

Field Efficacy Study of Gamithromycin for the Treatment of Bovine Respiratory Disease Associated with *Mycoplasma bovis* in Beef and Non-lactating Dairy Cattle

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ABSTRACT

Efficacy of gamithromycin, an azalide 15-membered semi-synthetic macrolide antibiotic, was evaluated as a treatment for bovine respiratory disease (BRD) associated with *Mycoplasma bovis* in purebred and crossbred beef cattle and non-lactating dairy cattle in two randomized, negative control, blinded studies in the United States. A total of 502 cattle 5 to 10 months of age (260 at Site 1 and 242 at Site 2) were obtained from sale barns and shipped directly to the study site. Animals enrolled were those displaying BRD (depression score or respiratory char-

acter score ≥ 2 and rectal temperature $\geq 40^{\circ}\text{C}$ [104.0°F]). Replicate pairs were formed and cattle within each replicate were randomized to treatment (sterile saline administered at 2 mL/50 kg body weight or gamithromycin at 6 mg/kg body weight [2 ml/50 kg]) administered by subcutaneous injection. All animals were observed for adverse experiences once daily post-treatment from Days 0 to 10. Rectal temperatures were recorded on Day 10 for each animal. Cattle responding to treatment for BRD were declared treatment successes on Day 10, based on depression score ≤ 1 , respiratory character score ≤ 1 , and rectal temperature $< 40^{\circ}\text{C}$. Gamithromycin provided significantly better proportion of treatment successes compared with saline control (74.4% versus 24.0%, $p < 0.001$ at

Site 1 and 67.4% versus 46.2%, $p=0.002$ at Site 2). Of the 73 cattle positive for *M. bovis* immediately prior to treatment, 68.5% of the cattle treated with gamithromycin were treatment successes on Day 10 compared with 12.8% of cattle treated with saline.

INTRODUCTION

Mycoplasma bovis is a member of the class Mollicutes, bacteria that are distinguished by the lack of cell walls, having instead a complex plasma membrane.^{1,2} *Mycoplasma bovis* is an important pathogen of young dairy and veal calves and is a causative agent of mastitis in adult cows and respiratory disease and arthritis in feedlot and stocker calves.³ Although most cases of bovine respiratory disease (BRD) involve more than one pathogen, including *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*, there are reports of *M. bovis* being the predominant bacteria isolated from the lungs of calves with BRD.^{1,2} *Mycoplasma bovis* is often the causative pathogen when pneumonia is chronic and unresponsive to antibiotics.^{1,3-5} Chronic pneumonia and polyarthritis caused by *M. bovis* in calves are often refractory to antibiotic treatment.²⁻⁶ In particular, *M. bovis* is resistant to antibiotics of the β lactam family, which express their activity by inhibiting synthesis of the bacterial cell wall (a feature that is lacking in the Mollicutes).^{1,2} Other antibiotics representing various classes found to be ineffective against *M. bovis* include neomycin, erythromycin, and flumequin.² Although streptomycin administered at 20 mg/kg significantly reduced *M. bovis* counts in the lungs, treatment did not provide significant reduction in lung lesions.²

Gamithromycin is an azalide 15-membered semi-synthetic macrolide antibiotic that has been developed for treatment and prevention of BRD. Pharmacokinetic, pharmacodynamic and clinical studies of gamithromycin showed that a single subcutaneous dose at 6 mg/kg provides rapid therapeutic and persistent activity in the control and treatment of infections. This study was conducted to evaluate the efficacy

of gamithromycin for treatment of BRD associated with *Mycoplasma bovis* in beef and non-lactating dairy cattle.

MATERIALS AND METHODS

Two randomized, negative control, blinded clinical field efficacy studies, identified as Site 1 and Site 2, were conducted at two different locations in the United States (Central States Research Centre, Inc., Oakland, NE and Agri-Research, Inc., Canyon, TX, respectively). The studies were conducted from November 2 through November 15, 2009 (Study 1) and March 21 through April 8, 2010 (Study 2). Animals in each 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 regulations and requirements of local IACUC.

