Efficacy of Gamithromycin Injectable Solution for Control of Pneumonia in Cattle Challenged with *Histophilus somni* after Treatment

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ABSTRACT

Recently weaned calves transported to the feedlot are at risk of infection, illness and even death from *Histophilus somni* and other pathogens associated with bovine respiratory disease (BRD) throughout the feeding period. Gamithromycin is an azalide 15-membered semi-synthetic macrolide antimicrobial that is licensed in the European Union and Canada for therapeutic and preventative treatment of BRD associated with *Mannheimia haemolytica*, *Pasteurella multocida*, and *H. somni*. This placebo-controlled study evaluates the efficacy of gamithromycin injectable solution administered subcutaneously to cattle at 6.0 mg/kg body weight for control of the development of pneumonia in cattle challenged with *H. somni* approximately 6 hours post-treatment. Mean depression and respiratory scores were significantly lower (P<0.01) for the gamithromycin treated group than for the saline control group throughout the 7-day evaluation period. Mean rectal temperatures were significantly lower (P<0.01) for gamithromycin treated calves from Day 1 through Day 6. Total weighted percentage lung consolidations were significantly (P<0.01) lower in the gamithromycin group than in the saline group. In total, 16 calves in the saline control group, and seven from the gamithromycin group had one or more bacterial pathogens cultured from lung samples collected at necropsy. In this study, gamithromycin treated calves had an excellent safety and tolerability profile compared to those calves receiving saline. Results of this study indicate that gamithromycin administered subcutaneously at 6.0 mg/kg was significantly efficacious in the prevention of clinical and pathological disease induced by *H. somni*.

INTRODUCTION

Histophilus somni (formerly *Haemophilus somnus*) is a common pathogen in North American cattle feedlots and can cause an acute, often fatal, septicemic disease that can involve the respiratory, cardiovascular, musculoskeletal, or nervous systems.[1] Respirato-

ry infection by *H. somni* results in a chronic suppurative bronchopneumonia. Other respiratory pathogens, such as *Mannheimia haemolytica*, *Pasteurella multocida*, or *Mycoplasma bovis*, may complicate the infection further.

Feedlot cattle are at risk of infection by *H. somni* throughout the feeding period. However, recently weaned calves are at higher risk of infection and death from this infection than are older weaned calves, yearlings, or mature animals.[2] The risk of infection with *H. somni* peaks early in the feeding period, around 21 to 23 days on feed. Calves that succumb to the infection generally die 30 to 60 days into the feeding period. Sudden death is often the first indication of *H. somni* infection in a feedlot animal. Fever, anorexia, and profound depression are also typical of *H. somni* infection.

Gamithromycin is an azalide 15-membered semi-synthetic macrolide antimicrobial developed for treatment and prevention of BRD.[3-5] Studies of the pharmacokinetic and pharmacodynamic properties of gamithromycin show that a single subcutaneous dose at 6 mg/kg body weight provides rapid therapeutic lung concentrations and persistent activity in the treatment and prevention of BRD infections, due to the low level of plasma protein binding and high availability of the drug in lung tissue.[4] Gamithromycin is licensed in the European Union and Canada for therapeutic and preventative treatment of BRD associated with *M. haemolytica, P. multocida*, and *H. somni*.[6]

This study evaluates the efficacy of gamithromycin injectable solution administered subcutaneously to cattle at 6.0 mg/kg body weight compared with efficacy of saline in the control of development of pneumonia in cattle challenged with *H. somni* approximately 6 hours post-treatment.

MATERIALS AND METHODS

Animals

Forty crossbred beef calves, including 34 males and 6 females, 3 to 8 months of age, and weighing 134 to 285 kg , were obtained from Logan Valley Feeders of Oakland, Nebraska. All calves were determined to be in good general health and were deemed suitable for the study. The animals were healthy, colostrum-deprived calves that had been raised at the source facility. Animals were individually identified by means of duplicate ear tags with unique numbers and began acclimation to the study facilities 6 days prior to treatment. All calves were evaluated by the clinical observer at least once daily for health problems beginning on Day -5. No disease control measures were performed for calves during the study except for the provision of monensin sodium for prevention and control of coccidia.

