Bovine respiratory disease complex is the most common and costly health problem in beef cattle today. By some accounts, feedlot deaths associated with BRDC are increasing. Economic losses associated with BRDC primarily occur during the feeding phase and include a reduction in mean daily gain and carcass quality and an increase in days on feed.

When prevention and treatment costs are included, the estimated cost to the US cattle industry is >$3 billion annually. Additionally, the impact on animal well-being is considerable because BRDC is clearly the most common cause of illness and death in beef cattle after weaning. Bovine respiratory disease complex is a multifactorial disease, the development of which is influenced by preweaning and postweaning factors. Some causative agents associated with BRDC are considered ubiquitous commensal organisms in cattle. The most commonly isolated organism from BRDC-affected lungs, Mannheimia haemolytica, is considered commensal in cattle. Successful treatment outcomes require early disease recognition, accurate prognostication, and application of appropriate therapeutics. Treatment failures increase economic losses through a decrease in performance and increase in mortality rate. Therefore, an accurate and timely diagnosis is important to allow informed economic and animal welfare decisions to be made.

Subjective measures such as attitude, appetite, and degree of activity are used to determine whether calves require additional examination or treatment for BRDC. These observations are performed on individual animals, yet commercial beef production systems involve management of cattle in populations or herds. Because herd animals tend to conceal clinical signs,

**Objective**—To determine the usefulness of physiologic, behavioral, and pathological changes as objective indicators of early respiratory disease in calves with Mannheimia haemolytica pneumonia.

**Animals**—14 crossbred beef steers.

**Procedures**—Disease was experimentally induced in healthy calves through endoscopic pulmonary inoculation of M haemolytica. Calves were necropsied on days 1, 2, 3, 5, 7, and 9 after inoculation. Physical examination variables (rectal temperature, heart rate, and respiration characteristics), clinical illness score, and degree of activity were assessed 3 times daily beginning 4 days prior to inoculation and continuing throughout the study. Twice before inoculation and on days 1, 2, 3, 5, 7, and 9, arterial blood gas measurements, serum biochemical analyses, and CBCs were performed. Pedometers and accelerometers were used to monitor cattle behavior and activity throughout the trial.

**Results**—All calves became clinically ill after inoculation and had gross and histopathologic signs of bronchopneumonia. No variable was a reliable indicator of disease progression as judged by percentage of pulmonary involvement. However, activity as measured by total steps taken in a 24-hour period was lower after versus before disease induction.

**Conclusions and Clinical Relevance**—This single-pathogen challenge model successfully yielded clinical signs and pathological effects consistent with naturally acquired respiratory disease. Routine laboratory variables and subjective measures were not reliable indicators of lung involvement or the progression of pneumonia. However, activity, objectively measured with pedometers and accelerometers, appeared to be a promising indicator for early recognition of bovine respiratory disease.
overt signs of illness are often lacking early in the disease process.\textsuperscript{11} Improvements in diagnosis could be made through identification of repeatable, accurate measures associated with early stages of BRDC. We hypothesized that changes in hematologic, behavioral, and physical examination findings would be helpful for diagnosis and monitoring of BRDC. The purpose of the study reported here was to evaluate changes in physiologic, pathologic, and behavioral variables in beef calves at multiple points following inoculation with \textit{M haemolytica}. 

**Materials and Methods**

Calves—Calves were selected from a population of 34 crossbred beef steers that were purchased by an order buyer through a local auction and that participated in a previous, as yet unreported, 14-day observational trial during which calves were observed for clinical signs of respiratory disease. The calves were observed for clinical illness for a total of 21 days prior to experimental \textit{M haemolytica} inoculation. All 34 calves in the population were tested for persistent infection with bovine viral diarrhea virus by use of antigen-capture ELISA.\textsuperscript{a} Additionally, a blood sample was collected from each calf, and serum was harvested for measurement of antibodies against \textit{M haemolytica}. Results of the \textit{M haemolytica} ELISA for the study calves ranged from an optical density of 0.137 to 0.571.

Nine of the initial 34 (26\%) calves were not eligible for the study for the following reasons: 3 participated in another study, 1 had a poor temperament, 1 was missing results of anti-\textit{M haemolytica} antibody testing, 3 had respiratory disease, and 1 had keratoconjunctivitis. Of the remaining 25 calves, the 11 with the highest \textit{M haemolytica} optical density readings were initially excluded from the study to reduce challenge-response variability. On day 0, prior to inoculation, 4 calves were removed from the study and pen for keratoconjunctivitis (n = 1 calf) or shoulder injury (3) and replaced by 4 randomly selected healthy calves from the 11 calves excluded because of the \textit{M haemolytica} assay results. Therefore, in total, 14 calves from the original pool of 34 calves with a mean weight of 199.2 kg were enrolled in the study. The study calves were housed in a 45.7 X 45.7-m drylot, fed commercial grain mix and grass hay, and allowed free access to water. The protocol for this study was approved by the Kansas State University Institutional Animal Care and Use Committee. This protocol included an approved method for euthanasia to be used if a calf became moribund at any point during the study.

