Association of hematologic variables and castration status at the time of arrival at a research facility with the risk of bovine respiratory disease in beef calves

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Objective—To determine the association of CBC variables and castration status at the time of arrival at a research facility with the risk of development of bovine respiratory disease (BRD).

Design—Retrospective cohort study.

Animals—1,179 crossbred beef bull (n = 588) and steer (591) calves included in 4 experiments at 2 University of Arkansas research facilities.

Procedures—Calves underwent processing and treatments in accordance with the experiment in which they were enrolled. Castration status and values of CBC variables were determined at the time of arrival at the facilities. Calves were monitored to detect signs of BRD during a 42-day period.

Results—The areas under the receiving operator characteristic curves for CBC variables with significant contrast test results ranged from 0.51 (neutrophil count) to 0.67 (eosinophil count), indicating they were limited predictors of BRD in calves. The only CBC variables that had significant associations with BRD in calves as determined via multivariable logistic regression analysis were eosinophil and RBC counts. The odds of BRD for bulls were 3.32 times the odds of BRD for steers.

Conclusions and Clinical Relevance—Results of this study indicated that low eosinophil and high RBC counts in blood samples may be useful for identification of calves with a high risk for development of BRD. Further research may be warranted to validate these variables for prediction of BRD in calves. Calves that were bulls at the time of arrival had a higher risk of BRD, versus calves that were steers at that time. (*J Am Vet Med Assoc* 2013;243:1035–1041)

Bovine respiratory disease in beef calves that have been recently weaned and received at a stocker cattle operation or feedlot facility is an important concern for the US beef cattle industry; BRD causes 70% of beef cattle morbidity and 50% of beef cattle deaths in feedlots[1] and has a higher cost for beef cattle in North America than any other disease.[2] Predisposing causes for BRD in calves that have been recently received at a stocker cattle operation include physical and psychological causes of stress, immune system dysfunction, dehydration, and poor nutritional status.[3,4] Specific stressors include weaning, castration, shipping, social restructuring attributable to commingling of animals, novel types of feed, and handling. Environment-associated disease risk factors for calves include climate, ambient temperature, airborne dust particles, exposure to noxious gases, housing density of animals, humidity, and ventilation.[5] Microbial organisms that cause BRD include viruses (bovine herpesvirus-1, bovine viral diarrhea virus, bovine respiratory syncytial virus, parainfluenza-3 virus, and bovine coronavirus), bacteria (*Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*), and *Mycoplasma* spp.[5]

Abbreviations

AUC	Area under the curve
BRD	Bovine respiratory disease
CI	Confidence interval
ROC	Receiver operating characteristic

The physiologic stress in calves resulting from weaning, marketing, transportation, and arrival at a novel facility can negatively affect feed intake, growth rate, carcass quality, behavior, metabolism, immune system function, and overall health.[6,7] Potential biomarkers for detection of disease in cattle after transportation have been investigated; results of such studies suggest that a rapidly performed, objective, repeatable, and cost-effective method for prediction or diagnosis of BRD in cattle would be useful.

The objectives of the study reported here were to determine the association between values of CBC variables determined at the time of arrival at a research fa-

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cility and the risk of subsequent development of clinical signs of BRD in beef calves and to determine the accuracy of CBC variable threshold values for prediction of the risk of BRD for such animals. Another objective of the study reported here was to determine the association between castration status (steer vs bull calf) at the time of arrival at a research facility and the risk of development of clinical signs of BRD.

Materials and Methods

Animals—Data for the present study were obtained from records of calves in 4 other studies (experiments 1–4).[8–10] Procedures in those studies were approved by the University of Arkansas Animal Care and Use Committee. Data for 591 steers and 588 bull calves (mean ± SD body weight at the start of the study, 197 ± 2.4 kg [433.4 ± 5.3 lb]) were included in the present study. The mean ± SD age of calves at the start of the study was unknown because birth records for individual animals were not available; however, the estimated age range of calves included in the present study was 200 to 365 days. In each of those other studies, male beef calves were procured from Arkansas auction barns or ranches and transported by truck (transport duration, ≤ 5 hours) to the University of Arkansas Agricultural Experiment Station (located near Savoy; calves in experiments 1 and 2) or the University of Arkansas Livestock and Forestry Branch Station (located near Batesville; calves in experiments 3 and 4). Castration status of calves for the present study was determined on the day of arrival at one of the research facilities. The BRD status of calves in the present study was classified in accordance with detection of clinical signs consistent with that disease; calves did not have BRD (signs of BRD were not detected), had a diagnosis of BRD at least once (treated for BRD at least once; BRD1), or had a diagnosis of BRD twice (treated for BRD twice; BRD2). All calves included in the group of animals that had a diagnosis of BRD twice were included in the group of animals that had a diagnosis of BRD at least once. Treatments for BRD1 included 40 mg of florfenicol/kg (18.2 mg of florfenicol/lb; experiments 1 and 3), SC; 10 mg of enrofloxacin/kg (4.5 mg of enrofloxacin/lb; experiment 2), SC; or 2.5 mg of tulathromycin/kg (1.1 mg of tulathromycin/lb; experiment 4), SC, in the neck. A second diagnosis of BRD (BRD2) was made for calves that were observed to have a second BRD event. Treatments for BRD2 included 6.6 mg of ceftiofur crystalline free acid/kg, SC, in the caudal aspect of the ear (3 mg of ceftiofur crystalline free acid/lb; experiment 1); 40 mg of florfenicol/kg (experiments 2 and 4), SC, in the neck; or 10 mg of tilmicosin/kg (experiment 3), SC, in the neck.

