Clinical disease and lung lesions in calves experimentally inoculated with *Histophilus somni* five days after metaphylactic administration of tildipirosin or tulathromycin

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OBJECTIVE
To compare clinical disease and lung lesions in calves experimentally inoculated with *Histophilus somni* 5 days after metaphylactic administration of tildipirosin or tulathromycin.

ANIMALS
Twenty-four 3-month-old Holstein and Holstein-crossbreed steers.

PROCEDURES
Calves were randomly allocated to 3 groups of 8 calves. On day 0, calves in group 1 received tildipirosin (4 mg/kg, SC), calves in group 2 received tulathromycin (2.5 mg/kg, SC), and calves in group 3 received isotonic saline (0.9% NaCl) solution (1 mL/45 kg, SC; control). On day 5, calves were inoculated with 10 mL of a solution containing *H somni* strain 7735 (1.6×10^9 CFUs/mL, intrabronchially; challenge). Calves were clinically evaluated on days 5 through 8 and euthanized on day 8. The lungs were grossly evaluated for evidence of pneumonia, and bronchial secretion samples underwent bacteriologic culture.

RESULTS
The mean clinical score for each group was significantly increased 12 hours after challenge, compared with that immediately before challenge, and was significantly lower for tildipirosin-treated calves on days 6, 7, and 8, compared with those for tulathromycin-treated and control calves. The mean percentage of lung consolidation for tildipirosin-treated calves was significantly lower than those for tulathromycin-treated and control calves. *Histophilus somni* was isolated from the bronchial secretions of some tulathromycin-treated and control calves but was not isolated from tildipirosin-treated calves.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that metaphylactic administration of tildipirosin to calves 5 days prior to *H somni* challenge prevented subsequent culture of the pathogen from bronchial secretions and was more effective in minimizing clinical disease and lung lesions than was metaphylactic administration of tulathromycin. (*Am J Vet Res* 2016;77:358–366)

Respiratory tract disease, particularly pneumonia, is the primary cause of morbidity and economic losses in beef cattle, and it is second only to diarrheal diseases as a cause of morbidity and economic losses in dairy calves.[1,2] Bovine respiratory disease is caused by numerous viruses, pathogenic bacteria, and stressful conditions that interact to overcome the respiratory tract defenses.[3,4] Even though viral pathogens and stress have major roles in the development of BRD, severe pneumonia is typically the result of infections caused by bacterial pathogens such as *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, *Mycoplasma bovis*, and, in chronic disease, *Trueperella pyogenes*.[5]

Although *M haemolytica* is the bacteria most commonly isolated from the lungs of cattle with severe pneumonia, *H somni* is widespread and possibly represents an emerging pathogen of cattle throughout the world.[6–12] Aside from pneumonia, *H somni* also causes thrombotic meningoencephalitis, myocarditis, synovitis, and infertility in cattle.[6,13] Commercial vaccines against *H somni* are available; however, their efficacy is limited, and some induce antibodies against IgE that can enhance respiratory tract hypersensitivity.[14–16]

Because of concerns regarding the efficacy and safety of *H somni* vaccines, cattle are often administered antimicrobials to control respiratory tract infections caused by *H somni* and other bacterial pathogens at feedlot entry (metaphylaxis) or to treat

ABBREVIATIONS

BRD	Bovine respiratory disease
BVDV	Bovine viral diarrhea virus
LHS	Lung histopathology score

BRD.[16–18] Results of a 10-year (2000–2009) study[19] of the antimicrobial susceptibility of bacterial isolates obtained from North American feedlot cattle indicate that the percentage of *H somni* isolates susceptible to tetracycline, tulathromycin, enrofloxacin, and florfenical declined during that period to < 50%, 81%, 86%, and 92%, respectively, whereas all *H somni* isolates remained susceptible to ceftiofur. Because the livestock industry demands efficacious long-acting antimicrobials and the susceptibility of bacterial pathogens to various antimicrobials is constantly changing, the development and marketing of new antimicrobials are important. In 2011, tildipirosin, a long-acting macrolide, became commercially available for the prevention and treatment of BRD in cattle.[20] Results of a study[21] conducted to determine the pharmacokinetics of tildipirosin in cattle indicate that it is rapidly distributed to the respiratory tract and is slowly eliminated. In an experimental study,[22] feedlot cattle that were metaphylactically treated with tildipirosin 10 days prior to inoculation with *M haemolytica* had fewer clinical signs of disease and less pulmonary damage than did similar cattle that were metaphylactically treated with tulathromycin, another macrolide. In a clinical study,[23] beef heifers considered at high risk for development of BRD that were treated with tildipirosin at feedlot arrival had a lower morbidity rate and greater average daily gain than did similar heifers treated with tulathromycin at feedlot arrival. The objective of the study reported here was to compare clinical disease and lung lesions in calves experimentally inoculated with *H somni* 5 days after metaphylactic administration of tildipirosin or tulathromycin.