Inoculation—\textit{Mannheimia haemolytica} serotype A1 (OSU strain) isolated from a bovine with naturally developing BRDC was used as the inoculum. The isolate was grown on brain heart infusion agar containing 5\% bovine blood in a CO\textsubscript{2} incubator for 18 to 22 hours. Colonies were then suspended in brain heart infusion broth and incubated at 37°C for 5.5 hours. Bacteria were pelleted by centrifugation, washed 3 times in sterile PBS solution, and resuspended in PBS solution. The final concentration of approximately 1 X 10\textsuperscript{9} organisms/mL was estimated on the basis of a standard curve of CFUs versus optical density. Plate counts were used to confirm bacterial concentration. Four days after trial initiation, each calf was individually restrained in a portable cattle chute without sedation. The external nasal passage was cleaned, and a fiberoptic endoscope (length, 100 cm; diameter, 6.6 mm) was passed through the nares into the trachea and then the tracheal bronchus, into the right cranial lung lobe. Forty milliliters of the final concentration of \textit{M haemolytica} inoculant in PBS solution was injected through a polyethylene tube (length, 160 cm; diameter, 1.95 mm) into the tracheal bifurcation, followed by a flush with 40 mL of PBS solution.

Pathological evaluation—A random number generator was used to randomly assign calves to be euthanized by means of captive bolt and exsanguination on day 1, 2, 3, 5, 7, or 9 after experimental inoculation (day 0). Necropsy and microbial culture and histologic evaluation of tissue from each set of lungs were completed immediately after euthanasia. Percentage of pulmonary involvement in the disease process was determined by palpation, visual inspection, and measurement of lesion cubic area by a board-certified pathologist (DM). The percentage of total lung consolidation was calculated according to the percentage of each lobe identified as pneumatic and the following formula: (0.53 X percentage consolidation of the apical aspect of the left cranial lobe) + (0.049 X percentage consolidation of the apical aspect of the left caudal lobe) + (0.319 X percentage consolidation of the diaphragmatic aspect of the left caudal lobe) + (0.043 X percentage consolidation of the accessory lobe) + (0.352 X percentage consolidation of the diaphragmatic aspect of the right caudal lobe) + (0.061 X percentage consolidation of the right middle lobe) + (0.60 X percentage consolidation of the apical aspect of the right caudal lobe) + (0.063 X percentage consolidation of the apical aspect of the right cranial lobe).\textsuperscript{12}

Data collection—Rectal temperature, heart rate, respiratory rate (measured by auscultation), respiratory character (unremarkable vs rapid), behavior, degree of activity, and CIS were assessed 3 times daily beginning 4 days prior to inoculation until study completion. Physical examinations and all other assessments were completed by the same experienced veterinarian at each measurement point. Degree of activity was recorded as unremarkable or decreased relative to the typical degree. Clinical illness scores were recorded while calves were being walked to the manual livestock restraining chute. Scores were assigned as follows: 1 = clinically normal; 2 = slightly ill; 3 = moderately ill; and 4 = severely ill.

Arterial blood gas and chemical measurements, serum biochemical analyses, and CBCs were performed daily, 4 days prior to disease induction, on inoculation day, and on days 1, 2, 3, 5, 7, and 9 after inoculation. Samples of arterial blood were collected from the medial branch of the caudal auricular artery into non-heparinized syringes and immediately transferred into 1.3-mL plastic lithium heparin tubes\textsuperscript{b} for analysis. The interval from blood collection to completed blood gas
and chemical analysis with a chute-side portable analyzer was estimated at < 3 minutes. Variables measured included pH, arterial partial pressure of carbon dioxide (Paco₂), arterial partial pressure of oxygen (Pao₂), base excess, bicarbonate concentration (HCO₃⁻), TCₐ, arterial oxygen saturation (SaO₂), and lactate concentration. Venous blood samples were collected from a jugular or coccygeal vein into 1.3-mL lithium heparin tubes. Serum was harvested and analyzed for concentrations of sodium, potassium, chloride, urea nitrogen, and glucose. Venous blood samples were also collected into 2-mL EDTA tubes for CBCs.

Animal behavior was continuously measured by use of accelerometers and pedometers. Four days before inoculation, each calf was fitted with an accelerometer and pedometer on the distal lateral aspect of the right metatarsus. These devices were housed in a plastic container and attached to the limb with 2 self-adhesive straps. Each call wore the devices until euthanized. The accelerometer measured acceleration (g) in the x, y, and z axes at the rate of 100 times/s. Commercial software was used to calculate the mean force (g) and maximum and mean vector magnitude over each 2-second period. Vector magnitude was calculated 100 times/s with the following equation:

\[ r = \sqrt{x^2 + y^2 + z^2} \]

The mean and maximum vector magnitudes were calculated on the basis of all vector magnitude calculations for each 2-second period. Accelerometers were removed each morning, and data were downloaded on days 0, 1, 2, 3, 5, 7, and 9; accelerometers were then replaced. Data were not recorded between the time of accelerometer removal and replacement (approx 1 minute/d).

Calves were videotaped in a group setting within the pen for approximately 0.5 hours each day beginning 4 days prior to inoculation and continuing through the 9-day trial period. The video recordings were analyzed and logged by an investigator (unaware of the accelerometer data) to record cattle behavior as 1 of 3 types: lying down, standing in place, or walking. Video data were used to generate an algorithm to classify accelerometer data in a manner reported elsewhere. The combined video and accelerometer data were partitioned into training (70%) and test (30%) sets. The training set was used to generate a behavior classification algorithm by use of a classification tree. The test set was run through the classification algorithm and compared with actual logged video values to evaluate the accuracy of the classification algorithm by comparing \( \kappa \)-statistic values for each categorical behavior variable. The classification algorithm was used to assign a predicted behavior for accelerometer readings at each measurement point for each calf.