Animals

At Site 1, 242 purebred and crossbred beef cattle, including one bull, 91 steers, and 150 heifers approximately 5 to 10 months of age, and weighing 160 to 286 kg on Day 0 were enrolled. These multi-origin commingled calves were obtained from sale barns in Missouri, Kentucky, Tennessee, and Iowa. Animals were shipped directly to the study site.

At Site 2, 260 multi-origin commingled purebred and crossbred beef cattle, including 118 bulls, 23 steers, and 119 heifers approximately 6 to 10 months of age, and weighing 165 to 264 kg on Day 0 were obtained from sale barns in Kentucky, Tennessee, Alabama, and Arkansas and shipped to the study site in Canyon, Texas. At both sites, transit time was estimated at no more than 24 hours.

Inclusion/Exclusion criteria

To be enrolled, animals had to be showing signs of naturally occurring BRD as defined by the following criteria: depression score or respiratory character score ≥ 2 and rectal temperature $\geq 40^{\circ}\text{C}$ (104.0°F).

Animals that were debilitated, suffering from systemic disease other than BRD, injured, fractious or moribund (depression

score = 4) were excluded from enrollment. The cattle had no known history of previous bacterial vaccinations for BRD or antimicrobials within 30 days of enrollment.

Following arrival, one nostril of each animal was wiped clean and a nasopharyngeal swab sample for microbiologic culture was collected for a presumptive determination of *M. bovis* status. Animals positive for *M. bovis* were observed daily for the presence of BRD clinical signs, and rectal temperatures. On days 1 – 5 following presumptive determination of *M. bovis* calves with a depression or respiratory character score of ≥ 2 and a rectal temperature $\geq 40^{\circ}\text{C}$ were enrolled in the study. On the final two days of the enrollment period, animals that were presumptively determined to be negative for *M. bovis* were also included at each study site to meet the minimum number of animals required in the study design. Enrolled animals were weighed and randomly allocated to treatments. Additional nasopharyngeal swab samples were obtained immediately pre-treatment for efficacy determination for microbiologic culture for *M. bovis*. Results were confirmed by polymerase chain reaction (PCR) testing of the samples.

Management

Cattle at both sites were delivered 3 to 6 days prior to the first day of enrollment (Day 0 was not the same calendar day for all animals) and processed on their respective day of arrival. Uniquely numbered ear tags were applied to each animal for identification. Each calf received a viral respiratory vaccine, a clostridial bacterin, and an endectocide for internal/external parasites. No antibiotics or vaccines containing antigens/toxins/toxoids of *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and/or *M. bovis* were administered. No concurrent medications were administered during the study, other than these disease control products that were administered pre-treatment.

Animals were housed in open air, concrete-floored pens measuring 12.5' X 48' and containing 8 calves per pen at site 1. At

site 2 animals were housed in outdoor dry lot pens measuring 18' X 52' with 10 calves per pen. Cattle were penned by replicate following allocation, with multiple replicates contained within a pen up to the acceptable pen capacity.

Animals were fed a total mixed ration ad libitum, containing monensin, once daily. Fresh water was available ad libitum via automatic water tanks.

Allocation and Treatment

Cattle that met the enrollment criteria were grouped in replicates of two within pens, based on order of appearance at the restraint chute. Treatments were randomly assigned to animals within each pair using a randomization schedule prepared by a biostatistician. Only the monitor and the person administering treatments knew the group assignments of the animals.

Treatments assigned in the studies were:

- Group 1 - gamithromycin injectable solution; administered at 6.0 mg/kg body weight (2.0 mL/50 kg)
- Group 2 - Sterile saline for injection (0.9% NaCl); administered at 2.0 mL/50 kg body weight.

The dose of the assigned treatment for each animal was administered according to the body weight determined at the time of treatment and reference to a dose chart prepared by the sponsor. Treatments were administered subcutaneously, in the left lateral neckline, with a maximum volume of 10.0 mL per injection site. If more than 10.0 mL was required, the dose was divided approximately equally and given in two sites.

Efficacy Endpoints

The pivotal endpoint was the proportion of cattle that were treatment successes on Day 10. Cattle responding to treatment for BRD were declared treatment successes on Day 10, based on a depression score ≤ 1 , respiratory character score ≤ 1 , and rectal temperature $< 40^{\circ}\text{C}$.

