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# Influence of tilmicosin on quantified pulmonary concentrations of three bacterial pathogens in calves with naturally-occurring bovine respiratory disease

**Travis H. Allen,**[1] *DVM;* **Edward G. Johnson,**[2] *DVM;* **Matthew D. Edmonds,**[2] *DVM, PhD;* **Jared A. Gould,**[3] *DVM, MS*
[1]*College of Veterinary Medicine, Washington State University, Pullman, WA 99164*
[2]*Johnson Research LLC., Parma, ID 83660*
[3]*Private Veterinary Consultant, Nampa, ID 83687*
*Corresponding author: Dr. Travis Allen, 3432 Pioneer Rd., Homedale, ID 83628, 208-989-7830, cattledvm@gmail.com*

**Abstract**

A randomized trial was conducted to determine the lower respiratory tract pathogen load in cattle at risk of developing bovine respiratory disease (BRD). The objectives were to characterize and quantify concentrations of respiratory pathogens in the main-stem bronchi before and after administration of tilmicosin as a treatment for BRD.

Ninety-three beef steers and heifers were selected from a group of 421 high-risk calves based on a BRD case definition that included clinical score and rectal temperature parameters. Three calves meeting the BRD case definition constituted a cohort; 1 animal within a cohort served as an untreated control, whereas the remaining 2 received 6.75 mg/lb (14.85 mg/kg) of body weight tilmicosin. An aliquot of solution obtained via lavage of the main-stem bronchi upon enrollment and at 72, 144, and 216 hours post-enrollment was serially diluted and cultured for respiratory bacteria.

Treated animals had significant reductions ($P<0.05$) in *Mannheimia haemolytica* concentrations at each post-enrollment time point compared to untreated controls, while concentrations of *Histophilus somni* at 144 and 216 hours post-enrollment were lower than untreated controls. Rectal temperature was also reduced ($P<0.05$) at all post-enrollment sampling times in calves treated with tilmicosin. An improvement ($P<0.05$) in clinical score during the post-enrollment period was noted for calves receiving tilmicosin.

**Key words**: bovine respiratory disease, tilmicosin, bronchial lavage, respiratory pathogen load

**Résumé**

Un essai randomisé a été menée dans un parc d'engraissement de recherche en Idaho afin d'étudier la charge de pathogènes dans le tractus respiratoire inférieur chez des bovins à risque de développer le complexe respiratoire bovin (CRB). Les objectifs de l'étude étaient de caractériser et de quantifier la concentration de pathogènes respiratoires dans les bronches souches avant et après l'administration de tilmicosine pour traiter le CRB.

Un total de 93 bouvillons et de taures de boucherie ont été sélectionnés à partir d'un groupe de 421 veaux à haut risque selon la définition d'un cas de CRB basée sur le score clinique et la température rectale. Trois veaux identifiés selon la définition d'un cas de CRB formaient une cohorte. Dans chaque cohorte, un veau servait de témoin non-traité alors que les deux autres recevaient une dose de tilmicosine de 6.75mg/lb (14.85 mg/kg) de poids corporel. Une aliquote de solution obtenue à partir d'un lavage des bronches souches au début de l'étude et à 72, 144 et 216 heures suivant le traitement a été diluée en série pour culture des bactéries respiratoires.

La concentration de *Mannheimia haemolytica* était moins élevée chez les animaux traités que chez les animaux témoins ($P < 0.05$) à chaque période de temps suivant le traitement alors que la concentration de *Histophilus somni* était moindre seulement à 144 et à 216 heures suivant le traitement. La température rectale était moins élevée ($P < 0.05$) chez les veaux traités à la tilmicosine à chaque période d'échantillonnage suivant le traitement. Les veaux du groupe traité à la tilmicosine avaient aussi un meilleur score clinique ($P < 0.05$) dans les périodes suivant le traitement.