Experiment 1[8] included 133 calves (103 bull and 30 steer calves). These calves were acquired from regional auction barns and received 1 of 4 vaccination regimens (clostridial bacterin, pentavalent respiratory vaccine, or both administered on day 0 or 14). Calves undergoing experiment 1 arrived at the research facility at 1 of 3 times (ie, 3 shipments).

Experiment 2[9] included 528 calves (211 bull and 317 steer calves) that underwent different prearrival management procedures in the presence or absence of a persistently infected bovine viral diarrhea virus pen mate during the 42-day receiving period. Calves in experiment 2 arrived at the research facility at 1 of 4 times (ie, 4 shipments).

Experiment 3[10] included 381 calves (230 bull and 151 steer calves) acquired from a regional auction barn. These calves received 4 experimental treatments (pentavalent respiratory virus vaccination on day 0 or 14 with or without administration of a growth implant containing 36 mg of zeranol on day 0). Calves in experiment 3 arrived at the research facility at 1 of 2 times (ie, 2 shipments).

Experiment 4 included 137 calves (44 bull and 93 steer calves) acquired from a regional auction barn. These calves received 4 experimental treatments (administration of a growth implant containing 200 mg progesterone with or without 20 mg of estradiol benzoate and 200 mg progesterone on day 0, 14, or 28). Calves in experiment 4 arrived at the research facility at 1 of 3 times (ie, 3 shipments).

For each calf, study day –1 was defined as the day the calf arrived at the research facility. During that day, calves were weighed and ear tags with unique identification numbers were placed. Calves were palpated to determine castration status (bull vs steer calf). Each calf was assigned to an experimental treatment group in accordance with the protocol for each experiment. Calves in each of the 4 experiments were housed together overnight. Calves had ad libitum access to hay and water. On study day 0 for each calf, vaccines, anthelmintics, and drugs intended to control infections were administered in accordance with the protocol for each of the 4 experiments. All calves received 1 of 2 pentavalent (bovine herpesvirus-1, bovine viral diarrhea virus type 1 and 2, bovine respiratory syncytial virus, and parainfluenza-3 virus) modified-live virus vaccines.[a,b] Calves in experiment 1 or 2 received a multivalent *Clostridium* spp bacterin toxoid.[c] Calves in experiment 3 or 4 received a multivalent *Clostridium* spp bacterin with tetanus toxoid.[d] Calves received 1 of 2 medications[e,f] for treatment of internal and external parasites. Bull calves were castrated by means of surgery or a banding method.[g] Calves in experiment 1 received an antimicrobial drug (10 mg of tilmicosin/kg, SC, in the neck) metaphylactically. Calves in experiment 3 received an antimicrobial drug (40 mg of florfenicol/kg, SC, in the neck) metaphylactically, if rectal temperature was ≥ 40°C (104.0°F) during initial processing.

Blood sample analyses—Blood samples (6 mL) were collected on study day 0 from the right jugular vein of each calf into 6-mL evacuated tubes containing 10.8 mg of K_2-EDTA[h]; the tubes were inverted several times, stored at 5°C, and analyzed within 24 hours by personnel at the USDA Agricultural Research Service Poultry Production and Product Safety Research Unit, Fayetteville, Ark. Whole blood samples for all animals were analyzed with the same automated hemocytometer[i] that was validated for analysis of bovine blood samples. The laboratory technicians who performed the CBC analysis were unaware of the experimental treatment and castration status of the calves. Variables determined by means of the CBC analysis included Hct, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, RBC distribution width, and

total WBC, lymphocyte, neutrophil, monocyte, eosinophil, basophil, platelet, and RBC counts. In addition, neutrophil-to-lymphocyte count ratios were determined for each calf.

