Efficacy of Gamithromycin Injectable Solution for the Treatment of *Mycoplasma bovis* Induced Pneumonia in Cattle

Kelly Lechtenberg, DVM, PhD[1]

Ronald K. Tessman, DVM, PhD, DACVIM[2]

S. Theodore Chester, PhD[2]

[1]*Midwest Veterinary Services, Oakland, NE 68045*

[2]*Merial, 3239 Satellite Blvd., Duluth, GA 30096-4640*

KEY WORDS: colostrum-deprived, cross-bred beef calves,gamithromycin,clinical pneumonia

ABSTRACT

Forty colostrum-deprived, cross-bred beef calves, including males and females 3 to 8 months of age, were procured and transported to the test facility 6 days before initiation of the study. On Day -2, each calf received 60 mL of challenge material by endobronchial infusion. Calves were randomly assigned to either Group 1 (saline at 2.0 mL/50 kg body weight by subcutaneous injection) or Group 2 (gamithromycin 6.0 mg/kg [2.0 mL/50 kg body weight] by subcutaneous injection).

Clinical assessments of depression scores, respiratory character scores, and rectal temperatures were conducted daily from Day -2 (prior to challenge) through Day 10. Calves were humanely euthanized and necropsy examinations were conducted on Day 10. Lungs were harvested and scored according to the percentage of pneumonic tissue. Each lung lobe was palpated and the percentage consolidation on the dorsal and ventral surface of each lung lobe was estimated and recorded. Calves treated with gamithromycin 6 mg/kg had lower mean depression scores, respiratory scores, and rectal temperatures than control calves from Day 1 through Day 10. On several post-treatment days, between-treatment differences were significant ($P \leq 0.05$). Treatment with gamithromycin also significantly reduced lung consolidation scores compared to untreated controls. Adverse reactions attributable to treatment were not observed from any calf treated with gamithromycin. It was concluded that a single subcutaneous injection of gamithromycin at 6 mg/kg body weight was effective in treating clinical pneumonia induced in young calves by endobronchial challenge with *M. bovis*.

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 likely to be the causative pathogen when pneumonia is chronic and unresponsive to antibiotics.[1,3-5] *Mycoplasma bovis* is resistant to antibiotics of the β lactam family, which express their activity by inhibiting synthesis of the bacterial cell wall. Although treatments have been effective against *M. bovis* in vitro, it is not uncommon to have treatments for this bacteria fail to resolve respiratory disease, arthritis, and mastitis in cattle.[1-5]

Gamithromycin is an azalide 15-membered semi-synthetic macrolide antibiotic that has been developed for treatment and prevention of BRD. Studies of the pharmacokinetic and pharmacodynamic properties and clinical effect of gamithromycin showed that a single subcutaneous dose at 6 mg/kg body weight provides rapid therapeutic and persistent activity in the control and treatment of BRD infections.[6-9] This study was conducted to evaluate the efficacy of gamithromycin in the treatment of pneumonia induced in young calves by endobronchial challenge with *M. bovis*.

MATERIALS AND METHODS

Animals

Forty colostrum-deprived, cross-bred beef calves, including males and females 3 to 8 months of age, were obtained from Logan Valley Feeders Colostrum Deprived Calf Unit in Oakland, NE. Calves were moved to the test facility (Midwest Veterinary Services, Oakland, NE) 6 days before challenge with *M. bovis*, individually identified by uniquely numbered duplicate ear tags, and housed as a single group.

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.

The housing area at the test facility was ventilated using high-efficiency particulate air filters (HEPA) on both the in-flow and out-flows. Heat was provided as needed using gas fired propane heaters located outside the room. Room temperature in the housing area during the acclimation and study periods ranged from 12.2°C (54ºF) to 23.3°C (74ºF). From the day of arrival at the test facility, animals were housed together in a single group and were fed a complete, pelleted calf ration containing monensin at the rate of 30 g/ton for coccidia prevention and control. Water was supplied ad libitum by an automatic water tank.