## Introduction

Bovine respiratory disease (BRD) is a multifactorial disease process in which cattle, often with a suppressed immune system due to stress, succumb to challenge with 1 or more respiratory pathogens.[8] *Mannheimia haemolytica, Histophilus somni,* and *Pasteurella multocida* are 3 frequently implicated bacterial pathogens, although others certainly exist.[5] Viral agents, including bovine herpes virus 1 (infectious bovine rhinotracheitis), bovine parainfluenza virus type 3, bovine respiratory syncytial virus, and bovine viral diarrhea virus, often precipitate the disease process which culminates as bacterial bronchopneumonia, the primary focus of this research.[5]

Evaluating therapeutic effectiveness of interventions for BRD (e.g. antimicrobial therapy) can be challenging.[3] A decrease in rectal temperature is frequently used to indicate a favorable response to therapy, although increased body weight and improved clinical appearance may be more appropriate measures and may reduce likelihood of re-treatment of recovering animals.[3] Limited information is available comparing improvement in these parameters to quantifiable changes in respiratory pathogen load. The objectives of this study were 1) to develop a model to quantify pathogen load in the lower respiratory tract during acute BRD, and 2) to compare changes in pathogen load in animals treated or untreated for BRD at set time points during the disease process.

## Materials and Methods

*Study Animals*

Three hundred ninety-four heifers and 27 steers were purchased from livestock auctions in California and Idaho during June and July 2011. Cattle arrived by truck to the research facility near Parma, ID on June 7 (79 head), June 27 (87 head), July 12 (83 head), July 23 (79 head), and July 30 (93 head). Cattle were commingled within truck load and placed in receiving pens with access to fresh water. Cattle were allowed to rest overnight until the following morning, at which time cattle were moved through a cattle handling facility for in-processing. Each animal received uniquely numbered duplicate visual ear tags, a modified-live viral respiratory vaccine,[a] multivalent clostridial bacterin-toxoid,[b] and an injectable anthelmintic.[c] Heifers were administered a growth-promoting implant containing 80 mg trenbolone acetate and 8 mg estradiol,[d] whereas steers received an implant containing 200 mg trenbolone acetate and 45 mg estradiol.[e]

Cattle were housed in open-air, dirt floor pens holding 14 to 16 head each. Pens were rectangular with 25 linear feet (7.62 m) of bunk space facing the feed alley, and 70 feet (21.3 m) deep, resulting in 1750 square feet (163 sq m) of pen space. Rectangular constant-flow concrete water tanks were along one fence line and shared with the adjacent pen, providing 4 linear feet (1.22 m) of tank space per pen. Cattle were fed a ration (65% concentrate, 35% roughage) comprised of alfalfa, corn earlage, wheat, dry distillers grain, corn syrup, and a liquid supplement containing monensin sodium[f] and tylosin.[g] The ration provided 53.01Mcal/cwt NEg and 14.79% crude protein on a dry matter basis. Cattle were provided fresh feed once daily.

*Cohort Groups*

Calves were monitored once daily in their home pen for signs of BRD using a scoring system outlined in Tables 1 and 2. Calves exhibiting a clinical score of 1 in both depression and respiratory categories or 2 or greater in either the depression or respiratory score categories were moved from their home pen to a treatment chute in the feedlot hospital for further evaluation. A case definition for BRD was met when an animal meeting the aforementioned criteria was also febrile,

**Table 1.** Clinical depression score definitions.

| Depression score | Clinical signs |
|---|---|
| 0 | Clinically normal animal |
| 1-mild | Stands or lies isolated from other cattle with head drooped. Responds to visual stimulation, moves away from observer. |
| 2-moderate | Drooped head, slower to respond to the observer. Difficulty standing, lack of stretching and may knuckle when walking. |
| 3-severe | Moribund |

**Table 2.** Clinical respiratory score definitions.

| Respiratory score | Clinical signs |
|---|---|
| 0 | Clinically normal animal |
| 1-mild | Serous nasal or ocular discharge, dry cough and/or elevated respiratory rate, subtle excessive salivation, dehydration. |
| 2-moderate | Purulent or muco-purulent nasal and/or ocular discharge, productive cough and forced respiratory effort, excessive salivation, pronounced dehydration. |
| 3-severe | Open mouth breathing, dyspnea, excessive salivation. |

characterized by a rectal temperature equal to greater than 104°F (40°C). Animals not meeting the case definition were returned to their respective home pen, and remained eligible for enrollment at a later date.

Of the 421 calves received, 93 calves (mean body weight 578 lb; 263 kg) met the case definition for BRD. A cohort group enrolled on the same day was comprised of 3 calves meeting the BRD case definition; 1 animal served as an untreated control, whereas the remaining 2 animals within each cohort were treated with tilmicosin[h] at 6.75 mg/lb (14.85 mg/kg) body weight. The dose of tilmicosin was rounded up to the nearest 0.5 ml and administered with a repeating variable dose syringe and 16 gauge x 5/8 inch (7.9 mm) needle subcutaneously in the right lateral neck. As animals presented through the chute, a treatment was assigned based on a pre-determined randomization worksheet, organized by random number assignment. A dangle tag indicating cohort group identification was placed into the left ear of each animal within a cohort group. Animals within a cohort group were commingled in pens containing a maximum of 3 cohort groups (9 animals).