BRD definition—Calves were observed during each morning to detect signs of respiratory illness until day 42 after arrival at the research facilities. Diagnoses of BRD were determined by trained experiment-station personnel. Personnel evaluating calves in experiments 1, 3, and 4 were not aware of the experimental treatments that calves were receiving; personnel evaluating calves in experiment 2 were aware of the experimental treatments calves were receiving. For calves with ≥ 2 clinical signs consistent with BRD (signs of depression, nasal discharge, ocular discharge, cough, gaunt appearance, and inappetence), rectal temperature was determined with a digital thermometer[j] (instrument readability, ± 0.1°C). Bovine respiratory disease was diagnosed for calves with such clinical signs of BRD and a rectal temperature ≥ 40.0°C (104.0°F). An antimicrobial drug was administered to calves with a diagnosis of BRD in accordance with the predetermined treatment protocol for each experiment. The procedure was repeated, and calves were eligible for subsequent BRD diagnosis after an appropriate posttreatment interval that depended on the antimicrobial that had been used (variables defined as BRD1 and BRD2, respectively). All calves were immediately returned to their pen after evaluation; calves with BRD were not separated from calves without BRD during the study.

Data collection and statistical analysis—The association between values of various CBC variables on day 0 and castration status at the time of arrival at the research facilities (day –1) and subsequent risk for development of BRD was determined. To control analyses for the inclusion of data for calves that may have been ill at the time of arrival, data for animals with total WBC count values less that the 10th percentile value for the study population or values greater than the 90th percentile value for the study population were not included in the analyses.

Data were recorded in spreadsheets[k] and subsequently analyzed with statistical software.[l] Each calf was considered an experimental unit for all analyses.

Univariable associations between a diagnosis of BRD and all calf-level covariate variables (experiment in which calves were enrolled, experimental treatment [4 treatments/experiment], shipment number, and castration status at the time of arrival) were tested via a χ^2 test and logistic regression analysis. Variables with a value of $P \leq 0.20$ were included in multivariable analyses.

The association between values of CBC variables and a diagnosis of BRD in calves was determined by use of 2 procedures. By use of a logistic procedure,[m] ROC curves and the AUC were used to determine the accuracy of each CBC variable for prediction of a diagnosis of BRD and for assessment of the CBC variable threshold values that resulted in the maximum combined sensitivity and specificity for prediction of a diagnosis of BRD. An ROC curve is a graphical method to determine the performance of a diagnostic test and represents a plot of the sensitivity of a test versus the false positive rate (1 – specificity) computed for various cutoff values to select the optimum cutoff value for distinguishing between diseased and nondiseased individuals.[11] The AUC is a global summary statistic of diagnostic accuracy and is equivalent to the probability that a randomly selected individual with a positive status has a greater test value than a randomly selected individual with a negative status.[12] Threshold values were determined via testing of the sensitivity and specificity values for multiple cutoff values for each variable. In addition, a χ^2 test and multivariable logistic regression models (generalized linear mixed models with logit link function[n]) were used for prediction of a diagnosis of BRD in calves. Each CBC variable was analyzed via a 2-level categorization (low or high values [determined on the basis of the optimal threshold values determined via the ROC curve analysis]) and a 3-level categorization (low [values less than the 25th percentile value], medium [values within the interquartile range], and high [values greater than the 75th percentile value]). Multivariable logistic regression models were tested independently for each categorization type (2- or 3-level categorization) for each CBC variable; included covariate variables were experiment, experimental treatment, shipment number, castration status, and the biologically plausible 2-way interactions (ie, CBC variable × castration status). Experimental treatment (nested within experiment) and shipment number were included as random effects; castration status was included as a fixed effect in each model.

Covariate variables and interactions with a value of $P > 0.10$ were removed from the final model via manual backward selection. All analyses were performed separately for calves that had a diagnosis of BRD at least once and for calves that had a diagnosis of BRD twice. Values of $P \leq 0.05$ were considered significant.

Results

For the 133 calves in experiment 1, a diagnosis of BRD was made once for 95 (71.4%) calves, and a diagnosis of BRD was made twice for 44 (33.1%) calves; BRD was not detected in 38 (28.6%) calves in this experiment. For the 528 calves in experiment 2, a diagnosis of BRD was made once for 217 (41.1%) calves, and that diagnosis was made twice for 120 (22.7%) calves; BRD was not detected in 311 (58.9%) calves in this experiment. For the 381 calves in experiment 3, a diagnosis of BRD was made once for 308 (80.8%) calves, and that diagnosis was made twice for 205 (53.8%) calves; BRD was not detected in 73 (19.2%) calves in this experiment. For the 137 calves in experiment 4, a diagnosis of BRD was made once for 73 (53.3%) calves, and that diagnosis was made twice for 16 (11.7%) calves; BRD was not detected in 64 (46.7%) calves in this experiment. Of the 1,179 calves in the present study, 658 (55.8%) calves had a diagnosis of BRD once, and 386 (32.7%) had a diagnosis of BRD twice. After removal of data for calves with outlier total WBC counts, data for 943 of the 1,179 (80.0%) calves were included in analyses. Descriptive statistics for CBC data were summarized (**Table 1**).