Inclusion/Exclusion Criteria

Animals selected for participation in the study had no history of bacterial pneumonia, vaccination against *M. bovis*, or history of receiving any therapeutic antibiotics in the 7 days prior to challenge.

Evidence of clinical pneumonia from 1 to 3 days after *M. bovis* challenge qualified an animal for inclusion in the study. Criteria for enrollment included the following:

Depression score ≥1, and

Respiratory character score ≥1, and

Rectal temperature ≥39.7°C (103.5°F)

Animals that did not meet protocol criteria were not enrolled for evaluation of the efficacy of gamithromycin. Thirty-one of the 40 animals met enrollment criteria 2 days after challenge (Day 0). One of the 31 animals was not enrolled due to its overall condition and attitude; leaving 30 calves eligible for the study. Animals that were not enrolled were treated with tulathromcyin according to label recommendations and returned to source of acquisition.

Mycoplasma bovis Challenge Preparation

Challenge material was prepared in the laboratory at the test site. Friis broth media was prepared and checked for sterility according

to the standard operating procedure at the laboratory. No contamination was noted for either the agar plates or the broth. Frozen stock culture of *M. bovis* (KL100) was thawed at room temperature. Once thawed, stock culture was added to Friis broth and streaked onto two Friis agar plates. All were incubated at 37ºC for 48 hours in a 5% CO2 incubator. The broth–stock culture was checked for contamination and was determined to be free of contamination. The Friis agar plates developed for the growth and determination of *M. bovis* bacterial colonies revealed typical morphology of *M. bovis*. The broth-stock culture was transferred to approximately 2800 mL of Friis broth and again incubated as described above. A 10-mL aliquot of this material, after incubation, was taken for titrations. Two plates were prepared in the dilution of 10^6 to determine the number of colony forming units (CFU) present in 60 mL of challenge material. Individual plate counts were 43 for plate 1 and 36 for plate 2, providing 79 colonies for the two plates. The number of bacteria per mL of challenge material was determined to be 39.5×10^7 CFU.

Prior to infection of calves, the *M. bovis* isolate used (KL 100, isolated in 2005 at Logan Valley Feeders), was cultured to determine the strain present. Minimum inhibitory concentration (MIC) testing was performed to obtain an indication of the in vitro susceptibility of the challenge strain to gamithromycin. The MIC tests were performed according to standard operating procedures prepared by the testing laboratory to comply with CLSI (Clinical Laboratory Standards Institute) standard methods, although at the time of this writing, specific CLSI recommendations had not been published for testing *Mycoplasma* spp. Briefly, the isolates were subcultured in Hayflick agar to ensure purity and each was susceptibility tested using MIC plates prepared at the test facility laboratory. The KL 100 isolate had an MIC of 4 µg/mL for gamithromycin. The results indicated that the challenge strain was adequately susceptible to gamithromycin and therefore suitable for use in this efficacy study.

***Mycoplasma bovis* Challenge**

On Day -2, all 40 calves each received 60 mL of challenge material (2.37×10^{10} CFU *M. bovis*) by endobronchial infusion. An additional 60 mL of sterile saline and 60 mL of air was used to flush the endoscope port following challenge administration. Signs of clinical pneumonia were not observed from any animal prior to challenge or on the day of challenge administration. Animal observations began immediately prior to challenge on Day -2, and continued until animals met enrollment criteria (depression score ≥1, respiratory character score ≥ 1 and a rectal temperature ≥39.7°C [103.5ºF]).

Allocation

Day 0 was the same for all animals in the study, and all animals in a replicate were enrolled and treated on the same day. On Day 0, animals were ranked by increasing body weight and allocated consecutively to replicates of two animals each. Within a replicate, animals were allocated by a randomization schedule prepared by a Merial biostatistician in a 1:1 ratio of animals treated with gamithromycin to animals treated with saline. A randomized complete block design was applied for the randomization using the PROC PLAN procedure of SAS® version 8.2.