Bronchial lavage and nasopharyngeal swab samples were obtained from each animal within a cohort at the time of enrollment (time 0, immediately prior to tilmicosin treatment) and at approximately 72, 144, and 216 hours post-enrollment. In the event that an incomplete cohort group was formed (i.e., less than 3 BRD case definitions on a given day), the animal(s) within the incomplete cohort group were treated and removed from the candidate pool.

*Bronchial Lavage and Nasopharyngeal Swab Technique*

Cattle were restrained using a hydraulic squeeze chute and nose tongs when sampled using bronchial lavage and a nasopharyngeal swab. Deep nasopharyngeal swabs were obtained from each animal for identification of respiratory pathogens present in the upper respiratory tract. A sterile 33 inch (84 cm) double-guarded culture swab[i] was inserted through a ventral nare to a depth of approximately 8 inches (20 cm). The swab was exposed and brushed against the nasopharyngeal mucosa, withdrawn into the guard, and removed from the animal. Swabs were placed in charcoal transport media and stored on ice until further processing.

Immediately following completion of nasopharyngeal swab sampling, bronchial lavage was conducted by placing a 1.5 inch (3.8 cm) diameter by 17 inch (43 cm) speculum in the oropharyngeal cavity. The guards of a 33 inch (84 cm) double-guarded culture swab[i] were passed through the speculum and between the arytenoid cartilages. The inner guard was subsequently advanced, and a sterile 3/16 inch (4.8 mm) by 36 inch (91 cm) round, rigid plastic pipette[j] was fully advanced through the inner guard. This procedure, verified via endoscopy in 3 clinically normal animals selected randomly from the candidate pool, provided access to the distal trachea, approximately 3.9 inches (10 cm) rostral to the bronchus of the right cranial lung lobe and approximately 8 inches (20 cm) rostral to the tracheal bifurcation. Sixty mL of phosphate-buffered saline were injected through the pipette and immediately aspirated. Improved recovery of lavage fluid was accomplished by withdrawing the pipette approximately 2 to 4 inches (5 to 10 cm) while aspirating. The recovered lavage fluid was placed into a 50 mL centrifuge tube and stored on ice until further processing.

*Characterization and Quantification of Bacterial Pathogens*

Recovered bronchial lavage fluid was diluted logarithmically and cultured at each dilution. On each culture media plate, 100 µl of dilution was evenly distributed over the surface of the media using sterile glass beads. Dilutions were plated on Columbia blood agar, chocolate agar, and blood agar containing selective agents for the isolation of Pasteurellaceae. The plates were incubated at 98.6°F ± 5.4°F (37°C ± 3°C) in 10% CO2 (± 3%) for 18 to 24 hours. Potential isolates of *M. haemolytica, H. somni*, and *P. multocida* were confirmed via research facility standard operation procedure (LAB-003). Tests included potassium hydroxide preparation, oxidase, catalase and indole biochemical tests, and colony morphology. Final colony forming units (CFU) were extrapolated from the dilution and reported as CFU/mL of recovered bronchial lavage. Select isolates were frozen at -94°F (-70°C) for potential later retrieval. Nasopharyngeal swabs were streaked on culture media for isolation of bacterial colonies. These isolates were tested using the same biochemical testing, and the presence of *M. haemolytica, H. somni*, or *P. multocida* was recorded as a positive isolation of the pathogen from the upper respiratory tract.

*Post-enrollment Health Observations*

Immediately prior to post-enrollment sampling (time 0), each animal was assigned a visual and respiratory clinical score in its home pen using the previously described scale (Tables 1 and 2). Rectal temperature was taken using an electronic thermometer. Visual and respiratory scores and rectal temperatures were taken again from study animals at 72, 144, and 216 hours post-enrollment. Animal caretakers making observations were not blinded to treatment assignments.

A postmortem examination was performed on each animal that died during the trial. Grossly normal and abnormal lung were collected for bacteriologic testing; sterile swabs were used to sample a freshly cut section of lung. Swabs were streaked on growth media in a similar fashion to the processing of the nasopharyngeal swabs.

