
</tr>
</tbody>
</table>

a,b Means within a row without a common superscript are different ($P < 0.05$).

1AMCON = auction market, control; AMPI = auction market, exposed to a PI-BVDV pen mate; PCCON = preconditioned, control; PCPI = preconditioned, exposed to a PI-BVDV pen mate.

2SE is log transformed.

3Management = main effect of weaning management (auction market vs. preconditioned); Exposure = main effect of PI-BVDV exposure (control vs. persistently infected bovine viral diarrhea virus exposure); Interaction = management × exposure.

4Treatment × day, $P < 0.001$.

5Statistical analysis was performed on log-transformed data; geometric means are reported.
appears that stress and exposure to a PI-BVDV pen mate are additive. Marked differences in Hp concentration in newly received beef calves of PC vs. AM origin indicate differences in secondary bacterial infection or trauma from surgical castration existed for the 2 management systems. The association observed between RT and Hp suggests that Hp may have potential as a biomarker for BRD diagnosis, but further validation is warranted. Further research is needed to more clearly understand the complex interactions of physiological stress and respiratory pathogens and their impact on inflammation and BRD pathogenesis.

**LITERATURE CITED**