Materials and Methods

Animals

Twenty-four Holstein and Holstein-crossbreed steers that were approximately 3 months old were purchased from a commercial dairy. Prior to being purchased, calves were vaccinated with a multivalent clostridial vaccine and administered a broad-spectrum anthelmintic. Calves were transported to the Oklahoma State University Bovine Research Park. At arrival to the research facility, each calf was individually weighed, identified with an ear tag (numbers 1 to 24), and randomly assigned to 1 of 6 pens (ie, 4 calves/pen) in a biosecurity-level-2 barn. An ear notch specimen was also obtained from each calf and fixed in formalin for evaluation of BVDV antigen by means of an immunohistochemical staining method as described.[24] All calves tested negative for BVDV antigen, which suggested that they were not persistently infected with BVDV.

The calves had ad libitum access to water and were fed a commercial pelleted complete calf ration[a] at a rate of 3% of body weight daily, which equated to 2.2 to 2.6 kg of pelleted ration per calf twice daily. The calves were acclimated to the facility for 8 days prior to initiation of the study. Calf care was overseen by the Oklahoma State University Animal Resources Unit, an Association for Assessment and Accreditation of Laboratory Animal Care–accredited facility. All study procedures were reviewed and approved by the Oklahoma State University Institutional Animal Care and Use Committee.

Experimental design

On day 0, each pen of 4 calves was randomly assigned by means of drawing numbers from a hat to receive 1 of 3 treatments; thus, each treatment group consisted of 8 calves (ie, 2 pens). Calves in group 1 received tildipirosin[b] (4 mg/kg, SC), calves in group 2 received tulathromycin[c] (2.5 mg/kg, SC), and calves in group 3 received saline (0.9% NaCl) solution (1 mL/45 kg, SC; control). The doses of tildipirosin and tulathromycin administered were in accordance with those recommended by the respective manufacturers. The volume of saline solution administered to the calves in group 3 approximated the volume of the assigned antimicrobial administered to the calves of groups 1 and 2. The personnel that administered the treatments and evaluated the calves on a twice daily basis remained unaware of (ie, were blinded to) which treatment was assigned to the calves of each pen for the duration of the observation period.

On day 5, all calves were experimentally inoculated (challenged) with 10 mL of PBS solution supplemented with 5% bovine fetal serum containing 1.6×10^9 CFUs of *H somni*/mL instilled via a flexible bronchoalveolar lavage tube (length, 3 m; external diameter, 11 mm; internal diameter, 3 mm) that was passed through the nasal passage and nasopharynx to the level of the tracheal bifurcation. Proper placement of the tube at the tracheal bifurcation was verified on the basis of qualitative observations that included an elicited cough, absence of evidence of esophageal or ruminal placement as determined by smell and lack of tension and failure to observe the tube within the esophagus during placement, the presence of resistance at the carina, and the passage of the tube to a predetermined mark that approximated the distance from the nares to the carina. Following experimental inoculation, the tube was flushed with 60 mL of saline solution and 120 mL of air before it was removed from the calf.

On day 8, all calves were weighed, sedated with xylazine[d] (0.25 mg/kg, IM), and transported in a trailer in groups of 4 to 6 calves from the research facility to the Oklahoma Animal Disease Diagnostic Laboratory, where they were euthanized by means of a captive bolt followed by exsanguination. Immediately after euthanasia, a necropsy was performed on each calf.

Histophilus somni strain used for experimental inoculation

Histophilus somni strain 7735[e] that was isolated from a calf naturally infected with pneumonia was used to challenge the study calves. The isolate was cultured in brain-heart infusion broth at 37°C for 12 hours, washed twice with sterile PBS solution, and

resuspended in PBS solution supplemented with 5% bovine fetal serum to achieve a bacterial density of approximately 1.6×10^9 CFUs/mL. The minimum inhibitory concentration was 2.0 µg/mL for both tildipirosin and tulathromycin, and the strain was considered highly susceptible to both antimicrobials.

Clinical evaluation

Calves were observed twice daily by study personnel. Each calf was individually weighed at arrival to the research facility, on day 5 immediately prior to *H somni* challenge, and on day 8 before euthanasia. Rectal temperature was recorded for each calf during initial processing at arrival to the research facility and once daily between 8 and 9 AM on days 5 through 8. Each calf was assigned a clinical score approximately 12 hours after *H somni* challenge, twice daily on days 6 and 7, and on the morning of day 8. This clinical score could range from 0 to 10 and represented the summation of the subjective scores assigned to each of 3 aspects of the calf's health (general behavior, appetite, and respiratory quality). General behavior was scored on a scale of 0 to 4 where 0 = normal, 1 = slight depression, 2 = moderate depression, 3 = severe depression, and 4 = severe prostration or recumbent. Appetite at the time of feeding was scored on a scale of 0 to 3 where 0 = normal (calf readily approached feed), 1 = slightly reduced, 2 = markedly reduced, and 3 = no appetite. Respiratory quality was scored on a scale of 0 to 3 where 0 = normal, 1 = slight dyspnea, 2 = moderate dyspnea, and 3 = severe dyspnea. All the scores were assigned by 1 investigator (TAS) who was blinded to the treatment group assignment of each calf.