If present, *M. haemolytica, H. somni*, or *P. multocida* was reported as postmortem isolation from the lower respiratory tract. No further diagnostics were pursued postmortem.

*Statistical Analysis*

Statistical analysis was done by an independent statistician. Individual animal served as the experimental unit. A repeated measures analysis of variance was conducted on log transformed bacterial count data (CFU•mL+1) using the MIXED procedure of SAS.[k] The statistical model included the fixed effects of treatment, time, and the treatment-by-time interaction. Categorical data (i.e., clinical scores) were analyzed as categorical data by time point. Continuous data (i.e., rectal temperature) were analyzed using RMANOVA as described above. Logistic regression was used to compare CFU/mL of respiratory pathogens to a positive nasopharyngeal swab culture.

## Results

Of 421 cattle received, 93 were enrolled in the study, including 62 treated and 31 untreated controls. All 62 treated cattle completed the 216-hour trial period, during which time data were collected at the 4 different sample times. Six of 31 untreated cattle died of BRD within 216 hours, representing a case fatality rate of 19.4%. For statistical analysis of pathogen load, only cattle with an accurate diagnosis of BRD were included, defined as the presence of any respiratory pathogen in the lower respiratory tract at the time of enrollment (time 0). Of the 62 treated cattle, 50 had a positive culture for *M. haemolytica*, while 8 were positive for *H. somni* at time 0; 4 of 62 treated cattle were infected with both pathogens. Of 31 negative controls, 25 were culture-positive for *M. haemolytica*, and 3 were positive for *H. somni*; 1 of 31 negative control cattle was infected with both pathogens. A single negative control animal had a positive lavage culture for *P. multocida* at the time of enrollment. Although collection technique did not vary between animals or treatment groups, there was a significantly greater volume ($P<0.05$) of lavage fluid collected at the time of enrollment (time 0) in treated cattle compared with controls. This difference was not observed at any post-treatment collection time.

A significant reduction ($P<0.05$) in *M. haemolytica* concentration was observed at each time point post-treatment in the treated cattle, but not in controls (Figure 1). In cattle that cultured positive for *H. somni* infection, there was a significant reduction ($P<0.05$) in pathogen load at 144 and 216 hours post treatment, but not in controls (Figure 2). Treated cattle also had a significant reduction ($P<0.05$) in mean body temperature at each time point post treatment compared to controls (Figure 3). In addition, cattle with a positive culture for *M. haemolytica* and treated with tilmicosin had significantly improved ($P<0.05$) clinical respiratory and depression scores compared to untreated controls (Figures 4 and 5).

Acute respiratory disease followed by death occurred in 6 control animals prior to the end of the study (216 hours). Lower respiratory concentrations (CFU/mL) of pathogens isolated from those animals were frequently found to rise until death (Figure 6). Bacterial culture of pulmonary tissue collected postmortem from these animals revealed pathogens of the same species as the final lung lavage prior to death.

The presence of bacterial pathogens in lower respiratory tract samples (lavage fluid) was compared to nasopharyngeal swab samples to estimate the sensitivity,

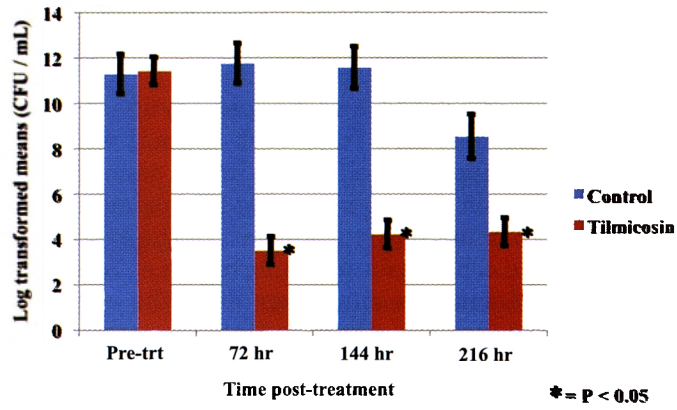


**Figure 1.** Bronchial lavage *M. haemolytica* concentrations in cattle with naturally occurring BRD treated with tilmicosin vs untreated control cattle.