Pedometers recorded the number of steps taken in a 24-hour period (midnight to midnight). These data were collected for each day, beginning 4 days before inoculation through to euthanasia.

Statistical analysis—All data were imported into a statistical software program for analysis. General linear mixed models were used to analyze continuous data on hematologic values, pedometer readings, rectal temperature, body weight, and heart and respiratory rates while accounting for repeated measures on individual calves to determine changes in variable values from before to after inoculation and day-to-day variation and associations with percentage of lung consolidation. Fixed effects evaluated included trial day in all models and examination time (morning, noon, or evening) for rectal temperature, heart rate, and respiratory rate data. All measurements obtained prior to pneumonia induction were grouped into a single period (day –1), and mean values for preinduction data were considered as baseline values for each calf. When significant differences by trial day were detected, comparisons among days were made by use of model-generated least squares mean values and corresponding SEMs. Mean ± SEM values are reported. A value of \( P \leq 0.05 \) was considered significant for all analyses.

Agreement between video-recorded behavior data and the algorithm used to classify accelerometer data was tested by calculating an overall \( \kappa \) value. The proportion of time standing and lying was modeled by use of logistic regression models with a random effect included to account for repeated measurements on calves and a fixed effect for trial day of the experiment. To assess potential changes in behavior patterns with time, the interaction between the percentages of time spent in each behavior across trial days was also tested. Results from the sample collection period closest to the necropsy were used in models as described to compare relationships between the percentage of time spent lying down and standing and the percentage of lesioned lung in each calf.

Results

Calves—All 34 calves in the original population had negative results for bovine viral diarrhea virus infection as evaluated with the antigen-capture ELISA. Two calves each were euthanized on trial days 1, 2, 3, and 5 after inoculation (day 0). On each of days 7 and 9, 3 calves were euthanized.

Pathological evaluation—All lung sets contained gross lesions consistent with a diagnosis of bronchopneumonia; the extent of total pulmonary involvement ranged from 1.2% to 23.4% (Table 1). Gross lesions were consistent with fibrinous bronchopneumonia with pleuritis on days 1 through 3. Histopathologic findings on those 3 days included multifocal atelectatic lobules containing large numbers of neutrophils within the alveolar spaces. Additionally, some lobules contained large amounts of serofibrinous fluid intermixed with alveolar neutrophils. Bronchi and bronchioles were cuffed by small- to moderate-sized sheaths of lymphocytes, and the lumens were always filled with neutrophils. Interlobular septae and perivascular tissues were commonly widened by serofibrinous fluid containing few neutrophils. Atelectasis with resolving pneumonia was evident in lungs harvested on days 5, 7, and 9. Histopathologically, lung tissue on days 3 through 9 contained large numbers of neutrophils with eosino-
philic, fibrillar fluid filling most alveoli. Some fibrin was dense and well formed. In other areas, there was extensive parenchymal destruction and necrosis characterized by loss of architecture. Bordering these areas, there were moderate numbers of neutrophils and a few aggregates of densely basophilic, spindle-shaped leukocytes. A few small airways contained intralumenal granulation tissue (bronchiolitis obliterans). Thirteen of the 14 calves had lesions on the right side of the lung. The overall mean percentage of lobe involvement for the target areas of the right cranial apical, right caudal apical, and right middle lobes was 40%, 55%, and 26%, respectively.

Statistical analysis revealed day of euthanasia was significantly ($P=0.03$) associated with the overall percentage of pulmonary lesions. The percentage of pulmonary involvement was significantly higher on day 2 than on days 3 and 7 but was not different from that on the other days (Figure 1). The percentage of pulmonary involvement on day 7 was significantly lower than on days 2 and 5. There was no apparent increase or decrease in the percentage of pulmonary involvement with time.

Mannheimia haemolytica was isolated from 10 of 14 lung sets. Of the 4 sets of lungs from calves from which M haemolytica was not recovered, Arcanobacterium pyogenes was isolated from 2 and Histophilus somnus from another. One lung set had no bacterial growth.

### Table 1—Percentage consolidation of lung lobes measured at necropsy in 14 calves euthanized at various points after intrabronchial inoculation (day 0) with Mannheimia haemolytica.

<table>
<thead>
<tr>
<th>Calf No.</th>
<th>Day of euthanasia</th>
<th>Rt crn apical</th>
<th>Rt cd apical</th>
<th>Rt mid</th>
<th>Rt diaph</th>
<th>Acc</th>
<th>Lft crn apical</th>
<th>Lft cd apical</th>
<th>Lft diaph</th>
<th>Total lung score*</th>
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<tbody>
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<td>1</td>
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<td>20</td>
<td>90</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
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<td>2</td>
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<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>14.0</td>
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<td>12</td>
<td>9</td>
<td>0</td>
<td>25</td>
<td>0</td>
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<td>9</td>
<td>30</td>
<td>90</td>
<td>100</td>
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<td>0</td>
<td>0</td>
<td>30</td>
<td>40</td>
<td>16.9</td>
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</table>

Mean value NA 40.0 51.8 26.1 2.1 21.4 5.7 12.9 2.9 11.8

*Total percentage lung consolidation = (0.53 \times \text{Lft crn apical}) + (0.049 \times \text{Lft cd apical}) + (0.319 \times \text{Lft diaph}) + (0.043 \times \text{Acc}) + (0.352 \times \text{Rt diaph}) + (0.061 \times \text{Rt mid}) + (0.60 \times \text{Rt cd apical}) + (0.083 \times \text{Rt crn apical}).