Treatment

Treatments were assigned as follows:

Group 1 = saline (2.0 mL/50 kg body weight)

Group 2 = Gamithromycin 6.0 mg/kg; (2.0 mL/50 kg body weight)

Animals were dosed at 2.0 mL/50 kg body weight, with doses rounded up to the nearest 0.1 mL when necessary. All treatments were administered subcutaneously on the right side of the neck. No drug was lost at treatment administration. No adverse experiences/health issues related to drug administration were observed for any animal on Day 0.

Primary Endpoints

Clinical assessments were conducted daily from Day -2 (prior to challenge) through

Day 10 at approximately the same time of day. Assessments were conducted by the same individual, except for Days 8 and 9 when, due to scheduling conflicts, another trained individual assessed the animals. The following 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 daily using calibrated thermometers from Day -2 (pre-challenge) through Day 10. Temperatures on Day 0 were recorded before treatments were administered.

Percentage Lung Consolidation

Calves were humanely euthanized and necropsy examinations were conducted on Day 10. Lungs were harvested and scored according to the percentage of pneumonic tissue. Each lung lobe was palpated and the percentage consolidation on the dorsal and ventral surface of each lung lobe was estimated and recorded. The percentage of consolidation for the total lung was calculated by multiplying the percentage consolidation of each lobe by the contribution of that specific lobe to the total lung weight. The percent weight of each of the lobes 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). The same person made the consolidation estimates for all calves.

Secondary Endpoints

Microbiology

Samples were collected from one of the affected lobes that had lung lesions present for microbiological determinations. Lung/pneumonic lobe samples were transferred directly from the necropsy facility to the microbiology laboratory. Tissue samples were tested for *H. somni*, *P. multocida*, *M. bovis*, and *M. haemolytica*. All samples were processed on the same day they were collected.

The microbiology lab made swabs from all 30 lung tissue samples, which were streaked onto blood agar, chocolate agar, and Friis agar plates. All plates were incubated at 37ºC overnight in a 5% CO2 incubator. All plates were screened the next day for bacterial growth to determine if further incubation was required. This procedure was followed until there was either bacterial growth determined or a determination of no bacterial growth found (3 days after initial inoculation). All isolates positive for *M. bovis* were confirmed by PCR. *Mycoplasma bovis* isolates were stored frozen at -70ºC for possible future sensitivity determinations to gamithromycin and/or other antimicrobials.

Statistical Analysis

The arcsine-square root transformed percent total lung consolidation scores were analyzed as a general linear mixed model using the MIXED procedure from SAS® version 9.1.3. Treatment group was the fixed effect and replicate was the random effect.

Depression and respiratory character

scores were compared between treatment groups by the Wilcoxon rank sum test using the NPAR1WAY procedure in SAS® version 9.1.3 separately for each day. Rectal temperatures were analyzed as a repeated measures general linear model using the MIXED procedure. Treatment, day, and treatment-by-day interaction were fixed effects, and replicate and replicate-by-treatment were random effects. The first-order autoregressive covariance structure was used with the subject identified by the replicate-by-treatment interaction. The Kenward-Roger option was used to estimate the degrees of freedom.

The null hypothesis that the two treatment groups shared the same expected mean versus the alternative hypothesis that their expected means were different was tested using the (two-sided) α=0.05 significance level.

RESULTS

Clinical endpoints

Mean depression scores are plotted in Figure 1. The mean depression score was numerically lower for the gamithromycin group than for the control group throughout the entire study, and the difference between groups was significant on Days 3–6 ($P \leq 0.05$).

Mean respiratory scores are plotted in Figure 2. The mean respiratory character score for the group treated with gamithromycin was lower than for the control group on each day following initiation of treatment, and the difference between groups was significant on Days 3–6, 8, and 10 ($P \leq 0.01$).

The mean rectal temperature for the gamithromycin group was lower than the control group throughout the post-treatment period (Figure 3). Differences between treatments were significant on Days 1, and 3–9 ($P \leq 0.05$).