Measurement of serum *H somni*–specific IgG concentration

A blood sample (10 mL) was obtained by jugular venipuncture from each of 10 randomly selected calves (3 each from groups 1 and 2 and 4 from group 3) on day 0 and from those same calves on day 8 for determination of serum *H somni*–specific IgG concentration by the use of an ELISA in a manner similar to that described for determination of *M haemolytica*–specific IgG concentration.[25,26] Briefly, *H somni* strain 7735 was cultured in brain-heart infusion broth plus 10% bovine fetal serum at 37°C overnight (approx 12 hours) and fixed in formalinized saline solution. The formalin-killed bacteria were then suspended in a coating buffer solution to achieve bacterial density of approximately 5.0×10^7 CFUs/mL, and 100 µL of the resulting suspension was added to each well of a microtiter plate. The plates were incubated overnight at 37°C and then washed 3 times with a PBS-0.5% Tween20 solution. Plates were blocked by adding 100 µL of 3.0% alkali-soluble casein and incubated at 37°C for 60 minutes. Then, 100 µL of serum (diluted 1:400 to be within the linear range of the titration curve) was added to each well, and the plates were incubated at 37°C for 60 minutes and then washed 6 times with a PBS-0.5% Tween20 solution. To each well, 100 µL of a 1:1,000 dilution of horseradish peroxidase–conjugated goat anti-bovine IgG[f] was added as the secondary antibody, and the plates were incubated at 37°C for 60 minutes and then washed 6 times with a PBS-0.5% Tween20 solution. Subsequently, 100 µL of a substrate solution that contained *o*-phenylenediamine[g] was added, and the plates were incubated at room temperature (approx 22°C) in a dark environment for 5 minutes. Results were recorded from measurements obtained by an automated plate reader at a wavelength of 490 nm. Convalescent and naïve calf sera served as positive and negative controls, respectively. Each serum sample was assayed in triplicate, and the mean result was calculated and used for analysis. All assays were performed on the same day to minimize plate-to-plate variation, which was < 1% for control samples. Results were reported as the nanograms of IgG bound in each sample, compared with that for a set of IgG standards that were run simultaneously with the samples.

Necropsy

During necropsy, the pluck (tongue, trachea, esophagus, heart, and lungs) was removed from each calf, and the heart, bronchial lymph nodes, abdominal viscera, and stifle and carpal joints were examined for gross lesions by 1 investigator (NJS). The right and left lung lobes were removed from the pluck by severing the main bronchi and pulmonary arteries and evaluated by an investigator (AWC) who was blinded to the treatment group assignment of each calf. Sterile swabs were used to collect bronchial mucus samples from deep within the left and right main bronchi for bacteriologic culture. The percentage of consolidated tissue within each lung lobe was estimated. Diagrams of the consolidated areas were made, and the lungs were photographed for morphometric analysis of consolidation. The lung lobes were collectively weighed, and the lung weight as a percentage of body weight at the time of *H somni* challenge was calculated.

Histologic evaluation

Tissue specimens from right and left lungs were collected, fixed in formalin, and processed for histologic examination in a routine manner. If gross lesions were not observed in the lungs, a tissue specimen from the middle lung lobe was obtained for examination. Each specimen was assigned a LHS by 1 investigator (AWC) who was blinded to the treatment group assignment of each calf, without the benefit of the accompanying report of gross findings. This LHS was scored on scale of 0 to 4 where 0 = no lesions (normal lung); 1 = minimal pathological changes such as multifocal small numbers of neutrophils within alveoli or bronchioles, mild edema manifested as fine proteinaceous to fibrinous intra-alveolar exudate, and dilatation of lymphatics or loosening of peribronchial connective tissue; 2 = mild pathological changes that were similar to the minimal pathological changes except more widespread and intense; 3 = moderate path-

ological changes such as large multifocal to coalescing inflammatory cell infiltrates, coagulated intra-alveolar fibrin, vascular thrombosis, and fibrinous pleuritis; and 4 = severe pathological changes such as diffuse areas of hemorrhage, inflammation, and pleuritis with vasculitis and thrombosis. A necrosis score was also assigned to each specimen and was scored on a scale of 0 to 4 where 0 = no necrosis, 1 = minimal (single or a few small random foci of necrosis), 2 = mild (multiple small random foci of necrosis), 3 = moderate (multiple moderately sized foci of necrosis), and 4 = severe (multiple large foci of necrosis that often involved entire lobules). The total histologic score for each calf was the summation of the LHSs for the left and right lung lobes and the mean necrosis score and thus could range from 0 to 12.