Acc = Percentage consolidation of the accessory lobe. Lft cd apical = Percentage consolidation of the apical aspect of the left caudal lobe. Lft crn apical = Percentage consolidation of the apical aspect of the left cranial lobe. Lft diaph = Percentage consolidation of the diaphragmatic aspect of the left caudal lobe. NA = Not applicable. Rt cd apical = Percentage consolidation of the apical aspect of the right caudal lobe. Rt crn apical = Percentage consolidation of the apical aspect of the right cranial lobe. Rt diaph = Percentage consolidation of the diaphragmatic aspect of the right cranial lobe. Rt mid = Percentage consolidation of the right middle lobe.

The percentage of consolidation of the left cranial lobe was significantly higher on day 2 than on days 3 and 7 but was not different from that on the other days (Figure 1). The percentage of pulmonary involvement on day 7 was significantly lower than on days 2 and 5. There was no apparent increase or decrease in the percentage of pulmonary involvement with time.

CISs—All calves had a CIS of 1 (clinically normal) during the 4 days prior to inoculation, but all were scored as ill after inoculation. Statistical analysis was not completed for CISs, respiratory character, or degree of activity because the observer was not blinded to the time relative to disease induction. All calves had a CIS...
of 3 at the first postinoculation observation. On day 1 after inoculation, 32 of 37 measurements yielded a CIS of 2 (slightly ill) and 6 yielded a CIS of 3 (moderately ill). For the rest of the study, 151 measurements yielded a CIS of 2 and 28 yielded a CIS of 3. No calves had a CIS of 1 between inoculation and euthanasia.

**Rectal temperature**—Least squares mean rectal temperatures for all preinoculation and postinoculation days (range, 39.8° to 40.9°C) were greater than the published upper reference limit (39.5°C). Only the inoculation-day rectal temperature was significantly (P < 0.05) different (higher) than values for all preinoculation and postinoculation days (Figure 2). Rectal temperature, averaged over all study days, increased throughout the day. There were significant (P < 0.05) differences among measurements collected in the morning (mean ± SEM, 39.6 ± 0.1°C), at noon (39.4 ± 0.1°C), and in the early evening (40.2 ± 0.1°C).

**Respiratory rate**—Mean respiratory rate on day 0 was significantly (P = 0.05) greater than that on day 1 but not higher than that any other day. Respiratory rates were greater than the upper published reference limit (60 breaths/min) on all trial days and significantly greater than baseline (day –1) values on days 5 through 8 (Figure 3). Respiratory rates were less (P < 0.01) in the morning (58.0 ± 2.4 breaths/min) relative to noon (75.3 ± 2.7 breaths/min) or the evening (76.7 ± 2.5 breaths/min).

**Heart rate**—Heart rates were greater than reference values (60 to 80 beats/min) on all trial days and were significantly greater on inoculation day relative to all other days in the trial. Heart rates appeared to decrease throughout the trial (Figure 4). Out of all daily measurement points, heart rates were greatest (P < 0.05) at noon (112.7 ± 2.8 beats/min). Evening heart rates (106.0 ± 2.7 beats/min) were significantly (P < 0.05) less than noon heart rates but greater (P < 0.05) than morning heart rates (101.1 ± 2.6 beats/min).