Nasopharyngeal swabs collected following arrival and immediately prior to treatment on Day 0 were transferred to an

Table 1. Summary of treatment successes for bovine respiratory disease regardless of pathogen

Site	Treatment Group	Treatment Successes n/N (%)	P-value
1	Gamithromycin	90/121 (74.4)	<0.001
	Saline Control	29/121 (24.0)	--
2	Gamithromycin	87/129 (67.4)	0.002
	Saline Control	60/130 (46.2)	--

The proportion of calves that were treatment successes were analyzed using the GLIMMIX procedure of SAS® Version 9.1.3. Treatment group was the fixed effect, and pen and pen × treatment interaction were the random effects. Two-sided P-value determined by F-test comparing the expected proportion of successes.

off-site microbiology laboratory for culture determination of *M. bovis*. Nasopharyngeal swabs collected immediately prior to treatment on Day 0 were also submitted for PCR (confirmation) of *M. bovis*. In addition, the nasopharyngeal swabs taken prior to treatment (Day 0) were cultured for other BRD pathogens (*M. haemolytica*, *P. multocida*, *H. somni*). All samples were maintained frozen ($\leq -60^{\circ}\text{C}$) during storage.

Animals were not declared treatment failures or otherwise removed before Day 10 unless necessary for humane reasons (severe BRD cases, such as animals with depression or respiratory character scores ≥ 3).

Cattle treated with gamithromycin that were declared BRD treatment successes and were within the subset of animals that had positive results for *M. bovis* prior to treatment (Day 0, pretreatment swab), were declared treatment successes for BRD associated with *M. bovis*.

Clinical Scores

Animals were observed at approximately the same time of day (AM) from Days 1 to 10. Cattle were declared treatment successes on Day 10 if they met the following criteria: depression score ≤ 1 , and respiratory character score ≤ 1 , and rectal temperature $< 40^{\circ}\text{C}$. The following scales for scoring depression and respiratory character were used:

The depression scores use the scale:

- 0 = Normal
- 1 = Mild depression

- 2 = Moderate depression
- 3 = severe depression
- 4 = Moribund

The respiratory character scores used the scale:

- 0 = Normal
- 1 = Mild respiratory distress
- 2 = Moderate respiratory distress
- 3 = Severe respiratory distress.

Safety Endpoints

All animals were observed for adverse experiences once daily after treatment on Days 0 to 10. Animals that were removed from their respective pen(s) for humane reasons were examined to determine the reason for removal. Animals were observed for visible injection site reactions daily.

Statistical Analyses

Comparisons were made between groups with regard to the proportion of treatment successes using the generalized linear mixed model in the GLIMMIX procedure in SAS® version 9.1.3. The proportion of treatment successes within each pen was the variable analyzed, with treatment group the only fixed effect and pen and pen × treatment interaction the two random effects. The logit link was used.

Rectal temperatures were compared on Day 10 using the general linear mixed model in the MIXED procedure in SAS®, with treatment group the only fixed effect, and the randomization replicate pair the only random effect.

Table 2. Treatment successes and failures for gamithromycin or saline to treat bovine respiratory disease in calves positive for *Mycoplasma bovis* at the time of enrollment

Site	Total Animals Positive for <i>M. bovis</i>	Treatment Group	Treatment Success on Day 10	Treatment Failure on Day 10
1	57	Gamithromycin	45 (79.0%)	12 (21.1%)
		Saline control	5 (13.9%)	31 (86.1%)
2	6	Gamithromycin	5 (83.3%)	1 (16.7%)
		Saline control	0 (0.0%)	3 (100.0%)

RESULTS

At Site 1, cattle were evenly allocated such that there were 121 in each group. At Site 2, data were collected and analyzed for 129 calves in the gamithromycin group and 130 in the control group.

Pretreatment Testing for *M. bovis*

Ninety-three of the 242 cattle (38.4%) at Site 1 had positive results for *M. bovis* on the microbiologic culture test and confirmed by PCR for the nasopharyngeal swabs taken immediately prior to treatment, including 57 in the gamithromycin treatment group (47.1%) and 36 in the saline control group (29.8%).