Inclusion Criteria

Enrolled animals had no history of bacterial pneumonia, vaccination against bacterial pathogens that cause respiratory disease (including *Histophilus somni*), or a history of therapeutic antibiotic administration during the 7 days prior to treatment. To be enrolled in the study, acceptable calves (ones that met these criteria) also met the following clinical criteria:

- Depression Score <1, and
- Respiratory Character Score ≤1, and
- Rectal Temperature <40°C (104.0°F)

Animal Management

Animals in the study were managed similarly and with due regard for their well-being. Animals were handled in compliance with Merial Institutional Animal Care and Use Committee (IACUC) approvals, and all applicable local regulations and requirements of local IACUC. The study monitor and investigator ensured that these procedures were in compliance with the protocol.

Calves were housed in pens, each measuring 10 × 50 feet, inside animal rooms in the BL-2 Cattle Facility at Midwest Veterinary Services, Oakland, NE (an environmentally modified confinement building) beginning 6 days prior to day of treatment administration. Two replicates were housed in each pen, providing 62.5 ft^2 of floor space

per animal. The rooms were ventilated using HEPA filter air (in and out) and heated as needed using gas-fired propane heaters located outside the room. Starting at acclimation (Day -6), animals were provided ad libitum a commercial, complete, pelleted calf ration, containing monensin sodium at 30 g/ton for coccidia prevention and control. Suitable well water was available ad libitum via automatic waterers. Samples of the feed supply and water were taken and stored frozen for analysis of nutrients and contaminants.

Allocation

On Day 0, cattle were ranked by increasing body weights determined on Day -1 and allocated consecutively to replicates of two animals each. Within a replicate, animals were allocated to one of two treatment groups by a randomization schedule prepared by a Merial biostatistician. A randomized complete block design was applied for the randomization using the PROC PLAN procedure of SAS® version 8.2. Treatments were commingled within a pen such that all animals within a replicate (one calf of each treatment group) were penned together, and two replicates were penned together within a single room (four calves per room).

Treatment

Animals were weighed on Day 0 to facilitate dose calculations. All scales were verified for accuracy prior to each weighing using verified test weights that represented the weight range of the cattle in the study. Each test substance was administered to the assigned animal once on Day 0. Both gamithromycin and sterile saline were administered by subcutaneous injection in the right side of the neck with a 16-gauge, ¾-inch needle. A maximum volume of 10.0 mL was administered per injection site. Gamithromcyin was administered at a dose rate of 2.0 mL/50 kg body weight. Saline was administered at a dose rate of 2.0 mL/50 kg body weight. The dose administered to each animal was derived from the dose chart provided for the study.

***Histophilus somni* Challenge**

The *Histophilus somni* used in the study for challenge purposes was isolated from the field by Midwest Veterinary Services in Oakland, NE and had been maintained under laboratory conditions for 1 year. Prior to infection of calves, the *H. somni* isolate used was cultured for determination of strain. Minimum inhibitory concentration (MIC) testing was performed by Microbial Research Inc, Fort Collins, CO, to obtain an indication of the challenge strain's in-vitro susceptibility to gamithromycin. MIC tests were conducted using veterinary fastidious medium with incubation at approximately 37°C under 6% CO2 for 24 hours. Quality control testing of the organisms deemed the MIC tests acceptable and in control. The isolate, MVS T2, had an MIC of 1 µg/mL for gamithromycin. These results indicated the challenge strain was adequately susceptible to gamithromycin and was therefore suitable as a challenge strain for evaluating the efficacy of gamithromycin.

Challenge procedure

All calves received *H. somni* challenge material approximately 6 hours after treatment on Day 0. Each calf received a 60-mL (approximately 4.8×10^{10} colony-forming units [CFU]) suspension of *H. somni* endobronchially. An additional 60 mL of saline and 60 mL of air were used to flush the endoscope port following challenge administration of each calf. Challenge administration was facilitated by intranasal passage of an endoscope to the level of the tracheal bifurcation.