ROC curve analysis—Threshold values for CBC variables for prediction of development of BRD in

Table 1—Values of CBC variables for 943 calves* (483 bull and 460 steer calves) at the time of arrival at a research facility.

Variable	Overall		Mean ± SD		Least squares mean ± SE†	
	Mean ± SD	Range	Bulls	Steers	Bulls	Steers
Total WBCs (× 10^3 cells/μL)	8.50 ± 2.1	5.25–12.9	8.33 ± 2.03	8.68 ± 2.09	8.41 ± 0.17	8.87 ± 0.18‡
Neutrophils (× 10^3 cells/μL)	2.81 ± 1.5	0.17–8.22	2.87 ± 1.64	2.73 ± 1.44	2.81 ± 0.39	2.88 ± 0.40
Lymphocytes (× 10^3 cells/μL)	4.47 ± 2.2	1.03–11	4.23 ± 2.10	4.70 ± 3.46	4.44 ± 0.33	4.85 ± 0.34§
Neutrophil-to-lymphocyte count ratio	0.86 ± 0.7	0.02–4.71	0.92 ± 0.77	0.79 ± 0.62	0.88 ± 0.13	0.80 ± 0.13
Monocytes (× 10^3 cells/μL)	1.04 ± 0.4	0.175–3.38	1.04 ± 0.43	1.03 ± 0.42	1.05 ± 0.04	1.02 ± 0.04
Eosinophils (× 10^3 cells/μL)	0.10 ± 0.2	0–1.7	0.08 ± 0.15	0.11 ± 0.18	0.07 ± 0.02	0.09 ± 0.02§
Basophils (× 10^3 cells/μL)	0.09 ± 0.1	0.001–0.88	0.09 ± 0.09	0.10 ± 0.09	0.08 ± 0.01	0.09 ± 0.01‡
RBCs (× 10^6 cells/μL)	10.3 ± 1.4	5.13–16.1	10.5 ± 1.44	10.1 ± 1.34	10.4 ± 0.13	10.2 ± 0.13§
Hemoglobin (g/dL)	12.5 ± 1.3	8.4–16.3	12.6 ± 1.30	12.3 ± 1.27	12.7 ± 0.16	12.3 ± 0.16‡
Hct (%)	36.9 ± 3.7	23.5–46.9	37.3 ± 3.77	36.4 ± 3.61	37.6 ± 0.37	36.6 ± 0.38‡
Mean corpuscular volume (fL)	36.1 ± 3.3	24.7–51.5	36.0 ± 3.29	36.1 ± 3.23	36.2 ± 0.39	36.2 ± 0.40
Mean corpuscular hemoglobin (pg)	12.2 ± 1.2	1.5–17.3	12.2 ± 1.22	12.2 ± 1.14	12.2 ± 0.08	12.2 ± 0.08
Mean corpuscular hemoglobin concentration (g/dL)	33.8 ± 1.2	28.9–39.3	33.8 ± 1.17	33.7 ± 1.26	33.8 ± 0.25	33.7 ± 0.24
RBC distribution width (%)	26.0 ± 2.6	19.3–36.9	25.7 ± 2.68	26.2 ± 2.54	25.9 ± 0.32	26.3 ± 0.32
Platelets (× 10^3 platelets/μL)	517 ± 339	23–5,807	546 ± 383	486 ± 282	562 ± 56	542 ± 55

*Data for 236 calves in the study with total WBC count values less than the 10th percentile value for the study population or values greater than the 90th percentile value for the study population are not included. †The model used to determine least squares mean values included the variables experimental group and shipment number as covariate variables. ‡Values for bull calves and steers are significantly ($P < 0.001$) different (determined with the Wilcoxon test). §Values for bull calves and steers are significantly ($P < 0.05$) different (determined with the Wilcoxon test).

calves determined via ROC curves analyses were summarized (**Table 2**). Contrast test results between parameter AUC and chance AUC were significant for the variables total WBC count, lymphocyte count, neutrophil-to-lymphocyte count ratio, eosinophil count, RBC count, hemoglobin concentration, Hct, mean corpuscular volume, and mean corpuscular hemoglobin concentration. However, the resulting AUC values for those variables with significant contrasts were low to moderate and ranged from 0.51 (neutrophil count) to 0.67 (eosinophil count), indicating low value for prediction of a diagnosis of BRD.

Univariable analyses—Results of univariable analyses indicated all evaluated calf-level covariate variables (experiment, experimental treatment, shipment number, and castration status) had significant effects on the risk of calves for a diagnosis of BRD at least once and for a diagnosis of BRD twice. Results of a χ^2 test for analysis of values of CBC variables (by use of 2- and 3-level categorization methods) and number of BRD diagnoses for calves were summarized (**Table 3**).