**Other clinical variables**—Respiratory character was unremarkable for all calves prior to inoculation and for 4 of 14 calves for the first reading after inoculation. From Table 2—Least squares mean values for results of CBCs performed in calves (initial n = 14)* before (day –1; a combination of several measurements for each calf) and at various points after intrabronchial inoculation (day 0) with M haemolytica. See Figures 1 and 2 for remainder of key.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference limits†</th>
<th>–1</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>9</th>
<th>P value‡</th>
</tr>
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<tbody>
<tr>
<td>Leukocytes (&lt;10^9 cells/µL)</td>
<td>7.00–14.00</td>
<td>9.58^a</td>
<td>12.57^b</td>
<td>9.82^c</td>
<td>8.41^d</td>
<td>8.67^e</td>
<td>10.19^f</td>
<td>11.24^g</td>
<td>&lt; 0.001</td>
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<tr>
<td>Segmented neutrophils (&lt;10^9 cells/µL)</td>
<td>1.00–5.00</td>
<td>4.20^a</td>
<td>7.89^b</td>
<td>4.86^c</td>
<td>3.96^d</td>
<td>3.73^e</td>
<td>4.89^f</td>
<td>6.96^g</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Band cells (&lt;10^9 cells/µL)</td>
<td>0–0.20</td>
<td>0.03</td>
<td>0.11</td>
<td>0.02</td>
<td>0.00</td>
<td>0.02</td>
<td>0.06</td>
<td>0.07</td>
<td>&lt; 0.01</td>
</tr>
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<td>Lymphocytes (&lt;10^9 cells/µL)</td>
<td>2.50–7.50</td>
<td>4.89</td>
<td>4.07</td>
<td>4.41</td>
<td>3.99</td>
<td>4.55</td>
<td>4.71</td>
<td>3.95</td>
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</tr>
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<td>Monocytes (&lt;10^9 cells/µL)</td>
<td>0.25–0.85</td>
<td>0.27</td>
<td>0.35</td>
<td>0.24</td>
<td>0.22</td>
<td>0.20</td>
<td>0.31</td>
<td>0.32</td>
<td>&lt; 0.05</td>
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<td>Eosinophils (&lt;10^9 cells/µL)</td>
<td>0–1.00</td>
<td>0.15</td>
<td>0.10</td>
<td>0.15</td>
<td>0.19</td>
<td>0.15</td>
<td>0.15</td>
<td>0.07</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td>Basophils (&lt;10^9 cells/µL)</td>
<td>0–0.20</td>
<td>0.08</td>
<td>0.04</td>
<td>0.02</td>
<td>0.06</td>
<td>0.03</td>
<td>0.04</td>
<td>0</td>
<td>&lt; 0.01</td>
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<tr>
<td>RBCs (&lt;10^12 cells/µL)</td>
<td>5.00–8.00</td>
<td>9.13^a</td>
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<td>8.33^c</td>
<td>8.04^d</td>
<td>7.71^e</td>
<td>7.16^f</td>
<td>7.53^g</td>
<td>&lt; 0.001</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
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<td>10.58^b</td>
<td>10.24^c</td>
<td>9.87^d</td>
<td>9.49^e</td>
<td>8.84^f</td>
<td>8.94^g</td>
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<td>Hct (calculated %)</td>
<td>26.00–42.00</td>
<td>31.60^a</td>
<td>29.99^b</td>
<td>28.95^c</td>
<td>28.19^d</td>
<td>27.07^e</td>
<td>25.66^f</td>
<td>26.66^g</td>
<td>&lt; 0.001</td>
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<td>Hct (spun %)</td>
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<td>33.41^a</td>
<td>32.15^b</td>
<td>30.22^c</td>
<td>29.52^d</td>
<td>28.12^e</td>
<td>27.23^f</td>
<td>28.98^g</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>32.00–51.00</td>
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<td>34.91</td>
<td>35.08</td>
<td>35.20</td>
<td>34.88</td>
<td>35.16</td>
<td>35.08</td>
<td>0.78</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>11.00–18.00</td>
<td>12.42</td>
<td>12.48</td>
<td>12.32</td>
<td>12.36</td>
<td>12.48</td>
<td>12.46</td>
<td>12.82</td>
<td>0.33</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.00–37.00</td>
<td>35.72^a, b</td>
<td>35.62^a, b</td>
<td>35.52^a, b</td>
<td>34.98^a, b</td>
<td>35.71^a, b</td>
<td>35.34^a, b</td>
<td>36.16^a</td>
<td>0.02</td>
</tr>
<tr>
<td>Plasma protein (g/dL)</td>
<td>7.00–9.00</td>
<td>7.29^a</td>
<td>7.08^b</td>
<td>7.06^c</td>
<td>7.34^d</td>
<td>7.22^e</td>
<td>7.54^f</td>
<td>7.97^g</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/dL)</td>
<td>0.30–0.70</td>
<td>0.63^a</td>
<td>0.77^b, c</td>
<td>0.67^b</td>
<td>0.87</td>
<td>0.68^b, c</td>
<td>0.68^b, c</td>
<td>0.87^b, c</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*Calves were randomly assigned to be euthanized on trial day 1, 2, 3, 5, 7, or 9. †As indicated by the Kansas State University Clinical Pathology Laboratory. ‡Indicates result of statistical comparison of variable values among trial days.

MCH = Mean corpuscular hemoglobin. MCHC = Mean corpuscular hemoglobin concentration. MCV = Mean corpuscular volume.

*Within a row, values with different superscript letters are significantly (P < 0.05) different.
day 1 through trial completion, findings of 17 of 179 respiratory evaluations were classified as unremarkable. All other evaluations revealed rapid respiration. Body weights were only recorded daily, and trial day was not significantly associated with changes in calf body weight.

Hematologic analysis—Results of CBCs were evaluated independently to determine whether certain variables changed according to trial day (Table 2). The effect of trial day was significant (P < 0.05) for total numbers of leukocytes, segmented neutrophils, and erythrocytes as well as for hemoglobin, Hct (spun and calculated), mean corpuscular hemoglobin concentration, and plasma protein concentration. Plasma fibrinogen concentration appeared to increase with trial day, but the association was not significant (P > 0.10). There was no overall effect of trial day on numbers of hand cells, lymphocytes, monocytes, eosinophils, and basophils or on mean corpuscular volume or mean corpuscular hemoglobin (P > 0.10 for all). Erythrocytes, hemoglobin, and Hct values appeared to decrease over the trial period, with the largest values detected prior to initiation of pneumonia and lower values detected later on (P < 0.01 for all). Plasma protein concentration decreased on days 1 and 2, compared with pretrial values, and concentrations on days 7 and 9 were greater than the preinoculation concentrations (P < 0.01). Numbers of segmented neutrophils and total leukocytes had a different pattern, with the greatest values evident on day 1 (P < 0.01 for both). Preinoculation plasma fibrinogen concentrations were significantly (P < 0.05) less than concentrations on days 1, 3, and 9.

Serum biochemical analysis—No effect of trial day was evident for serum concentrations of sodium.
Table 5—Percentage of time spent in various activities as categorized by accelerometer in calves (initial n = 14)* before (day –1; a combination of several measurements for each calf) and at various points after intrabronchial inoculation (day 0) with M haemolytica.