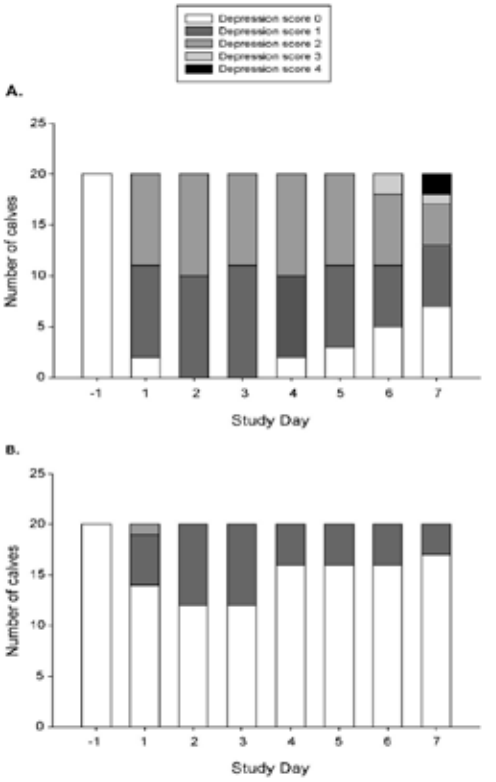
The challenge suspension was prepared according to procedures at the test facility. Briefly, the frozen isolate was thawed and regrown and checked for purity on a chocolate agar plate. The isolate was subsequently grown to challenge concentration in brain heart infusion broth.

Primary Endpoints

Clinical scores

Clinical evaluations were conducted on Day -1 at enrollment and then daily from Day 1 through Day 7. Evaluations were conducted by the same individual each day. The follow-

Figure 1. *Distribution of depression scores (0-4) for calves before and after treatment with saline (A) or gamithromycin 6 mg/kg (B) 5 to 9 hours before challenge with Histophilus somni. Scores were analyzed using Fisher's Exact Test (a chi-square procedure).*



ing scales were used for scoring depression and respiratory character:

Depression

0 = normal (no depression observed)

1 = mild depression (off-feed, moved when person entered pen)

2 = moderate depression (off-feed, moved when physically prompted)

3 = severe depression (pronounced; very reluctant to move when physically prompted)

4 = moribund (recumbent, near death)

Respiratory character

0 = normal (nothing unusual in respiratory character)

1 = mild respiratory distress (clinical signs included: mild cough, sneezing; mild increase in rate or shallow breathing, mild dyspnea)

2 = moderate respiratory distress (clinical signs included: increased cough, sneezing; moderate increase in rate or shallow breathing, moderate dyspnea)

3 = severe respiratory distress (clinical signs included: open-mouth breathing, or marked dyspnea or "thumping")

Rectal temperatures

Rectal temperatures were taken with a calibrated thermometer after animals were observed for their clinical scores on Day -1 (at enrollment), then daily from Day 1 through Day 7.

Necropsy

All calves were humanely euthanized on Day 7. At necropsy, lungs were harvested and scored for the percentage of consolidated pneumonic tissue. The same person scored pneumonic tissue and made the consolidation estimate for each calf evaluated. Each lung lobe was palpated and the percent consolidation on the dorsal and ventral surface of each lung lobe was estimated and recorded. Percentage consolidation for the total lung was calculated by multiplying the percent consolidation of each lobe by the percent weight (factor = %/100) for that specific lobe. The percent weight of each lobe is as follows: left cranial (apical) 5%, left posterior cranial (cardiac) 6%, left caudal (diaphragmatic) 32%, intermediate 4%, right cranial (apical and accessory) 11%, right middle (cardiac) 7% and right caudal (diaphragmatic) 35%. The products of each lobe calculation were added to obtain the total weighted lung percent consolidation (lung score).

Secondary Endpoints

Lung/pneumonic lesion samples were transported to an in-house microbiology diagnostic laboratory for culture after collection. Bacterial cultures were performed according

to procedures at the test facility. Briefly, all samples were plated simultaneously on three distinct types of growth agar (MacConkey, chocolate, and 5% sheep blood agar) and incubated overnight. Bacteria were identified as to genus and species based on the colonial appearance, microbiologic appearance, and biochemical tests.

Masking

Personnel who performed clinical observations and individual scoring of lungs/pneumonic lesions were masked to treatment group allocation. All individuals who collected study data were masked to treatment assignments.

Statistical analyses

All analyses were performed using SAS® version 9.2. The score variables were analyzed using Fisher's Exact Test (a chi-square procedure).Rectal temperature was analyzed using a mixed model repeated measures analysis of covariance including the fixed factor of treatment, the random factor of replicate, and day as the repeated effect. The Day -1 value was used as a covariate in the analysis. When the treatment-by-day interaction was found to be statistically significant ($P<0.05$), the treatments were compared separately for each day. Total weighted percentage of lung congestion was analyzed using randomized-block analysis of variance; the data were transformed to the arcsine of the square root of the proportion for analysis and calculation of treatment group means.