Multivariable analyses—Multivariable analyses included castration status at the time of arrival as a fixed effect and experimental treatment (nested within experiment) and shipment number as random effects. No interactions (ie, CBC variable × castration status) were found to be significant; consequently, they were removed from the models. Results indicated significant associations between a diagnosis of BRD (at least once or twice) and eosinophil and RBC counts (via 2- and 3-level categorization methods for values of CBC variables), except for a diagnosis of BRD at least once and RBC count analyzed via a 2-level categorization method for values of CBC variables (**Table 4**).

Castration status analysis—The effect of castration status at the time of arrival at one of the research facilities on subsequent determination of a diagnosis of BRD was initially tested via univariate logistic regression. Results of this analysis indicated male calves that were sexually intact (bulls) at the time of arrival had a significantly higher risk for development of BRD, compared with the risk for male calves that were castrated (steers) at that time. The odds of a diagnosis of BRD at least once or twice for bull calves were 8.63 (95% CI, 6.31 to 11.7; $P < 0.001$) and 4.02 (95% CI, 2.98 to 5.41; $P < 0.001$) times as high as the odds of such diagnoses for steer calves, respectively. When potential confounding variables (treatment [nested within experiment] and shipment number) were included in the model, the odds of a diagnosis of BRD at least once or twice for bull calves were 3.32 (95% CI, 2.23 to 4.93; $P < 0.001$) and 2.66 (95% CI, 1.85 to 3.80; $P < 0.001$) times as high as the odds of such diagnoses for steer calves, respectively.

Table 2—Threshold values for various CBC variables for prediction of BRD in calves determined via ROC curve analyses.

Variable	Threshold value	AUC	*P* value
Total WBCs (× 10^3 cells/μL)	8.06	0.59	< 0.001
Neutrophils (× 10^3 cells/μL)	2.16	0.51	0.69
Lymphocytes (× 10^3 cells/μL)	3.67	0.57	< 0.001
Neutrophil-to-lymphocyte count ratio	0.64	0.55	0.03
Monocytes (× 10^3 cells/μL)	0.90	0.53	0.13
Eosinophils (× 10^3 cells/μL)	0.05	0.67	< 0.001
Basophils (× 10^3 cells/μL)	0.06	0.54	0.11
RBCs (× 10^6 cells/μL)	10.0	0.60	< 0.001
Hemoglobin (g/dL)	12.2	0.59	< 0.001
Hct (%)	36.7	0.57	< 0.001
Mean corpuscular volume (fL)	36.4	0.56	0.001
Mean corpuscular hemoglobin (pg)	12.2	0.54	0.06
Mean corpuscular hemoglobin concentration (g/dL)	33.7	0.55	0.02
RBC distribution width (%)	25.9	0.52	0.39
Platelets (× 10^3 platelets/μL)	464	0.54	0.08

Values of *P* are for contrast between parameter AUC and chance AUC.

Table 3—Results of analysis for determination of the association between various variables and determination of a diagnosis of BRD for calves during a 42-day period after arrival at a research facility.

	2-level categorization		3-level categorization	
Variable	**BRD1**	**BRD2**	**BRD1**	**BRD2**
Castration status	< 0.001	< 0.001	< 0.001	< 0.001
Shipment number	< 0.001	< 0.001	< 0.001	< 0.001
Treatment	< 0.001	< 0.001	< 0.001	< 0.001
Total WBC count	0.01	< 0.001	0.03	0.13
Neutrophil count	0.68	0.97	0.12	0.26
Lymphocyte count	< 0.001	< 0.001	0.03	0.01
Neutrophil-to-lymphocyte count ratio	0.052	0.09	0.06	0.14
Monocyte count	0.31	0.72	0.31	0.75
Eosinophil count	< 0.001	< 0.001	< 0.001	< 0.001
Basophil count	0.09	0.29	0.31	0.90
RBC count	< 0.001	< 0.001	< 0.001	< 0.001
Hemoglobin	< 0.001	< 0.001	< 0.001	< 0.001
Hct	0.01	0.012	0.001	0.004
Mean corpuscular volume	0.005	0.016	0.02	0.01
Mean corpuscular hemoglobin	0.055	0.013	0.11	0.04
Mean corpuscular hemoglobin concentration	0.06	0.83	0.12	0.002
RBC distribution width	0.28	0.50	0.12	0.48
Platelet count	0.08	0.20	0.08	0.44

Data are *P* values determined via univariable analysis (χ^2 test). Each variable was analyzed via a 2-level categorization method (low or high values [determined on the basis of the optimal threshold values determined by means of the ROC analysis]) and a 3-level categorization method (low [values less than the 25th percentile value], medium [values within the interquartile range], and high [values greater than the 75th percentile value]).

BRD1 = Calves that had a diagnosis of BRD at least once (ie, treated for BRD once or twice) during the study. BRD2 = Calves that had a diagnosis of BRD twice (ie, treated for BRD twice) during the study.

Table 4—Results of multivariable logistic regression analysis for an association between values of various CBC variables and the risk of BRD for calves during a 42-day period after arrival at a research facility.