<table>
<thead>
<tr>
<th>Activity</th>
<th>–1</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>P value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying</td>
<td>41.1a</td>
<td>42.3a</td>
<td>41.8b</td>
<td>45.9a</td>
<td>52.9a</td>
<td>55.9a</td>
<td>50.9b</td>
<td>40.4a</td>
<td>40.4a</td>
<td>40.0b</td>
<td>0.08</td>
</tr>
<tr>
<td>Standing</td>
<td>54.2a</td>
<td>52.9a</td>
<td>53.9a</td>
<td>50.2a</td>
<td>43.1a</td>
<td>39.4a</td>
<td>45.1a</td>
<td>46.3a</td>
<td>55.9a</td>
<td>47.2ab</td>
<td>0.02</td>
</tr>
<tr>
<td>Walking</td>
<td>4.7</td>
<td>3.8</td>
<td>4.2</td>
<td>3.8</td>
<td>4.7</td>
<td>4.6</td>
<td>4.0</td>
<td>4.6</td>
<td>3.8</td>
<td>3.1</td>
<td>0.86</td>
</tr>
</tbody>
</table>

*Indicates result of statistical comparison of variable values among trial days. See Table 2 for remainder of key.

Figure 6—Least squares mean ± SEM percentage of daily time spent lying down (solid line) or standing (dashed line) measured by use of accelerometer in calves (initial n = 14) before (day –1; a combination of several measurements for each calf) and at various points after intrabronchial inoculation (day 0) with M haemolytica. Unlike the model used for the data in Figure 5, the model used for these data was an interactive logistic regression model that adjusted for repeated measures on individual calves as well as the time spent lying and standing each trial day. See Figures 2 and 5 for remainder of key.

and glucose or for the anion gap; however, trial day was associated with all other serum biochemical variables. All serum analytes except pH, Hct, and hemoglobin concentration remained within reference limits from 4 days prior to inoculation through to euthanasia. The mean serum pH value on day 5 (7.61) was higher than the upper reference limit (7.53) and was greater than that on days 1, 2, 3, and 9 (Table 3). Mean serum hemoglobin concentration was lower than the lower reference limit (8 mg/dL) on days 2 through 7, but only on days 3 and 7 were values significantly (P < 0.05) less than preinoculation values. Mean serum TCO₂ values were significantly (P < 0.05) less prior to inoculation than on days 1, 2, and 5. Mean serum potassium and chloride concentrations were also significantly (P < 0.05) less on day 1 than before inoculation. On day 5, the mean base excess value was significantly (P < 0.01) greater than on all other postinoculation days or before inoculation.

Blood gas analysis—Most measured blood gas variables were significantly (P < 0.05) associated with trial day (Table 4). Arterial oxygen saturation appeared to increase with trial day, but the increase was not significant (P = 0.08). Blood lactate concentration was greater before inoculation than on all postinoculation days except for day 1. Arterial pH, HCO₃⁻, TCO₂, and base excess were significantly (P < 0.05) less prior to inoculation than on all days after inoculation. Mean preinoculation PaO₂ was significantly (P < 0.01) less than that on days 5, 7, and 9. Mean preinoculation PaCO₂ was significantly (P < 0.05) less than that on days 1, 5, and 9.

Activity—Model-adjusted mean step counts were significantly (P < 0.01) lower for all days after inoculation, compared with values before inoculation (Figure 5). Before inoculation, the mean step count was 10,986 ± 693, and on day 0 it was 6,280 ± 693. At no time after inoculation did the least squares mean total step count within a 24-hour period exceed 7,137.

Videotapes of behavior of the 14 study calves were obtained for the 4 days prior to inoculation and for each calf from inoculation until the day of scheduled euthanasia (day –3 was not recorded), with a mean recording time of 2.2 min/d/calf. The overall agreement between the classification algorithm and the video-recorded data was high (κ = 0.91; 95% CI, 0.89 to 0.92). Therefore, the classification tree was used to generate behavior estimates for each datum point from the accelerometer data. Data from the first day an accelerometer was placed and the euthanasia date for each calf were eliminated from the analysis because these were not complete 24-hour periods. The percentage of time calves spent standing significantly (P < 0.05) decreased with increasing time after inoculation, and the percentage for lying down appeared to increase over the same period, although the increase was not significant (Table 5). Mean time spent walking was not associated with trial day (P = 0.86). A significant (P < 0.05) interaction was identified in the amount of time cattle spent either standing or lying as the trial progressed; calves spent more time standing relative to lying down from before inoculation to day 2, when the standing and lying down pattern inverted until day 7, when again calves spent more time standing (Figure 6). Prior to induction of pneumonia, cattle spent a greater percentage of time standing (33.0 ± 3.7%) than lying (42.3 ± 3.7%). After induction of pneumonia, there were few differences in the amount of time spent in each activity, and in fact, on day 4 after inoculation, cattle spent a greater percentage of time lying (56.0 ± 4.6%) than standing (39.6 ± 4.6%).

Discussion

The challenge model used in this study successfully resulted in clinical and pathological signs of respiratory disease consistent with those associated with BRDC due to M haemolytica. The distributions of pulmonary lesions in our study were similar to those of a bronchoscopy study in which M haemolytica lesions...
were restricted to 15% to 17% of the right lung lobes. Other researchers have reported difficulty reproducing *M haemolytica* pneumonia without preexposure to viral pathogens or stressors. Preweaning variables that can influence development of BRDC include colostrum intake, infection with bovine viral diarrhea virus, and preshipment management factors. Postweaning variables include transportation stress, castration and dehorning, sex of cattle, and nutritional management. In our study, transportation stress was not present, and we believe the acclimation period was of sufficient duration to decrease the likelihood of viral stress; however, other stressors such as heat stress and frequent restraint did occur. These stressors may have contributed to the success of our challenge model.