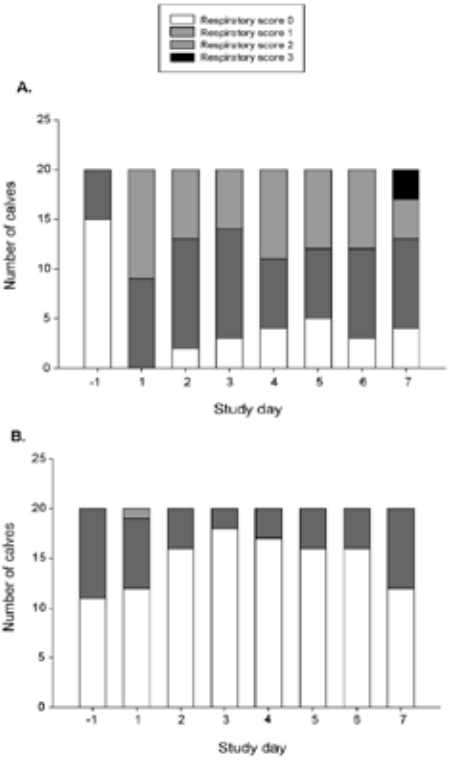
RESULTS

Dosing of the cattle was uneventful and all cattle received their assigned treatment. There were no treatment-related adverse events observed during the study. Housing environmental temperature and humidity measured on Day -4 through Day 6 varied between 45°F (7.2°C) and 72°F (22.2°C), and 30% and 71% relative humidity.

Clinical scores

Cattle treated with gamithromycin had significantly ($P<0.01$) lower depression scores (i.e., were less depressed) than controls from Day 1 through Day 7 (Figure 1). A majority of the cattle treated with gamithromycin (at least 12) had depression scores of 0 (normal) on each post-treatment day, while a majority of the control cattle (at least 13) had depression score of at least 1 on each day.

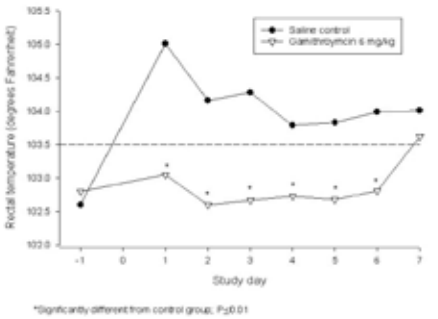
Figure 2. *Distribution of respiratory character scores (0-3) for calves before and after treatment with saline (A) or gamithromycin 6 mg/kg body weight (B) 5 to 9 hours before challenge with Histophilus somni. Scores were analyzed using Fisher's Exact Test (a chi-square procedure).*



Cattle treated with gamithromycin had significantly ($P<0.01$) lower respiratory scores (i.e., had less respiratory distress) than the controls from Day 1 through Day 7 (Figure 2). A majority of the cattle treated with gamithromycin (at least 12) had respiratory scores of 0 on each day, while a majority of the control (at least 15) had respiratory scores of at least 1 on each day.

Cattle treated with gamithromycin had significantly ($P<0.01$) lower rectal tempera-

Figure 3. *Mean rectal temperatures for calves before and after treatment with saline or gamithromycin 6 mg/kg body weight before challenge with Histophilus somni. The data were analyzed using a mixed model repeated measures analysis of covariance including the fixed factor of treatment, the random factor of replicate, and day as the repeated effect. The Day -1 value used as a covariate in the analysis.*



ture than control cattle from Day 1 through Day 6 (Figure 3). On Day 7, the difference between the groups was not significant (P>0.10).

Cattle treated with gamithromycin had significantly (P<0.01) less congested lung tissue at necropsy than the controls. The mean percentage (retransformed mean) of lung consolidation was 2.7% for the treated cattle and 26.7% for the controls (Figure 4).

Nine saline-treated control calves and five from the gamithromycin 6.0 mg/kg body weight group had pathological descriptors recorded at necropsy. These were most often fibrinous/fibrous adhesions and/or pleuritis, but one control calf did include swollen lymph node descriptions.

Two calves from the saline-treated control group were considered moribund on Day 7, were humanely euthanized, and a necropsy was performed after the clinical assessment and rectal temperatures were collected. Necropsy findings supported a diagnosis of fibrinous bronchopneumonia for both animals. The development of pneumonia was attributed to the challenge with *H. somni*.