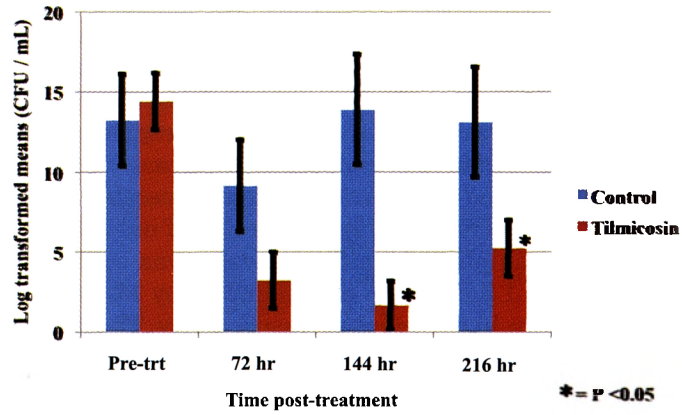


**Figure 2.** Bronchial lavage *H. somni* concentrations in cattle with naturally occurring BRD treated with tilmicosin vs untreated control cattle.

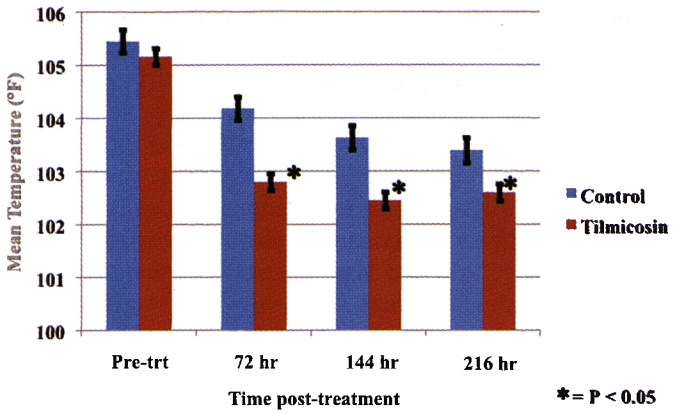


**Figure 3.** Mean rectal temperature of cattle with naturally occurring BRD treated with tilmicosin vs untreated control cattle.

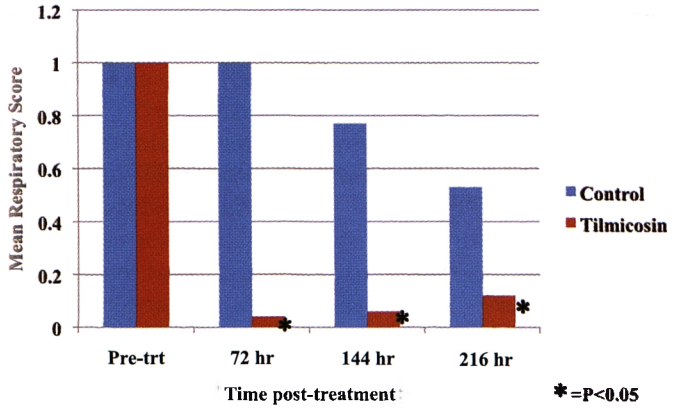


**Figure 4.** Clinical respiratory scores of cattle with naturally occurring BRD treated with tilmicosin vs untreated control cattle.

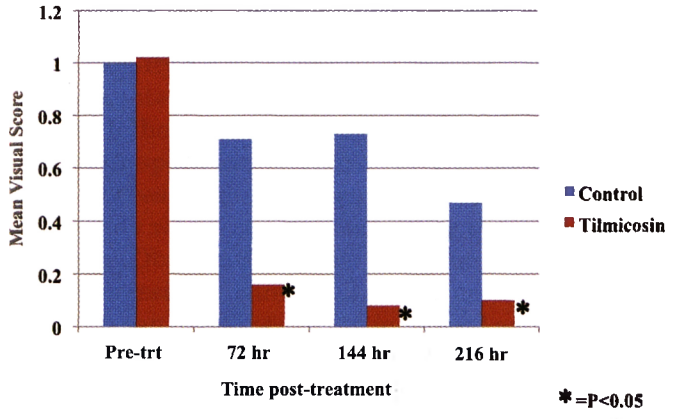


**Figure 5.** Clinical visual scores of cattle with naturally occurring BRD treated with tilmicosin vs untreated control cattle.

specificity, and predictive values of the presence of upper respiratory pathogens at predicting lower respiratory disease at the time of enrollment in the study (Table 3). Logistic regression of lower respiratory tract pathogen load was used to predict the likelihood of a positive nasopharyngeal swab throughout the study. For every log increase in *M. haemolytica* concentration in lower respiratory samples, a 31% increase in the likelihood of positive nasopharyngeal culture was observed (Figure 7). For every log increase in *H. somni* concentration, a 23% increase in the likelihood of a positive nasopharyngeal culture was observed (Figure 8).