Lung consolidation scores

Mean total lung consolidation scores were 14.0% for the control group and 4.6% for the gamithromycin group. The difference was statistically significant (P=0.024).

Microbiological endpoints

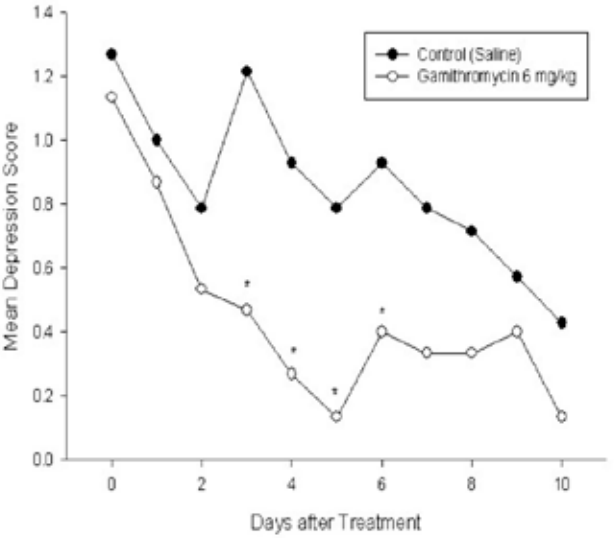
In the saline control group, 11 animals tested positive for one or more bacteria. Of these, eight tested positive for *M. bovis*, two for *H. somni*, seven for *P. multocida*, and one for *M. haemolytica*. Four control animals were negative for any bacterial growth. In the gamithromycin group, 11 animals tested positive, including seven that were positive for *M. bovis* and six for *P. multocida*. No animals were positive for *M. haemolytica* or *H. somni*.

Additional swabs were collected from the right carpal joint of animal #16 (control) and the right tarsal joint of animal #26 (gamithromycin) that became lame during the study. These swabs were handled and evaluated the same as for lung tissue swabs. Both samples tested positive for *M. bovis*.

Adverse experiences

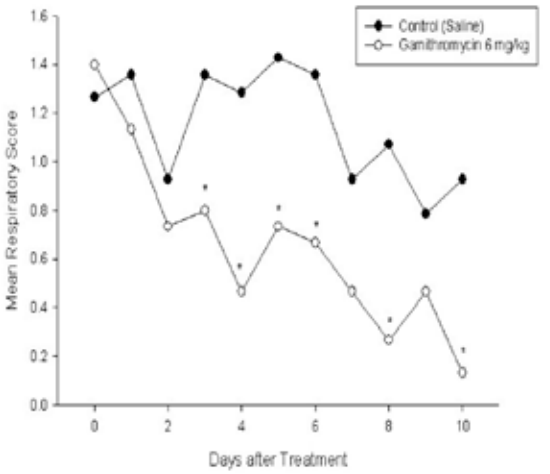
Lameness observed from two calves (one

***Figure 1**. Mean depression scores*



** Significantly different from control group; P ≤ 0.05*

Figure 2. *Mean respiratory character scores*



** Significantly different from control group; P ≤ 0.05*

from each treatment group) during the post-treatment period was noted by the investigator as being unrelated to treatment and was subsequently found to be caused by the presence of *M. bovis* in a leg joint. No deaths occurred in the group of calves treated with gamithromycin; however, one calf (#37) in the control group died 3 days after challenge (1 day after administration of saline). Calf #37 (control group) was found dead in the pen. A necropsy was performed, which provided a diagnosis of fibrous bronchopneumonia. It was determined that the animal suffered from an acute pneumonia. This death was determined to be a result of the study challenge and was not recorded as an adverse experience.