	BRD1			BRD2		
Variable	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
2-level categorization						
Eosinophils ($\times 10^3$ cells/μL)			< 0.001			< 0.001
< 0.054	1.98	1.31–2.89		2.04	1.41–2.97	
≥ 0.054	—	—		—	—	
RBCs ($\times 10^6$ cells/μL)			0.058			0.03
< 10.0	0.75	0.49–1.01		0.69	0.49–0.97	
≥ 10.0	—	—		—	—	
3-level categorization						
Eosinophils ($\times 10^3$ cells/μL)			0.002			< 0.001
< 0.011	2.65	1.54–4.57		2.51	1.51–4.17	
0.011–0.108	1.55	0.99–2.41		1.71	1.07–2.73	
> 0.108	—	—		—	—	
RBCs ($\times 10^6$ cells/μL)			0.002			0.007
< 9.46	0.62	0.36–1.05		0.63	0.41–0.98	
9.46–11.2	0.44	0.28–0.70		0.53	0.36–0.79	
> 11.2	—	—		—	—	

Only CBC variables with significant ($P \leq 0.05$) effects on determination of a diagnosis of BRD at least once in calves are included.

— = Referent.

See Table 3 for remainder of key.

Discussion

A diagnosis of BRD can be difficult to determine for cattle in a production setting because the etiology of the disease is complex and animals may not show clinical signs of illness. Furthermore, because the medical history of cattle arriving at a production facility is typically unknown, a rapid, objective, repeatable, cost-effective method for prediction of BRD risk of calves may be useful for decisions regarding metaphylactic use of antimicrobial drugs. Such potential methods include measurement of biomarkers via validated assays or detection of changes in animals in behavior or body temperature consistent with clinical disease. The authors are unaware of previously published data regarding the efficacy of use of determination of values of CBC variables for prediction of a diagnosis of BRD in high-risk cattle at the time of arrival at a production facility. Cir-

culating concentrations of acute-phase proteins such as ceruloplasmin,[13] haptoglobin,[14] and serum amyloid-A[15] increase ≥ 100-fold in response to infection and inflammation in cattle. Results of some studies[16,17] indicate that measurement of circulating haptoglobin concentrations is a useful method for detection of infectious disease in animals; however, results of other studies[14,18] indicate that haptoglobin has limited use as a biomarker for identification of animals with disease. Nevertheless, results of haptoglobin assays may be better suited for determination of a diagnosis of acute or chronic inflammation resulting from a secondary bacterial infection than they are for prediction of disease, because the acute-phase response in animals is transient.[19]

Prediction of BRD risk for particular shipments or sources of cattle is an important component of BRD management. Currently, personnel in stocker cattle operations and feedlot production facilities classify disease risk on the basis of limited information such as animal purchase origin, duration of transport, body weight loss during transport, and general appearance at the time of arrival at a facility. Other factors evaluated during disease risk assessment include anticipated temperature changes and precipitation amounts and aptitude or availability of personnel. Animals that are classified as having a high risk for BRD on the basis of such assessments are typically treated metaphylactically with an injectable antimicrobial drug at the time of initial processing at a stocker operation or feedlot facility to control that disease in the herd.[20] If the method is cost-effective and can be performed in a timely manner, determination of values of CBC variables could be an additional method for objective assessment of disease risk and determination of whether metaphylactic administration of antimicrobial drugs is warranted for cattle.

A limitation of this study was the potential inclusion of calves that had disease at the time day 0 blood samples were collected. For such calves, results of CBCs would not be predictive of the risk for BRD but would be diagnostic for that disease. To reduce the effects of this potential confounding factor, data for calves with outlier total WBC counts were removed from subsequent analyses; however, results were similar for analyses performed with and without data for calves with such outlier total WBC counts.

Results of the present study indicated there was a significant association between values of various CBC variables at the time of arrival at a facility and the risk of a subsequent diagnosis of BRD. Calves with low or intermediate eosinophil counts had a significantly higher risk for having a diagnosis of BRD at least once or twice, versus calves that had a high eosinophil count. In addition, calves with a high circulating RBC count had a significantly higher risk of having a diagnosis of BRD twice (determined via a 2-level categorization method for values) or at least once or twice (determined via a 3-level categorization method for values), versus calves with low RBC counts.