Statistical analysis

Rectal temperatures, clinical scores, serum *H somni*–specific IgG concentrations, percentage of consolidated lung tissue, lung weight as a percentage of body weight, and histologic scores were analyzed by either a nonparametric or general linear model approach. A Kruskal-Wallis ANOVA was performed to compare variables among treatment groups, and when post-hoc comparisons were necessary, a Mann-Whitney *U* test with a Bonferroni correction was used to account for multiple comparisons. For each calf, the change in body weight was calculated between arrival at the research facility and day 5 (*H somni* challenge), between days 5 and 8 (euthanasia), and between arrival at the research facility and day 8. The data distributions of the weight change for each interval were assessed for normality by use of the Kolmogorov-Smirnov test, and the data were further assessed for skewness and kurtosis. In a few instances, the data were not normally distributed, and because the sample size of each group was small (n = 8), weight change between intervals was compared among treatment groups by use of a nonparametric Kruskal-Wallis test. Clinical scores at various times after *H somni* challenge were compared with those immediately after *H somni* challenge by use of a paired *t* test. Clinical scores and rectal temperature were compared among treatment groups by means of separate mixed general linear models. The respective models included treatment group (1, 2, or 3) and data acquisition time (time) as fixed effects and calf identification as a random effect to account for repeated measures within each calf. When the interaction between treatment group and time was significant, an analysis of simple effects was performed to compare least square means among groups at each time. The number of calves from which *H somni* was cultured from bronchial swab specimens was compared among treatment groups by means of a χ^2 analyses. All analyses were performed with a commercially available statistical software program,[h] and values of $P < 0.05$ were considered significant.

Results

Clinical evaluation

Mean body weight did not differ among the treatment groups at arrival to the research facility, day 5 (immediately before *H somni* challenge), or day 8 (before euthanasia; **Table 1**). When the mean body weights for each group were graphed over the duration of the observation period, the lines for all 3 treatment groups had a similar slope (which suggested that the calves were gaining weight at a similar rate) until *H somni* challenge, after which the mean weight for the calves in the control group (group 3) trailed off substantially, compared with that for the calves in groups 1 and 2 (data not shown). The lines for the mean weights of the calves in groups 1 and 2 maintained a similar slope for the duration of the observation period.

None of the calves developed adverse systemic affects following administration of the assigned treatment on day 0. One calf in group 2 (tulathromycin treatment) developed a localized soft swelling (2.0 X 2.5 X 0.5 cm) at the injection site 24 hours after treatment, but that swelling resolved by 48 hours after injection.

At the time of *H somni* challenge on day 5, 1 calf in group 2 was assigned a respiratory quality

Table 1—Mean ± SD body weight, lung weight, lung weight as a percentage of body weight, and percentage of consolidated lung tissue for 3-month-old Holstein and Holstein-cross steers that were metaphylactically administered tildipirosin (4 mg/kg, SC; group 1; n = 8) or tulathromycin (2.5 mg/kg, SC; group 2; 8) or administered saline (0.9% NaCl) solution (1 mL/kg, SC; group 3 [control]; 8) 5 days before experimental inoculation with 10 mL of a solution containing *Histophilus somni* strain 7735 (1.6×10^9 CFUs/mL, intrabronchially).

	Group		
Variable	**1**	**2**	**3**
Body weight at arrival to research facility (kg)	78.9 ± 5.9	76.7 ± 9.3	74.3 ± 7.0
Body weight at *H somni* inoculation (kg)	81.9 ± 10.3	78.8 ± 8.6	78.7 ± 5.3
Body weight at euthanasia (kg)	79.0 ± 5.9	76.7 ± 9.4	74.2 ± 7.0
Lung weight (g)	1,065.8 ± 171.4	1,253.9 ± 274.1	1,270.5 ± 88.1
Lung weight as percentage of body weight at *H somni* inoculation (%)	1.31 ± 0.2[a]	1.61 ± 0.4[a,b]	1.61 ± 0.1[b]
Lung consolidation (%)	6.0 ± 2.3[a]	30.9 ± 21.5[b]	26.3 ± 17.6[b]

Calves were administered the assigned treatment on day 0, experimentally inoculated with *H somni* on day 5, and euthanized by captive bolt followed by exsanguination on day 8.

[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

Table 2—Mean ± SD clinical scores for the calves of Table 1 at various times after experimental inoculation with *H somni*.