## Discussion

Several techniques for measuring BRD severity and effectiveness of therapeutic intervention have been previously reported. DeDonder et al found that lung auscultation scoring and rectal temperature were useful for predicting the likelihood of retreating cattle diagnosed and treated for BRD.[7] In a study evaluating effectiveness of diagnosis based on clinical signs, Roof measured respiratory pathogen concentration in CFU

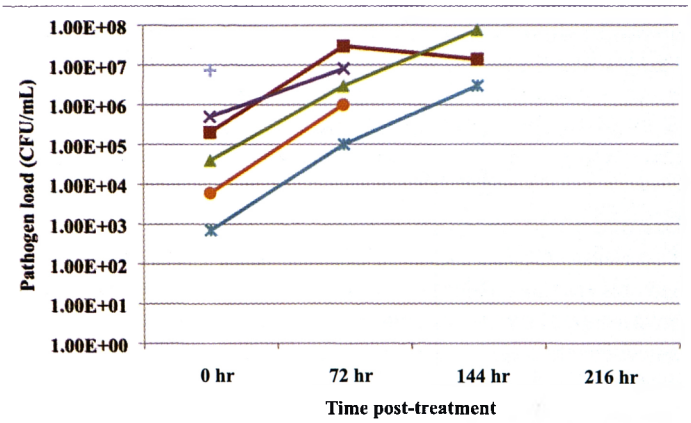


**Figure 6.** Concentrations of *M. haemolytica* in bronchial lavage (CFU/mL) for animals succumbing to BRD within 216 hours of diagnosis (6 head). Each symbol represents 1 animal.

**Table 3.** The ability of positive upper respiratory culture to predict lower respiratory disease at the time of enrollment in the study.

| | |
|---|---|
| Sensitivity | 69% |
| Specificity | 78% |
| Positive predictive value | 93% |
| Negative predictive value | 38% |

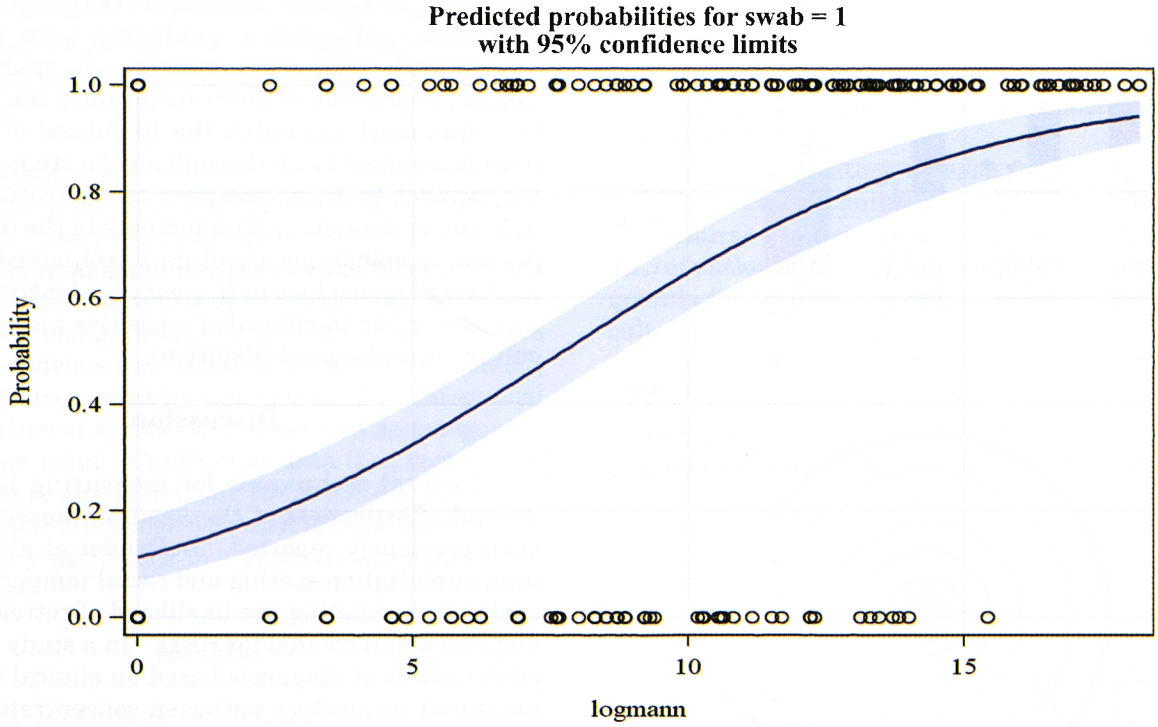


**Figure 7.** *Mannheimia haemolytica*-logistic regression of CFU/mL vs probability of positive nasopharyngeal swab culture in cattle with naturally occurring BRD (adapted from statistician results).