We did not determine a biological reason for the findings of this study; however, a plausible hypothesis could rely on the differences in chronic stress or inflammation among cattle where the hypothalamic-pituitary-adrenal axis is stimulated to a greater extent, resulting in endocrine-induced effects on hematopoiesis, which in turn result in a reduction in the eosinophil count,[21] and a reduction in the eosinophil count was a proxy for higher BRD risk in the present study. Furthermore, animals with chronic stress have a high risk for development of disease because of impairment of immune system function.[5–8,22] Results of another study[23] suggest that eosinophils are involved in the resolution of local inflammation in animals; eosinophils are recruited from circulating blood to an inflammatory site where they release mediators that block polymorphonuclear cell infiltration, promote clearance of phagocytes from the inflammatory site to draining lymph nodes, or both. Another cause for the association between a low circulating eosinophil count and a high risk for development of BRD in calves in this study may have been the effect of redistribution of immune system cells from circulating peripheral blood to immune system tissues during stressful events.[24] These findings may support our elucidation that decreased concentration of eosinophils in peripheral blood is indicative of preferential translocation to inflamed tissue and an association with increased risk for clinical BRD or that greater eosinophils overall may help resolve inflammation, resulting in less clinical disease. However, results of other studies[24,25] indicate that circulating eosinophil counts in calves do not change because of weaning or changes in environment, as evidenced by findings of similar counts before and after weaning and the fact that such counts were not substantially changed by the combined husbandry practices of weaning and housing.

High RBC counts typically indicate dehydration in animals. Calves that are dehydrated as a result of being transported long distances, or those subjected to an extended period of low water consumption for other reasons, are predisposed to BRD.[3–5]

Further investigation is warranted to determine the effects of various factors, such as castration status, on immune dysfunction and BRD risk for calves. Results of the present study indicated castration status of calves at the time of arrival at a facility was significantly associated with risk of a diagnosis of BRD at least once or twice. Castration reduces performance,[26,27] causes inflammation,[27,28] alters immune system function,[28] alters behavior,[29,o] and increases the risk of BRD[28,30] for beef cattle. The trauma associated with castration likely contributes directly to BRD pathogenesis; however, castration status at the time of arrival at a stocker operation or feedlot facility may also correlate with other preconditioning management procedures applied previously at the ranch of origin. Cattle purchased from auction barns as steers may be more likely to have been weaned, received a vaccine against organisms that cause respiratory disease, and received anthelmintic drugs at the facility of origin, versus bull calves[9]; these factors may decrease the risk of BRD for such animals. The finding of the present study that values of CBC variables seemed to differ between calves with a different castration status may support our belief that such animals had differences in management before arrival at the research facilities.

Results of the present study suggested that detection of a low circulating eosinophil count and a high

circulating RBC count may be useful for identification of calves that have a high risk for development of clinical signs of BRD. Results of this study also suggested that for male beef calves, those that were sexually intact at the time of arrival at a research facility had a higher risk of BRD, versus animals that were steers at that time. Further research may be warranted to determine the usefulness of determination of values of CBC variables for prediction of the risk of BRD diagnosis in beef calves.

a. Express 5, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
b. Bovi-shield GOLD 5, Pfizer Animal Health, New York, NY.
c. Alpha 7, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
d. Covexin 8, Merck Animal Health, Summit, NJ.
e. Ivomec, Merial, Iselin, NJ.
f. Cydectin, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
g. InoSol Co LLC, El Centro, Calif.
h. Vacutainer Plus plastic whole blood tube, 13 × 100 mm × 6.0 mL, BD Inc, Franklin Lakes, NJ.
i. Cell-Dyn 3500 system, Abbott Laboratories, Abbott Park, Ill.
j. GLA Agricultural Electronics, San Luis Obispo, Calif.
k. Excel 2010, Microsoft Corp, Redmond, Wash.
l. SAS, version 9.2, SAS Institute Inc, Cary, NC.
m. PROC LOGISTIC, SAS, version 9.2, SAS Institute Inc, Cary, NC.
n. PROC GLIMMIX, SAS, version 9.2, SAS Institute Inc, Cary, NC.
o. Sutherland MA, Davis BL, Brooks TA, et al. The effect of pain relief on the physiology and behavior of calves after castration and/or dehorning (abstr). *J Anim Sci* 2011;89(suppl 1):413.