Time after *H somni* inoculation (h)	Group 1	2	3
0	0.0 ± 0.0	0.13 ± 0.35	0.0 ± 0.0
12	4.4 ± 2.7†	4.6 ± 2.6†	6.3 ± 2.3†
24*	0.5 ± 1.0[a]	2.4 ± 0.5[b]	3.5 ± 0.9[c]
48*	0.3 ± 0.5[a]	1.7 ± 1.2[b]	3.4 ± 1.2[c]
72	0.3 ± 0.5[a]	1.9 ± 1.5[b]	2.8 ± 1.2[b]

At each assessment, the clinical score for each calf could range from 1 to 10 and represented the summation of the subjective scores assigned to each of 3 aspects of the calf's health (general behavior, appetite, and respiratory quality). General behavior was scored on a scale of 0 to 4 where 0 = normal, 1 = slight depression, 2 = moderate depression, 3 = severe depression, and 4 = severe prostration or recumbent. Appetite at the time of feeding was scored on a scale of 0 to 3 where 0 = normal (calf readily approached feed), 1 = slightly reduced, 2 = markedly reduced, and 3 = no appetite. Respiratory quality was scored on a scale of 0 to 3 where 0 = normal, 1 = slight dyspnea, 2 = moderate dyspnea, and 3 = severe dyspnea.

*Scores represent the mean for clinical scores assigned during the morning and evening assessments. †Value differs significantly ($P < 0.001$) from the corresponding value at 0 hours after *H somni* inoculation as determined by a paired *t* test.

See Table 1 for remainder of key.

score of 1 (slight dyspnea); however, its rectal temperature and appetite were clinically normal. None of the calves were assigned a general behavior score of 4 (severe prostration or recumbent) after challenge. Results of the mixed general linear model indicated that the interaction between treatment group and time was significantly associated with clinical score. On days 6 and 7, the median clinical score for calves in group 1 (tildipirosin treatment) was significantly ($P < 0.001$ for all comparisons) lower than that for the calves in group 2 and the control group, and the median clinical score for calves in group 2 was significantly (day 6, $P < 0.001$; day 7, $P = 0.002$) lower than that for the calves in the control group **(Table 2)**. On day 8, the median clinical score for calves in group 1 was again significantly lower than that for the calves in group 2 ($P = 0.008$) and the control group ($P < 0.001$); however, the median clinical scores for the calves in group 2 and the control group did not differ significantly ($P = 0.13$).

The mean ± SD rectal temperatures for each group after *H somni* challenge were summarized **(Figure 1)**. Within each group, the mean rectal temperature increased significantly between 0 and 24 hours after *H somni* challenge. At 48 hours after challenge (day 7), the mean rectal temperature for the calves in group 1 (38.4°C) was significantly lower than that for the calves in group 2 (39.1°C; $P = 0.025$) and the control group (39.4°C; $P = 0.003$); however, the mean rectal temperatures for the calves in group 2 and the control group did not differ significantly ($P = 0.39$).

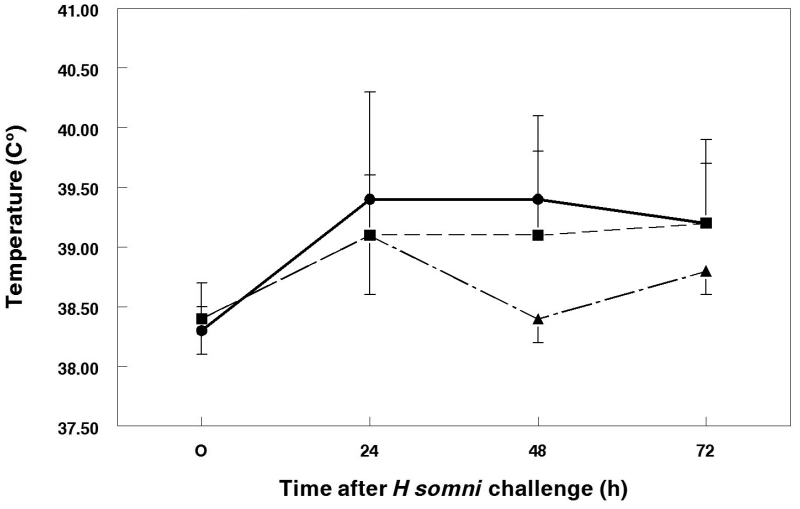

Figure 1—Mean ± SD rectal temperatures for 3-month-old Holstein and Holstein-cross steers that were metaphylactically administered tildipirosin (4 mg/kg, SC; group 1; n = 8; dashed and dotted line) or tulathromycin (2.5 mg/kg, SC; group 2; 8; dashed line) or administered saline (0.9% NaCl) solution (1 mL/kg, SC; group 3 [control]; 8; solid line) 5 days before experimental inoculation with 10 mL of a solution containing *Histophilus somni* strain 7735 (1.6×10^9 CFUs/mL, intrabronchially).

Serum *H somni*–specific IgG concentration

On day 0 (ie, day that the assigned treatment was administered), the serum *H somni*–specific IgG concentration was low in all 10 calves that were randomly evaluated and did not vary among treatment groups **(Table 3)**. The mean *H somni*–specific IgG concentration on day 8 was significantly ($P = 0.02$) greater than that on day 0 when results for all 10 calves were evaluated collectively; however, it did not differ significantly between days 0 and 8 for calves within a specific treatment group or among treatment groups.