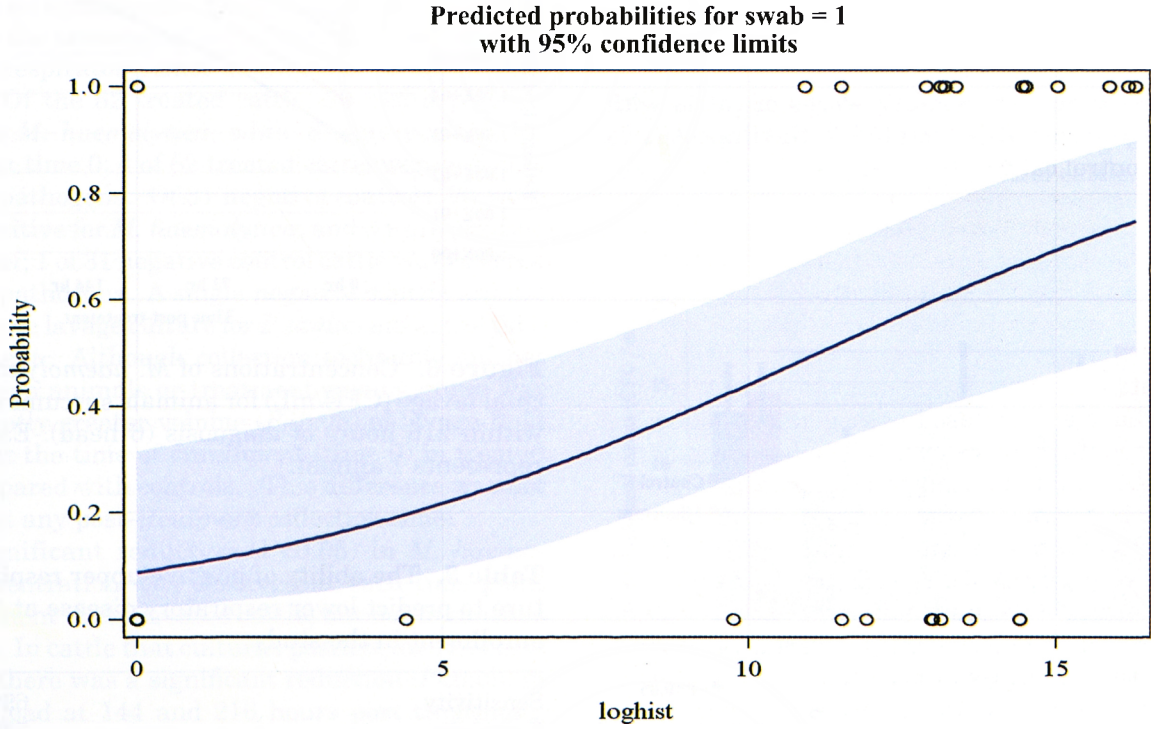


**Figure 8.** *Histophilus somni*-logistic regression of CFU/mL vs probability of positive nasopharyngeal swab culture in cattle with naturally occurring BRD (adapted from statistician results).

per gram of pulmonary tissue after euthanizing cattle diagnosed with acute BRD.[11] Although the techniques were very different, the investigators of the current study noted similarities when comparing pathogen load in pulmonary tissues (postmortem) to the antemortem techniques reported here.

Novel methods of identifying the effects of antimicrobial therapy on total pathogen load may help improve BRD treatment strategies and the ability to evaluate therapeutic effectiveness. A transtracheal method of obtaining lower respiratory samples was found to be useful in diagnosing respiratory disease in sheep[12] and calves.[9] Furthermore, it is desirable to have a minimally invasive and chute-side method of collecting similar samples. Angen et al compared transtracheal aspirates from clinically normal and diseased calves for the presence of respiratory pathogens.[2] Their technique using a semi-quantitative scale (pure culture, many bacteria in mixed culture, mixed culture, few bacteria in mixed culture, no growth) indicated 32% of clinically normal, and 100% of diseased calves had high numbers of respiratory pathogens as dominant flora in lower respiratory tract samples.[2] The methods in the present study allowed for full quantification of lower respiratory pathogens, and also provided post-treatment measure of response to antimicrobial therapy.