All calves had predominately right-side bronchopneumonia except one, which had lesions primarily on the left side. Presumably this was attributable to the calf coughing and potentially modifying the placement of the endoscope during introduction of *M haemolytica*. In another inoculation study, contrast bronchography revealed the dissemination of bacteria into other lung fields during coughing episodes. Gross necropsy and histologic evaluation of lung tissue revealed acute bronchopneumonia on the day after inoculation. However, by day 5 after inoculation, lung lesions appeared to be resolving grossly and histopathologically. This was unexpected given the extensive lung lesions observed within the first few days after inoculation. The finding may indicate that *M haemolytica* on its own is unable to sustain severe inflammatory responses for more than a few days. Values of other variables such as leukocyte and total neutrophil counts indicated a rapid but transient response to infection. In addition, in our study, calves had a high (> 39.7°C [103.5°F]) rectal temperature at inoculation, which may have been protective against bacterial growth. *Mannheimia haemolytica* was recovered from 10 of 14 lung sets, which is consistent with findings of a previous study in which *Pasteurella multocida* was recovered from 9 of 16 lungs after inoculation.

For all calves prior to inoculation, clinical illness scores, respiratory character, and degree of activity were as expected for healthy calves, but after inoculation, all measures were suggestive of disease. This validated the success of our challenge model and indicated that the subjective measures used may be helpful to recognize early pulmonary disease. Pyrexia and lethargy due to *M haemolytica* endotoxin and leukotoxin may precede measurable lung tissue damage. Clinical illness scores remained abnormal from inoculation to euthanasia, yet results of necropsy and histologic evaluation indicated a resolving bronchopneumonia starting on trial day 3. Because the observer was unblinded in our study, we were unable to evaluate the accuracy of CIS as a predictor of lung pathology severity. Our conclusion, which agrees with others, was that CISs do not provide an adequate assessment of disease progression as measured by percentage of pulmonary involvement.

In our study, we found that common clinical measurements (ie, rectal temperature, heart rate, and respiratory rate) may yield conflicting results and can vary by time of day. Rectal temperatures were higher than the upper reference limit throughout the trial. This finding was likely attributable to high environmental temperatures (> 32.2°C [90.0°F]) and frequent (3 times/d) restraint of the calves for physical examination during the trial period. The least squares mean rectal temperature was greater on inoculation day than on any other study day, and this transient increase may have been a direct result of the synergistic relationship between endotoxin and leukotoxin. Variation of rectal temperatures within any given day likely reflected daily changes in ambient temperature and agrees with findings that maximum heat dissipation occurs overnight. Mean rectal temperatures for the postinoculation morning readings were less than cutoff values (≥ 40°C [104°F]) used to determine sickness in some cattle production units. However, evening rectal temperature readings exceeded 40°C, suggesting that time of day should be taken into account when rectal temperature is used as a diagnostic tool.

Mean heart rates exceeded the published upper reference limit for all trial days. Interestingly, heart rate decreased and respiratory rate increased as the study progressed. This might have been a source of diagnostic conflict if respiratory rate and heart rate were used together as indicators of pulmonary disease. For heart rate but not rectal temperature or respiratory rate, the time of day at which a measurement was obtained was important, as the maximum heart rate was consistently obtained at noon.

Rectal temperature, respiratory rate, and heart rate values obtained immediately prior to euthanasia were not associated with percentage of pulmonary involvement. This finding agrees with that of a study in which rectal temperature, respiratory rate, and clinical score were associated with percentage of pulmonary involvement 7 days after inoculation. The difference between study results may be a reflection of different lung-scoring systems rather than a difference in study outcomes. The difference may also be explained by differences in inoculation methods, subject ages, breeds, and environmental conditions.

Complete blood counts revealed a transient increase in total numbers of leukocytes and a decrease in numbers of RBCs, although overall findings revealed few days when CBC values were not within reference limits. Absolute neutrophil numbers were high on days 1 and 9 after inoculation, which conflicts with reports that neutropenia develops with gram-negative bacterial pneumonia. We began our measurements early after bacterial insult, and our results may have indicated that neutrophilia develops initially before an influx of neutrophils into lung tissue is precipitated by leukotoxins and endotoxins in *M haemolytica* infections. This finding may be valuable in assessing progression of *M haemolytica* disease if neutrophilia exists early in the course of disease. Total leukocyte results in the present study were consistent with those of a similar study in which leukocyte counts increased the day after inoculation and remained high for 1 day. However, in another study, total leukocyte and absolute neutrophil counts were highest at the first measurement (72 hours) after tracheal inoculation in 2-week-old Holsteins. The conflicting evidence may indicate that multiple factors affect blood leukocyte numbers, and the usefulness of
leukocyte counts to consistently identify early BRDC is marginal.

Mean RBC counts in the present study were highest prior to inoculation and were higher than the laboratory-established upper reference limit before inoculation and on days 1, 2, and 3. Other researchers have reported a consistent decrease in RBC counts from inoculation through to the end of their study.\(^1\) In contrast, polycythemia has been detected in hypoxic calves with chronic pulmonary disease. As expected, mean serum fibrinogen concentrations were high and were significantly higher than baseline values on days 1, 3, and 9. However, fibrinogen values were not consistently higher than the upper reference limit after inoculation. Therefore, serum fibrinogen concentration was not a reliable predictor of BRDC in our trial. These collective findings suggested limited usefulness of CBCs as early, objective indicators of BRDC.