Bacteriologic culture

Histophilus somni was isolated from the main

Table 3—Mean ± SD serum *H somni*–specific IgG concentration (ng/mL) for 10 randomly selected calves (3 each from groups 1 and 2 and 4 from group 3) from Table 1 on days 0 and 8.

	Group			
Day	1	2	3	All sera
0	0.02 ± 0.01	0.03 ± 0.02	0.08 ± 0.03	0.04 ± 0.01
8	0.14 ± 0.07	0.06 ± 0.03	0.24 ± 0.06	0.18 ± 0.06*

Serum *H somni*–specific IgG concentration was determined by means of a single-dilution ELISA in which formalin-killed *H somni* strain 7734 was used as the primary antigen. Serum was obtained from the same calves on both days. All serum samples were assayed in triplicate.

*Value differs significantly ($P < 0.05$) from the corresponding value on day 0.

See Table 1 for remainder of key.

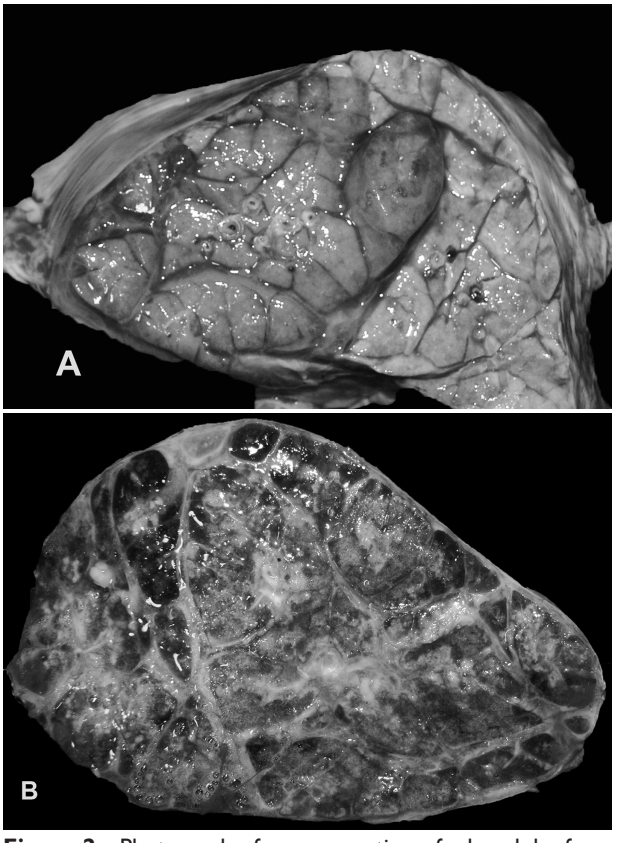

Figure 2—Photograph of a cross section of a lung lobe from a representative calf assigned to group 1 (A) and group 3 (control; B) of Figure 1. A—Bronchopneumonia is localized in the lung tissue to the left side of the photograph and is adjacent to lung tissue that appears grossly normal. B—The entire lung lobe is affected by severe diffuse necrosis and fibrinous pneumonia. ***See*** Figure 1 for remainder of key.

bronchi from 5 calves in the control group, 2 calves in group 2, and 0 calves in group 1. The number of *H somni* culture–positive calves in the control group was significantly ($P < 0.01$) greater than that in group 1 but did not differ significantly between the control group and group 2. The number of *H somni* culture–positive calves in group 2 did not differ significantly from that in group 1.

Lung lesions

The mean lung weight did not differ significantly ($P = 0.086$) among the 3 treatment groups (Table 1). However, the mean lung weight as a percentage of body weight at the time of *H somni* challenge for the calves of group 1 was significantly ($P = 0.022$) lower than that for the calves in the control group but did not differ significantly ($P = 0.29$) from that for the calves in group 2. The mean lung weight as a percentage of body weight at the time of *H somni* challenge did not differ significantly ($P = 0.92$) between the calves in group 2 and those in the control group.

The median percentage of lung consolidation for the calves in group 1 (5.25%) was significantly lower than that for calves in group 2 (25.0%; $P = 0.004$) and the control group (22.5%; $P = 0.014$; Table 1). The gross characteristics of the lung lesions also varied among the calves of the 3 treatment groups **(Figure 2)**. Many of the calves in the control group and group 2 had severe necrotizing fibrinous pleuropneumonia that was diffusely spread throughout the lung lobes, whereas the lung lesions in most of the calves of group 1 and some of the calves in group 2 lacked evidence of necrosis, were less severe, and were typically characterized as focal areas of acute bronchopneumonia that were surrounded by grossly normal lung tissue.