References

1. Smith RA. Impact of disease on feedlot performance: a review. *J Anim Sci* 1998;76:272–274.
2. Griffin D. Economic impact associated with respiratory disease in beef cattle. *Vet Clin North Am Food Anim Pract* 1997;13:367–377.
3. Callan RJ, Garry FB. Biosecurity and bovine respiratory disease. *Vet Clin North Am Food Anim Pract* 2002;18:57–77.
4. Duff GC, Galyean ML. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. *J Anim Sci* 2007;85:823–840.
5. Snowder GD. Genetics, environment and bovine respiratory disease. *Anim Health Res Rev* 2009;10:117–119.
6. Buckham Sporer KR, Burton JL, Earley B, et al. Transportation stress in young bulls alters expression of neutrophil genes important for the regulation of apoptosis, tissue remodelling, margination, and antibacterial function. *Vet Immunol Immunopathol* 2007;118:19–29.
7. Buckham Sporer KR, Weber PSD, Burton JL, et al. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. *J Anim Sci* 2008;86:1325–1334.
8. Richeson JT, Kegley EB, Gadberry MS, et al. Effects of on-arrival versus delayed clostridial or modified live respiratory vaccinations on health, performance, bovine viral diarrhea virus type I titers, and stress and immune measures of newly received beef calves. *J Anim Sci* 2009;87:2409–2418.
9. Richeson JT, Kegley EB, Powell JG, et al. Weaning management of newly received beef calves with or without continuous exposure to a persistently infected bovine viral diarrhea virus pen mate: effects on health, performance, bovine viral diarrhea virus titers, and peripheral blood leukocytes. *J Anim Sci* 2012;90:1972–1985.
10. Poe KD, Beck PA, Richeson JT, et al. Effects of respiratory vaccination timing and growth-promoting implant on health, performance, and immunity of high-risk, newly received stocker cattle. *Prof Anim Sci* 2013;29:413–419.
11. Dohoo I, Martin W, Stryhn H. *Veterinary epidemiologic research*. 2nd ed. Charlottetown, PE, Canada: AVC Inc, 2009.
12. Greiner M, Pfeiffer D, Smith RD. Principles and practical application of the receiver-operating characteristic analysis for diagnostic tests. *Prev Vet Med* 2000;45:23–41.
13. Conner JG, Eckersall PD, Wiseman A, et al. Acute phase response in calves following infection with *Pasteurella haemolytica, Ostertagia ostertagi* and endotoxin administration. *Res Vet Sci* 1989;47:203–207.
14. Burciaga-Robles LO, Holland BP, Step DL, et al. Evaluation of breath biomarkers and serum haptoglobin concentration for diagnosis of bovine respiratory disease in heifers newly arrived at a feedlot. *Am J Vet Res* 2009;70:1291–1298.
15. Eckersall PD, Young FJ, McComb C, et al. Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet Rec* 2001;148:35–41.
16. Richeson, JT, Kegley EB, Powell JG, et al. 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. *J Anim Sci* 2012;91:1400–1408.
17. Ceciliani F, Ceron JJ, Eckersall PD, et al. Acute phase proteins in ruminants. *J Proteomics* 2012;75:4207–4231.
18. Wittum TE, Young CR, Stanker LH. Haptoglobin response to clinical respiratory tract disease in feedlot cattle. *Am J Vet Res* 1996;57:646–649.
19. Orro T, Pohjanvirta T, Rikula U, et al. Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus. *Comp Immunol Microbiol Infect Dis* 2011;34:23–29.
20. Terrell SP, Thomson DU, Wileman BW, et al. A survey to describe current feeder cattle health and well-being program recommendations made by feedlot veterinary consultants in the United States and Canada. *Bovine Pract* 2011;45:140–148.
21. Griffin JFT. Stress and immunity: a unifying concept. *Vet Immunol Immunopathol* 1989;20:263–312.
22. Hodgson PD, Aich P, Stookey J, et al. Stress significantly increases mortality following a secondary bacterial respiratory infection. *Vet Res* 2012;43:21–32.
23. Isobe Y, Kato T, Arita M. Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of inflammation. *Front Immunol* 2012;3:270.
24. Lynch EM, Earley B, McGee M, et al. Effect of abrupt weaning at housing on leukocyte distribution, functional activity of neutrophils, and acute phase protein response of beef calves. *BMC Vet Res* [serial online]. 2010;6:39. Available at: www.biomedcentral.com/1746-6148/6/39. Accessed Aug 23, 2012.
25. Lynch EM, McGee M, Doyle S, et al. Effect of pre-weaning concentrate supplementation on peripheral distribution of leukocytes, functional activity of neutrophils, acute phase protein and behavioural responses of abruptly weaned and housed beef calves. *BMC Vet Res* [serial online]. 2012;8:1. Available at: www.biomedcentral.com/1746-6148/8/1. Accessed Aug 23, 2012.
26. Peterson AJ, Bass JJ, Payne E. Effects of neonatal adrenalectomy and castration on hormonal changes in pubertal bulls. *J Endocrinol* 1980;87:351–356.
27. Warnock TM, Thrift TA, Irsik M, et al. Effect of castration technique on beef calf performance, feed efficiency, and inflammatory response. *J Anim Sci* 2012;90:2345–2352.
28. Coetzee JF, Edwards LN, Mosher RA, et al. Effect of oral meloxicam on health and performance of beef steers relative to bulls castrated on arrival at the feedlot. *J Anim Sci* 2012;90:1026–1039.
29. Chase CC Jr, Larsen RE, Randel RD, et al. Plasma cortisol and white blood cell responses in different breeds of bulls: a comparison of two methods of castration. *J Anim Sci* 1995;73:975–980.
30. Daniels TK, Bowman JGP, Sowell BF, et al. Effects of metaphylactic antibiotics on behavior of feedlot calves. *Prof Anim Sci* 2000;16:247–253.