Although blood chemical values appeared to vary by trial day, values of most variables remained within reference limits throughout the trial and were therefore believed not to be useful for diagnosis of early BRDC. Mean blood glucose concentration for all postinoculation days was the only variable that was higher than the upper reference limit, but this variable was also high prior to disease induction. High blood glucose concentration is considered an indicator of stress.\(^30\) In our study, stress may have been induced from restraining and examining calves 3 times/d.

Hypoxemia can be a manifestation of severe respiratory disease when a decrease in both ventilation and tissue perfusion occur.\(^14,32\) Alveolar hypoxia as indicated by hypoxemia (low Sao\(_2\)) was not detected in our study after pneumonia induction. Although the cranioventral lung lobes had large areas of pathological change, the total lung involvement may not have been extensive enough to alter ventilation rates or reduce pulmonary gas exchange. On the other hand, arterial pH increased after induction. This finding indicated respiratory alkalosis associated with an increase in respiratory rate coupled with low percentage of pulmonary involvement. However, blood pH results remained within reference limits on all days except day 3, when alkalosis was detected. The alkalosis may have been attributable to increased sensitivity of arterial blood to pulmonary or metabolic changes, compared with the sensitivity of venous blood.\(^30\) An increase in respiratory rate may have contributed to increased blood pH, and although not evaluated in our study, septicemia caused by gram-negative bacteria can also contribute to an increase in blood pH.\(^15,32\) Low arterial blood pH values (respiratory acidosis) have been reported for calves with chronic respiratory disease.\(^33\) However, none of the calves in our study developed advanced stages of chronic pulmonary damage.

High blood lactate concentration has been considered a reliable prognostic indicator of death in cattle with acute bronchopneumonia.\(^34\) However, we did not find blood lactate concentration to be useful in identifying early BRDC, possibly because generalized body-tissue anaerobic metabolism did not have a chance to develop because of the low percentage of pulmonary involvement. Changes in blood gas and biochemical values are not unique to BRDC but can be affected by many factors and require extensive lung involvement before important changes do develop.\(^30,31\) Values of some variables (ie, arterial blood pH, HCO\(_3\), and Tco\(_2\)) changed consistently after pneumonia induction and may be useful for distinguishing disease states in a clinical setting.

Accelerometers were not useful in determining disease progression as measured by percentage of pulmonary involvement; however, these data provided evidence of a behavioral change after the disease inoculation (Figure 6). Prior to pneumonia induction, calves spent more time standing than lying, indicating the behavior pattern of healthy calves in the study environment. By day 4 after inoculation, calves spent more time lying than standing. These findings were consistent with the idea that one of the primary clinical signs of BRDC is depression, but the change in the relationship between time spent lying down and standing was not present until 2 days after induction of pneumonia. Other researchers suggest that animal health could be effectively monitored by use of accelerometers,\(^36\) and these devices have been used to evaluate changes in calf behavior following a specific event such as castration.\(^12\) In the present study, accelerometers objectively recorded behavior prior to disease induction and provided evidence of behavioral pattern changes in early cases of respiratory disease.

To the authors’ knowledge, the present study is the first in which the usefulness of pedometers as a potential indicator of respiratory disease in cattle has been demonstrated. In other studies,\(^35,37,39\) pedometers have been used to detect estrus and illness and to evaluate lameness in dairy cattle. We found that the total number of steps taken during a 24-hour period decreased after inoculation and remained lower than baseline values throughout the trial. Toxins from the M haemolytica inoculant may have induced lethargy and thus might explain the immediate activity decrease. Other plausible explanations for the decreased activity could include changes in weather, management, or the population within a pen. Calf management and feeding patterns were consistent prior to and after pneumonia induction; however, study calves were walked to and restrained in a livestock chute multiple times per day throughout the trial. This management could have changed activity patterns because of calf fatigue or acclimation to the system; yet, we would expect activity changes in such circumstances to be gradual rather than the abrupt, consistent change evident in pedometer findings (Figure 5). Additionally, the population within the pen was modified with the addition and removal of 4 calves prior to pneumonia induction. These calves had previously been housed together, yet a reordering of social hierarchy might have caused a transient impact on degree of activity. Although degree of activity decreased after disease induction, the number of steps on the day prior to harvest was not associated with percentage of pulmonary involvement at necropsy. Activity, as measured by total steps taken, may be an indicator of early respiratory disease, but it does not appear to be a reliable indicator of severity of disease as measured by percentage of pulmonary involvement.
In the study reported here, we used a challenge model to replicate clinical signs and pathological changes associated with early bronchopneumonia caused by *M. haemolytica* infection and investigated several variables as potential objective indicators of early BRDC. None of the measured variables predicted the percentage of pulmonary involvement in the study calves. Although physical and physiologic pattern changes from before inoculation through euthanasia were evident, often the numeric changes were too subtle to rely upon for consistent, accurate diagnosis in on-farm settings. Degree of total lung involvement for most calves in our study was low, and that might have minimized the numeric differences; however, our focus was early respiratory disease recognition when total lung involvement would be expected to be low. Although it is widely believed that calves with BRDC are less active, the present study is the first through which such a behavioral change was documented. The other disease-identification tools used in this study have been used for many years within the livestock industry, but results of our research indicated such tools are of limited value for early disease recognition. To reduce the effects of BRDC in beef calves, it is important to continue to evaluate tools for early recognition of disease.

### References

31. Corrigan ME, Drouillard JS, Spire MF, et al. Effects of me-