Antimicrobial levels in pulmonary tissue and serum have been measured to demonstrate presumed drug effect via volume of distribution. Pivotal studies have shown tilmicosin levels in lung tissue peak by 24 hours post-treatment, but remain above the MIC of *M. haemolytica* (3.12 mcg/mL) for at least 72 hours when given at label dosage (4.54 mg/lb; 10 mg/kg).[14] In the current study, the significant reduction in pathogen load for 216 hours post treatment suggests tilmicosin can have an extended duration of clinical efficacy without re-treatment. This supports other studies that found a 7-day post-treatment interval[6] or 10-day post-metaphylaxis period[10] following administration of tilmicosin resulted in a higher treatment success rate and lower BRD morbidity than a 3-day moratorium.

Untreated animals enrolled in this study were observed to have an increased pathogen load followed by death or spontaneous recovery. The high mortality rate in untreated animals suggests diagnosis of BRD in this study was accurate when using the defined case definition. Observations from this study indicate that errors of omission in clinical diagnosis, and subsequently treatment, can quickly result in increased pathogen load and damage to pulmonary tissues. Similar to previous studies, the insensitivity of nasopharyngeal swab cultures in diagnosing lower respiratory disease was observed here.[1,11,12] Results of the present study indicate that as lower respiratory pathogen load decreases, deep nasopharyngeal swab becomes a less reliable indicator of lower respiratory disease. It remains unknown if this discrepancy results from decreased shedding from the lower respiratory tract or from distribution of the antimicrobial.

In the present study, clinically recovered animals, both with and without antimicrobial therapy, were often found to have measureable concentrations of pathogens throughout the duration of the trial. Earlier studies also report clinically healthy animals frequently harbor respiratory pathogens in the lower respiratory tract.[2,11,12] Based on lung lesions present at the time of harvest, Thompson et al found the incidence of BRD in the combination of both treated and untreated study animals to be 52.5%.[13] Interestingly, 29.7% of these cattle had lung lesions at slaughter, but were not treated for BRD during the feeding period.[13] In a similar study, Bryant et al found 42% of all calves from a university herd had lung lesions present at the time of harvest.[4] Of the 17% diagnosed with acute BRD during the feeding period, 40% had pulmonary lesions compared to 42% of cattle that remained clinically normal.[4] The frequency of clinically normal animals shedding pathogens, the impact of those pathogens on the health of that animal, and the extent to which metaphylaxis reduces pathogen load are unknown and could potentially be studied using this model. Little is known about measureable differences in how different classes of antimicrobials affect pathogen load. These differences, as well as comparisons of onset and duration of treatment effect, could be identified using these techniques.

## Conclusion

Minimally invasive, antemortem quantification of lower respiratory pathogens is possible as demonstrated by methods used in this study. Samples obtained via nasopharyngeal swabs were a less sensitive indicator of lower respiratory tract disease as lower respiratory tract pathogen load decreased. This study also revealed that a single treatment with tilmicosin can decrease pathogen load for at least 9 days post-treatment. Further research is warranted using this technique to study impacts of lower respiratory pathogen load and response to therapeutic intervention. This may provide new information about current methods of BRD diagnosis and measurement of therapeutic effectiveness.

## Endnotes

[a]Bovi-Shield Gold 5®, Pfizer Animal Health, New York, NY

[b]Cavalry™ 9, Merck Animal Health, Summit, NJ

[c]Cydectin®, Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO

[d]Revalor® IH, Merck Animal Health, Summit, NJ

[e]Revalor® XS, Merck Animal Health, Summit, NJ
[f]Rumensin®, Elanco Animal Health, A Division of Eli Lilly & Co., Indianapolis, IN
[g]Tylan®, Elanco Animal Health, A Division of Eli Lilly & Co., Greenfield, IN
[h]Micotil® 300, Elanco Animal Health, A Division of Eli Lilly and Co., Greenfield, IN
[i]J-273, Double Guarded Culture Swab, Jorgensen Laboratories Inc., Loveland, CO
[j]16005 Thinwall Rigid Tubing, Lee's Aquarium and Pet Products, San Marcos, CA
[k]SAS, Version 9.2, SAS Institute Inc., Cary, NC


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