At Site 2, nine of the 259 cattle (3.5%) had positive results for *M. bovis* on the microbiologic culture test and confirmed on PCR test prior to treatment, including six in the gamithromycin group (4.7%) and three in the saline control group (2.3%).

Treatment Successes Overall

The analysis of treatment successes for each site is shown in Table 1. At Site 1, 74.4% of the 121 cattle treated with gamithromycin were considered treatment successes (no clinical signs of BRD) on Day 10 compared with 24.0% of the 121 cattle in the saline control group ($P < 0.001$).

At Site 2, 67.4% of the 129 cattle treated with gamithromycin were treatment successes compared with 46.2% for the saline control group ($P = 0.002$).

Treatment Successes for *M. bovis*-positive Animals

The distribution of treatment successes on Day 10 for animals at both sites with pre-treatment nasopharyngeal swabs positive for *M. bovis* by microbiologic culture and PCR tests are shown in Table 2. At Site 1, 45 of the 57 animals positive for *M. bovis* in the gamithromycin treatment group (79.0%) were treatment successes on Day 10 versus five of the 36 animals (13.9%) in the control group.

At Site 2, five of the six animals positive for *M. bovis* in the gamithromycin treatment group (83.3%) were treatment successes on Day 10, whereas none of the three animals in the control group were treatment successes (Table 2).

Pooled results from the two sites are presented in Table 3. Overall, at the two sites, 68.5% of the animals positive for *M. bovis* at the start of the study and treated with gamithromycin were treatment successes compared with only 12.8% of the control animals.

Clinical Findings

At Site 1, eight animals in the control group were found dead in their pens during the study after Day 0. Lung tissue culture and PCR results were positive for *M. bovis* for four of the eight animals. Twenty-two cattle, including two treated with gamithromycin and 20 controls, were removed from study

after Day 0 for severe BRD-related reasons. The affected animals were humanely euthanized, and necropsies revealed cardiopulmonary lesions consistent with a diagnosis of BRD. Nineteen of the 20 control animals euthanized were *M. bovis* positive by culture and PCR. *M. haemolytica*, *P. multocida*, and *H. somni* were found in lung tissue cultures of control animals, but only *M. haemolytica* was found in animals treated with gamithromycin. No deaths or early removal of cattle occurred at Site 2.

At Site 1, the least squares mean rectal temperature measured for the saline control group on Day 10 was 39.7°C, compared with 39.5°C for cattle in the gamithromycin group (P<0.001) (Table 4). At Site 2, the least squares mean rectal temperature was 39.4°C for the saline control group, compared to 39.3°C for the gamithromycin group (P=0.231).

Adverse experiences

No injection site reactions or clinical adverse experiences attributed to treatment were observed from any animal at either site throughout the study period.

DISCUSSION

Before treatments were initiated at Site 1, *M. bovis* was isolated from nearly half of the calves that were subsequently treated with gamithromycin and from approximately 30% of the control calves; however, only 5% of the calves in the gamithromycin group and 2% of the calves in the control group demonstrated *M. bovis* in nasopharyngeal swabs cultured before treatment at Site 2. Nevertheless, other BRD pathogens were

isolated from several cattle, and clinical signs that differentiated treatment successes from treatment failures were evident at both sites. At each site, the number of treatment successes was significantly higher in the gamithromycin treatment group than in the control group. Pooled data from the two sites revealed a remarkable difference in the percentage of treatment successes for cattle positive for *M. bovis* achieved by treating with gamithromycin compared to saline controls (68.5% vs. 12.8%).

Since first being isolated from cattle with severe mastitis in 1961, infection with *M. bovis* has been reported throughout the world.⁷ *Mycoplasma bovis* is an important target for treatment of BRD in beef calves during the early weeks in the feedlot, particularly since there is increasing evidence that *M. bovis* is immunosuppressive, compromising host defenses and predisposing the animal to invasion by other bacterial pathogens.^{7,8}

There is evidence in the studies described here that *M. bovis* might be a factor for the development of more severe signs of BRD in cattle. *Mycoplasma bovis* was isolated from only nine calves at Site 2 (3.5%), and although it is clear that other BRD pathogens were present in the cattle in that study, rectal temperatures and clinical scores were not as severely elevated as for cattle at Site 1, where *M. bovis* was detected in 38% of the cattle in the study. These observations are evidence for a potential synergistic effect of *M. bovis* with other BRD pathogens leading to more severe disease expression.