DISCUSSION

The *M. bovis* challenge method used in this study was successful in inducing clinical signs and lung lesions associated with clinical pneumonia in 31 of the 40 calves challenged on Day -2. The 30 calves that met the criteria for induction of clinical pneumonia and were otherwise suitable for inclusion in the study all had depression and respiratory scores ≥1 and rectal body temperatures ≥39.7°C [103.5ºF]) on Day 0, the designated day of treatment. *Mycoplasma bovis* and *M. haemolytica* were isolated from lung tissues collected from one calf that died on Day 1. Calves treated with gamithromycin 6 mg/kg had lower mean depression scores, respiratory scores, and rectal temperatures than control calves from Day 1 through to the end of the study (Day 10). On several post-treatment days, the mean values for these clinical signs of respiratory disease were significantly lower (P≤0.05) for calves treated with gamithromycin than for calves in the control group. Treatment with gamithromycin also significantly reduced lung consolidation scores compared with those for untreated controls (P≤0.05).

Pneumonia was the most prevalent manifestation of *M. bovis* infection in calves humanely euthanized and necropsied on Day 10; however, *M. bovis* also was isolated from a carpal joint in one calf and from the tarsal joint of a second calf that developed lameness during the study.

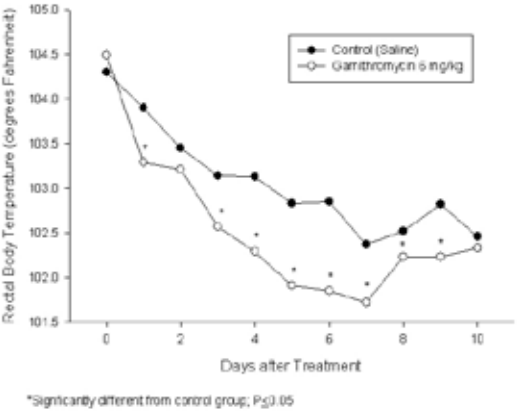
Mycoplasma bovis was first isolated from cattle with severe mastitis in 1961 in the United States.[10] Since that time, infection with *M. bovis* has been reported throughout the world.[10] *Mycoplasma bovis* is an important target for treatment of BRD in beef calves during the early weeks in the feedlot, particularly since there is increasing evidence that *M. bovis* is immunosuppressive, compromising host defenses and predisposing the animal to invasion by other bacterial pathogens.[10,11] It is expected that the prevalence of *M. bovis* 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.[10] *Mannheimia haemolytica*, *P. multocida*, and *H. somni* were the most common isolates associated with cases of bronchopneumonia in several studies in Canadian and US feedlots; however, following a 1989 report of an outbreak of pneumonia in the UK, there has been a noticeable increase in the prevalence of

chronic pneumonia and polyarthritis associated with *M. bovis*.[3,4,12-15]

Chronic pneumonia and polyarthritis caused by *M. bovis* in calves are often refractory to antibiotic treatment.[2-5,16] 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 streptomycin, neomycin, erythromycin, and flumequin.[2] Poumarat[2] 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 provide significant reduction in lung lesions.

Gamithroymcin is an azalide member of the macrolide antibiotics and is currently licensed for treatment and control of BRD pathogens *M. haemolytica*, *P. multicida*, and *H. somni* in Canada and Europe.[6,17,18] 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.[17,19,20] 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.[20] Although these compounds are generally well absorbed and reach effective concentrations in lung tissue, many of them bind extensively to plasma proteins, which restricts their extravascular distribution.[6,19,20] However, only 26% of gamithromycin binds to bovine plasma protein.[6,17] Maximum plasma concentrations of gamithromycin are reached 1 hour after subcutaneous dosing,

***Figure 3**. Mean rectal temperatures*



** Significantly different from control group; P ≤ 0.05*

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.[6]

Management procedures to prevent infection by *M. bovis* would be preferred to treatment; however, 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 in young calves.

CONCLUSION

A single subcutaneous injection of gamithromycin at 6 mg/kg body weight was effective in treating clinical pneumonia induced in young calves by endobronchial challenge with *M. bovis*.

REFERENCES

1. Maunsell FP, Donovan GA. *Mycoplasma bovis* Infections in young calves. *Vet Clin North Am Food Anim Pract* 2009;25:139-77, vii.
2. Poumarat F, Le GD, Philippe S et al. Efficacy of spectinomycin against *Mycoplasma bovis* induced

pneumonia in conventionally reared calves. *Vet Microbiol* 2001;80:23-35.