Histologic lung lesions ranged from mild suppurative bronchopneumonia to severe fibrinohemorrhagic pneumonia. The mean total histologic score for the calves of group 1 was significantly ($P = 0.003$) lower than that for the calves of the control group but did not differ significantly ($P = 0.10$) from that for the calves of group 2 **(Table 4)**. The mean total histologic score for the calves of group 2 did not differ significantly from that for the calves of the control group ($P = 0.79$). The median LHS for the right lung for the calves of group 1 was significantly ($P = 0.002$) lower than that for the calves of the control group but did not differ significantly ($P = 0.17$) from that of the calves of group 2. The median LHSs for the right lung did not differ significantly ($P = 0.45$) between the calves of group 2 and the control group. The median LHSs for the left lung did not differ significantly ($P = 0.79$) among the treatment groups. The median necrosis score for the calves of group 1 was significantly ($P = 0.023$) lower than that for the calves of the control group but did not differ significantly ($P = 0.25$) from that for the calves of group 2. The median necrosis scores for the calves of group 2 and the control group did not differ significantly ($P = 1.00$).

Table 4—Mean ± SD LHS for the right and left lung lobes, necrosis score, and total histologic score for the calves of Table 1.

Variable	Group 1	2	3
LHS			
Right lung lobe	2.9 ± 0.6[a]	3.5 ± 0.8[a,b]	4.0 ± 0.0[b]
Left lung lobe	0.5 ± 0.8	1.4 ± 1.2	1.9 ± 1.5
Necrosis score	0.3 ± 0.7[a]	1.4 ± 1.2[a,b]	2.1 ± 1.6[b]
Total histologic score	3.6 ± 1.5[a]	6.3 ± 2.6[a,b]	8.0 ± 2.2[b]

The LHS was scored on scale of 0 to 4 where 0 = no lesions (normal lung); 1 = minimal pathological changes such as multifocal small numbers of neutrophils within alveoli or bronchioles, mild edema manifested as fine proteinaceous to fibrinous intra-alveolar exudate, and dilatation of lymphatics or loosening of peribronchial connective tissue; 2 = mild pathological changes that were similar to the minimal pathological changes except more widespread and intense; 3 = moderate pathological changes such as large multifocal to coalescing inflammatory cell infiltrates, coagulated intra-alveolar fibrin, vascular thrombosis, and fibrinous pleuritis; and 4 = severe pathological changes such as diffuse areas of hemorrhage, inflammation, and pleuritis with vasculitis and thrombosis. Necrosis was scored on a scale of 0 to 4 where 0 = no necrosis, 1 = minimal (single or a few small random foci of necrosis), 2 = mild (multiple small random foci of necrosis), 3 = moderate (multiple moderately sized foci of necrosis), and 4 = severe (multiple large foci of necrosis that often involved entire lobules). The total histologic score for each calf was the summation of the LHSs for the left and right lung lobes and the mean necrosis score and thus could range from 0 to 12.

See Table 1 for remainder of key.

Discussion

Metaphylactic administration of antimicrobials to cattle, especially light-weight or otherwise stressed cattle (ie, high-risk cattle), at feedlot arrival is a common practice implemented by beef producers to decrease morbidity associated with BRD and thereby improve cattle performance.[27–29] Results of a study[30] that involved dairy calves between 2 and 16 weeks old indicate that metaphylactic administration of an immune modulator was associated with a decrease in the number of days those calves were subsequently treated for enzootic pneumonia. Long-acting antimicrobials such as tildipirosin and tulathromycin are desirable for the prevention (metaphylaxis) of BRD because they are readily distributed to the respiratory tract and are efficacious against many of the bacterial pathogens associated with BRD. In a study[31] conducted on a large commercial feedlot, the performance of steers with a mean body weight > 300 kg at feedlot entry that were administered tulathromycin or tilmicosin (another macrolide) for metaphylaxis was significantly better than that of similar steers that were not administered an antimicrobial for metaphylaxis; however, lung lesions were evenly distributed among the steers that did and did not receive metaphylaxis. Because of the widespread use of antimicrobials for metaphylaxis in beef cattle, we chose to compare the efficacy of tildipirosin with that of tulathromycin for the prevention of clinical BRD and lung lesions in calves experimentally inoculated with *H somni.*