Figure 4. *Weighted percentage of lung congestion. The data were transformed to the arcsine of the square root of the proportion and analyzed using randomized-block analysis of variance*

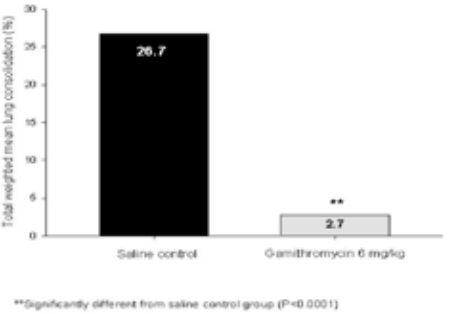
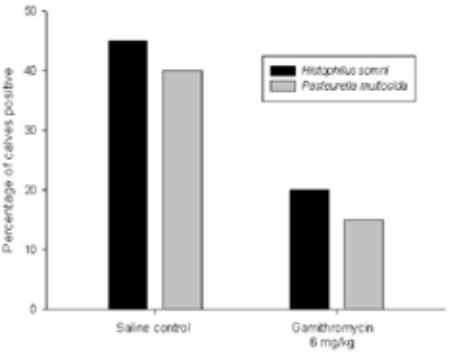


Figure 5. *Percentage of calves having positive cultures for Histophilus somni and Pasteurella multocida from samples taken at necropsy.*



Histophilus somni was isolated from lung samples collected from nine calves in the saline control group (45%) and 4 calves treated with gamithromycin 6 mg/kg (20%) (Figure 5). Pasteurella multocida also was isolated from at least one calf in each group, with the greater number isolated in samples from the saline control group (Figure 5). Mannheimia haemolytica was isolated from one sample collected from a control calf. In total, 16 calves in the saline control group and seven from the gamithromycin 6 mg/kg group had one or more bacterial pathogens cultured from lung samples collected at necropsy.

DISCUSSION

Histophilus somni, formerly known as Haemophilus somnus, can cause acute septicemic disease in young calves and transported cattle. Respiratory infection by *H. somni* results in a chronic suppurative bronchopneumonia, which is often complicated by other respiratory pathogens. Although the challenge in the present study was restricted to *H. somni*, co-infection with P. multocida was evident in several controls as well as in a lesser number of treated calves at necropsy. Mannheimia haemolytica also was isolated from a lung sample from one control calf.

Histophilus somni infection is responsible for the vast majority of deaths in large feedlots in western Canada.[1] Although vaccination of calves against *H. somni* has been successful in experimental challenge studies, it has met with limited success in the field, primarily because several strains or biotypes exist, and there is a high degree of antigenic variability among the strains.[1,7,8]

Successful antimicrobial treatment of *H. somni* infections is frequently hindered by the difficulty to identify affected animals and provide treatment early enough to save the animal from the rapidly fatal nature of these infections. In the present study, administration of gamithromycin 6.0 mg/kg administered approximately 6 hours before endobronchial challenge with *H. somni* was significantly effective in the prevention of clinical and pathological disease in young calves.

Clinical signs of respiratory disease in the control calves were evident throughout the 7 days following challenge with *H. somni*. Calves in the control group had significantly higher depression and respiratory scores, and rectal temperatures were significantly elevated in the control calves relative to temperatures of the calves treated with gamithromycin for 6 of the 7 days following treatment and challenge. Lung pathology in treated calves averaged less than 3% of the total lung volume compared with nearly 27% of the lung volume in control calves.

Gamithromycin is an azalide, 15-membered semi-synthetic macrolide class antimicrobial with uniquely positioned alkylated nitrogen at 7a-position of the lactone ring[4]. Gamithromycin accumulates rapidly and persists in lung tissue at concentrations at or above the MIC_{90} of the target pathogens through at least 10 days.[4] Macrolides in general have both bacteriostatic and bactericidal action mediated through disruption of bacterial protein synthesis.[9,10] The broad spectrum antimicrobial activity of gamithromycin includes M. haemolytica, P. multocida, and *H. somni*, the bacterial pathogens most commonly associated with BRD.[3-5] Field studies as well as challenge studies have provided evidence of the preventive and therapeutic efficacy of gamithromycin for treatment or control of BRD in cattle subjected to the stresses of transport, commingling, and other risk factors encountered in commercial cattle production.[3,5]

CONCLUSIONS

Results of this study indicate that gamithromycin 6.0 mg/kg administered approximately 6 hours before *H. somni* challenge was effective in the prevention of clinical and pathological disease induced by *H. somni* in young recently weaned calves transported to the feedlot. In this study, gamithromycin treated calves had an excellent safety and tolerability profile compared to those calves receiving saline.

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