3. Wiggins MC, Woolums AR, Sanchez S et al. Prevalence of *Mycoplasma bovis* in backgrounding and stocker cattle operations. *J Am Vet Med Assoc* 2007;230:1514-1518.
4. Gagea MI, Bateman KG, Shanahan RA et al. Naturally occurring *Mycoplasma bovis*-associated pneumonia and polyarthritis in feedlot beef calves. *J Vet Diagn Invest* 2006;18:29-40.
5. Gagea MI, Bateman KG, van DT et al. Diseases and pathogens associated with mortality in Ontario beef feedlots. *J Vet Diagn Invest* 2006;18:18-28.
6. Huang RA, Letendre LT, Banav N, Fischer J, Somerville B. Pharmacokinetics of gamithromycin in cattle with comparison of plasma and lung tissue concentrations and plasma antibacterial activity. *J Vet Pharmacol Ther* 2010;33:227-237.
7. Giguère S, Huang R, Malinski TJ, et al. Disposition of gamithromycin in plasma, pulmonary epithelial lining fluid, bronchoalveolar cells, and lung tissue in cattle. *Am J Vet Res.* 2011 March;72(3):326-330.
8. Lechtenberg K, Daniels CS, Royer GC, et al. Field efficacy study of gamithromycin for the control of bovine respiratory disease in cattle at high risk of developing the disease. *Intern J Appl Res Vet Med*. 2011;9(2):189-197.
9. Sifferman RL, Wolff WA, Holste JE, Smith LL, et al. Field efficacy evaluation of gamithromycin for treatment of bovine respiratory disease in cattle at feedlots. *Intern J Appl Res Vet Med*. 2011;9(2):171-180.
10. Nicholas RA, Ayling RD. *Mycoplasma bovis*: disease, diagnosis, and control. *Res Vet Sci* 2003;74:105-112.
11. Vanden Bush TJ, Rosenbusch RF. Characterization of the immune response to *Mycoplasma bovis* lung infection. *Vet Immunol Immunopathol* 2003;94:23-33.
12. Arcangioli MA, Duet A, Meyer G et al. The role of *Mycoplasma bovis* in bovine respiratory disease outbreaks in veal calf feedlots. *Vet J* 2008;177:89-93.
13. Duff GC, Galyean ML. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. *J Anim Sci* 2007;85:823-840.
14. Gevaert D. [The importance of *Mycoplasma bovis* in bovine respiratory disease]. *Tijdschr Diergeneeskd* 2006;131:124-126.
15. White BJ, Hanzlicek G, Sanderson MW, Anderson DE, Larson RL. Mollicutes species and *Mycoplasma bovis* prevalence and association with health outcomes in beef feeder calves at arrival and initial treatment for bovine respiratory disease. *Can Vet J* 2010;51:1016-1018.
16. Shahriar FM, Clark EG, Janzen E, West K, Wobeser G. Coinfection with bovine viral diarrhea virus and *Mycoplasma bovis* in feedlot cattle with chronic pneumonia. *Can Vet J* 2002;43:863-868.
17. Forbes AB, Ramage C, Sales J, Baggott D, Donachie W. Determination of the duration of antibacterial efficacy following administration of gamithromycin using a bovine Mannheimia haemolytica challenge model. *Antimicrob Agents Chemother* 2010.
18. Sgoifo Rossi CA, Vandoni SL, Bonfanti M, Forbes AB. Effects of arrival medication with gamithromycin on bovine respiratory disease in feedlot cattle in Italy. *Intern J Appl Res Vet Med* 2010;8:87-96.
19. Jain R, Danziger LH. The macrolide antibiotics: a pharmacokinetic and pharmacodynamic overview. *Curr Pharm Des* 2004;10:3045-3053.
20. Zhanel GG, Dueck M, Hoban DJ et al. Review of macrolides and ketolides: focus on respiratory tract infections. *Drugs* 2001;61:443-498.