It is expected that the prevalence of *M. bovis*

Table 3. Pooled distribution of treatment successes on Day 10 for Cattle Positive for *M. bovis* by Microbiologic Culture and PCR Tests

Treatment Group	Treatment Successes on Day 10	Treatment Failures on Day 10	Total Number of Animals Positive for <i>M. bovis</i>
Gamithromycin	50 (68.5%)	13 (20.6%)	63 (100%)
Saline Control	5 (12.8%)	34 (87.2%)	39 (100%)

is frequently underestimated because other respiratory pathogens are generally isolated first from calves with signs of pneumonia, and few laboratories routinely monitor for the presence of mycoplasmas.⁷ Although *M. haemolytica*, *P. multocida*, and *H. somni* are generally the most common isolates associated with cases of bronchopneumonia, there has been a noticeable increase in the prevalence of chronic pneumonia and polyarthritis associated with *M. bovis*.^{3,4,9-12}

Chronic pneumonia and polyarthritis caused by *M. bovis* in calves are often refractory to antibiotic treatment.²⁻⁶ In particular, *M. bovis* is resistant to antibiotics of the β lactam family, which express their activity by inhibiting synthesis of the bacterial cell wall (a feature that is lacking in the Mollicutes).^{1,2} Other antibiotics of various classes, such as streptomycin, neomycin, erythromycin, and flumequin have been found ineffective against *M. bovis*.² Poumarat² reported that spectinomycin, an aminocyclitol antibiotic, a class that is closely related to the aminoglycosides, administered at 20 mg/kg, significantly reduced *M. bovis* counts in the lungs, but the treatment did not significantly reduce in lung lesions.

Gamithromycin is an azalide member of the macrolide antibiotics and is currently licensed for treatment of BRD pathogens *M. haemolytica*, *P. multocida*, and *H. somni* in the United States, Canada and Europe, and for control of BRD pathogens *M. haemolytica*, *P. multocida*, and *H. somni* in Canada and Europe.^{6,13,14} Azalide macrolides are particularly effective for treatment of upper and lower respiratory infections because of their excellent potency against the organisms responsible for those infections and their ability to achieve high concentrations in lung macrophages and in epithelial lining fluid of the bronchioles, where BRD pathogens, such as *M. bovis*, multiply and cause extensive damage.¹⁴⁻¹⁶ Several macrolide antibiotics, including erythromycin, tylosin, tilmicosin, spiramycin, and tulathromycin, are approved for treatment and control of BRD in cattle in the United States and other

countries.¹⁵ Although these compounds are generally well absorbed and reach effective concentrations in lung tissue, many of them bind extensively to plasma proteins, which restrict their extravascular distribution.¹⁴⁻¹⁶ However, only 26% of gamithromycin binds to bovine plasma protein.^{6,14} Maximum plasma concentrations of gamithromycin are reached 30 minutes after subcutaneous dosing, and distribution into lung tissue is rapid and extensive, reaching peak concentrations by 24 hours. Concentrations of gamithromycin in lung tissue are prolonged as a result of its long elimination half-life (90.4 hours) in those tissues.¹⁴

Because there are still many unknowns about the epidemiologic features of this pathogen, it has been difficult to establish practices that will effectively prevent transmission and infection of cattle subjected to the stresses associated with handling and transport to the feedlot. Therefore, treatment with effective antibiotics, such as gamithromycin, remains an important method for control of *M. bovis* infection for young calves.

CONCLUSION

The results of these studies showed that gamithromycin provided a significantly higher percentage of bovine respiratory disease treatment successes than did treatment with saline control, ($P < 0.001$). Furthermore, the percentage of treatment successes was remarkably higher in the gamithromycin treatment group that tested positive for *M. bovis* by microbiologic culture and PCR immediately prior to treatment, compared to control group. Safety and tolerability of gamithromycin was similar to saline control.

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