Results of the present study suggested that metaphylactic administration of tildipirosin to 3-month-old calves 5 days prior to *H somni* challenge was generally superior to tulathromycin for minimizing clinical disease and pathological lung lesions. In this study, all calves had pyrexia 24 hours after *H somni* challenge, which was expected because of the large bolus of bacteria administered intrabronchially. However, the rectal temperatures of calves treated with tildipirosin (group 1) returned to within reference limits by 48 hours after challenge, whereas those of the calves treated with tulathromycin (group 2) and the control calves (group 3) remained abnormally increased. The mean clinical score for the tildipirosin-treated calves remained significantly lower than that for the tulathromycin-treated calves for the duration of the observation period following inoculation. During necropsy examination, the median percentage of lung consolidation for the tildipirosin-treated calves was significantly lower than that for the tulathromycin-treated calves and the control calves, and *H somni* was not cultured from any of the calves that were treated with tildipirosin. The minimum inhibitory concentration (as determined by in vitro methods by an independent diagnostic laboratory[i]) for both tildipirosin and tulathromycin was 2.0 µg/mL for the *H somni* strain 7735 that was used for the experimental inoculation; therefore, differences in the clinical efficacy between tildipirosin and tulathromycin could not be explained by differences in the susceptibility of the challenge bacterium to the 2 antimicrobials. The mean serum *H somni*–specific IgG concentration on day 0 (day that the assigned treatment was administered) did not vary among the 3 treatment groups, which suggested that pre-existing antibodies against *H somni* did not affect the susceptibility of the calves to the challenge inoculation. Additionally, none of the calves of the present study were exposed to cattle persistently infected with BVDV, which could have increased their susceptibility to *H somni* and skewed the data.[32] Consequently, on the basis of the results of the present study, we concluded that tildipirosin was a better choice for metaphylaxis than tulathromycin when calves were subsequently challenged with *H somni*.

Results of the present study were similar to those of another study[22] in which calves were experimentally inoculated with *M haemolytica*. In that study,[22]

the calves weighed more than the calves of the present study and were experimentally inoculated with *M haemolytica* at 10 instead of 5 days after treatment with tildipirosin or tulathromycin. Tildipirosin-treated calves had a lower incidence of clinical disease and fewer lung lesions than did tulathromycin-treated or untreated control calves.[22] It could be argued that the metaphylactic efficacy of an antimicrobial against 1 pathogen following experimental inoculation is not relevant for cattle with naturally occurring BRD because BRD is a multifactorial disease caused by the interaction of multiple pathogens following various initiating or stressful events (eg, weaning and transport). However, it is important to assess whether an antimicrobial has activity against an individual pathogen to gauge its potential efficacy should that pathogen act as a primary or secondary causative agent during a BRD outbreak.

In the present study, histologic evaluation of lung lesions did not substantially enhance the clinical or gross necropsy findings. The mean LHS and necrosis scores for the tildipirosin-treated and tulathromycin-treated calves were numerically lower than those for the control calves. Of particular interest was that the tildipirosin-treated calves had only minimal necrosis in the lungs, which might have implications for the treatment of cattle with naturally occurring BRD. Feedlot cattle with BRD that are treated with an antimicrobial and fail to respond are generally retreated. The performance of calves that require multiple treatments is often reduced because chronic BRD results in scarred or abscessed lung tissue, which impairs feed efficiency and weight gain.[5,33,34] In cattle with only mild BRD or that are treated early and rapidly respond to treatment, the affected lung tissue can be repaired and returned to nearly normal function with minimal scarring. Fibrosis is likely to develop in areas of necrosis because the body cannot regenerate necrotic lung tissue.[35] Macrolides inhibit the secretion of proinflammatory cytokines, phospholipase activity, and the release of leukotrienes and have anti-inflammatory effects in bovine macrophages and neutrophils.[36,37] In the present study, the tildipirosin-treated and tulathromycin-treated calves had less necrotic lung tissue 72 hours after *H somni* challenge than did the control calves, and we speculate that the extent of fibrotic lung tissue in the calves metaphylactically treated with an antimicrobial would likewise have been less than that in the control calves had they been allowed to finish the feeding period.

The findings of the present study indicated that metaphylactic administration of tildipirosin to 3-month-old calves 5 days prior to intrabronchial inoculation with *H somni* prevented subsequent culture of the pathogen from bronchial secretions and was more effective in minimizing clinical disease and lung lesions than was tulathromycin. At the time of *H somni* challenge, 1 tulathromycin-treated calf had slight dyspnea but was otherwise clinically normal. That calf might have been infected with a BRD pathogen that could have affected the study results; however, all the calves were housed in the same barn, and if 1 calf was infected with a pathogen other than *H somni*, it is likely that other calves would have been affected as well. Additional field studies of the efficacy of tildipirosin against *H somni* are warranted.

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Footnotes

a. Rumilab Maintenance Diet, LabDiet, St Louis, Mo.
b. Zuprevo, Merck Animal Health Inc, Summit, NJ.
c. Draxxin, Zoetis, Florham Park, NJ.
d. Rompun, Bayer Animal Health, Shawnee Mission, Kan.
e. Provided by Dr. Tom Inzana, Department of Biomedical Sciences and Pathobiology, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Va.
f. Horseradish peroxidase-conjugated, goat anti-bovine IgG (H+L), Kirkegaard and Perry Laboratories Inc, Gaithersburg, Md.
g. o-Phenylenediamine reagent grade, Amresco, Solon, Ohio.
h. SPSS, version 22, IBM Corp, Armonk, NY.
i. Kansas State Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, Kan.

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