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# Pharmacokinetics and pharmacodynamics of gamithromycin in pulmonary epithelial lining fluid in naturally occurring bovine respiratory disease in multisource commingled feedlot cattle

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## Pharmacokinetics and pharmacodynamics of gamithromycin in pulmonary epithelial lining fluid in naturally occurring bovine respiratory disease in multisource commingled feedlot cattle

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DeDonder, K. D., Apley, M. D., Li, M., Gehring, R., Harhay, D. M., Lubbers, B. V., White, B. J., Capik, S. F., Kukanich, B., Riviere, J. E., Tessman, R. K. Pharmacokinetics and pharmacodynamics of gamithromycin in pulmonary epithelial lining fluid in naturally occurring bovine respiratory disease in multisource commingled feedlot cattle. *J. vet. Pharmacol. Therap.* doi: 10.1111/jvp.12267.

The objectives of this study were to determine (i) whether an association exists between individual pharmacokinetic parameters and treatment outcome when feeder cattle were diagnosed with bovine respiratory disease (BRD) and treated with gamithromycin (Zactran®) at the label dose and (ii) whether there was a stronger association between treatment outcome and gamithromycin concentration in plasma or in the pulmonary epithelial lining fluid (PELF) effect compartment. The study design was a prospective, blinded, randomized clinical trial utilizing three groups of 60 (362–592 lb) steers/bulls randomly allocated within origin to sham injection or gamithromycin mass medication. Cattle were evaluated daily for signs of BRD by a veterinarian blinded to treatment. Animals meeting the BRD case definition were enrolled and allocated to a sample collection scheme consisting of samples for bacterial isolation (bronchoalveolar lavage fluid and nasopharyngeal swabs) and gamithromycin concentration determination (PELF and plasma). Gamithromycin susceptibility of *M. haemolytica* ( $n = 287$ ) and *P. multocida* ( $n = 257$ ) were determined using broth microdilution with frozen panels containing gamithromycin at concentrations from 0.03 to 16  $\mu\text{g/mL}$ . A two-compartment plasma pharmacokinetic model with an additional compartment for gamithromycin in PELF was developed using rich data sets from published and unpublished studies. The sparse data from our study were then fit to this model using nonlinear mixed effects modeling to estimate individual parameter values. The resulting parameter estimates were used to simulate full time–concentration profiles for each animal in this study. These profiles were analyzed using noncompartmental methods so that PK/PD indices ( $\text{AUC}_{24}/\text{MIC}$ ,  $\text{AUC}_{\infty}/\text{MIC}$ ,  $\text{C}_{\text{MAX}}/\text{MIC}$ ) could be calculated for plasma and PELF (also  $\text{T} > \text{MIC}$ ) for each individual. The calculated PK/PD indices were indicative that for both *M. haemolytica* and *P. multocida* a higher drug exposure in terms of concentration, and duration of exposure relative to the MIC of the target pathogen, was favorable to a successful case outcome. A significant association was found between treatment success and PELF  $\text{AUC}_{0-24}/\text{MIC}$  for *P. multocida*. The calves in this study demonstrated an increased clearance and volume of distribution in plasma as compared to the healthy calves in two previously published reports. Ultimately, the findings from this study indicate that higher PK/PD indices were predictive of positive treatment outcomes.

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## INTRODUCTION

Gamithromycin (ZACTRAN®; Merial Animal Health, Duluth, GA, USA), a macrolide of the azalide subclass, is approved for both treatment of BRD caused by *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* and the control of BRD caused by *M. haemolytica* and *P. multocida* (Food and Drug Administration (FDA), 2011). Macrolide antibiotics, in general, are bacteriostatic through the inhibition of bacterial RNA-dependent protein biosynthesis (Jain & Danziger, 2004). However, gamithromycin can also be bactericidal with minimum bactericidal concentrations only 1 dilution higher than the respective MIC (Huang *et al.*, 2010). The tendency for macrolides, especially azalides, to accumulate in inflamed tissue has been described previously (Amsden, 2001). This fact coupled with the known extensive tissue distribution of macrolides has sparked interest in the veterinary literature concerning the exposure–response relationship at the site of action as it pertains to the newer long-acting injectable macrolide formulations (Nowakowski *et al.*, 2004; Womble *et al.*, 2006; Venner *et al.*, 2010; Menge *et al.*, 2012; Villarino *et al.*, 2013a,b, 2014).

Recent work examining the concentrations of antibiotics in PELF of healthy animals has been performed to describe the disposition of gamithromycin in beef calves (Giguere *et al.*, 2011). Giguere *et al.* found that gamithromycin was rapidly absorbed and reached potentially therapeutic concentrations in PELF within 30 min after s.c. administration. To the authors' knowledge, all previous work describing the distribution of these drugs in cattle has been performed in healthy subjects and there are no publications describing the PK and PD of a macrolide class antibiotic in the PELF under the conditions of naturally occurring BRD. To that end, the objectives of this study were to (i) develop a compartmental PK model based upon existing PK data, (ii) use sparse data collected in this study to estimate parameter values for the animals in this study population, (iii) use the model estimated parameters to simulate complete concentration–time profiles of gamithromycin in the central and PELF effect compartments of each animal, (iv) determine if a relationship exists between plasma and/or PELF concentrations and treatment outcome, and finally (v) determine the PK/PD indices associated with treatment success in naturally occurring BRD.

## MATERIALS AND METHODS

### *Animals and husbandry*

One hundred and eighty cattle judged to be at high risk for BRD [overall average body weight of 470 pounds (362–592 lbs)] were sourced from Athens, Tennessee ( $n = 60$ ), Richmond, Kentucky ( $n = 60$ ), and Maryville, Missouri ( $n = 60$ ), as part of another study. Commingled steers and bulls of multiple origins and mixed breeds were acquired at each sale barn and transported to a small research feeding facility in Kansas where

they were housed in open air, dirt floor group housing pens for the duration of the trial.

### *Study design and treatment allocation*

The study design was a prospective, blinded, randomized clinical trial with masked subjective evaluators and was approved by the Kansas State University Institutional Animal Care and Use Committee.

Cattle were randomized to one of two treatments prior to arrival (by ear tag ID administered at the sale barn) so that each load was randomly allocated into two pens per source, one for each treatment (6 total pens in the study, 3 for each treatment). At initial processing, cattle assigned to treatment 1 served as untreated controls (CON) receiving saline at 2 mL/110 pounds subcutaneously in the neck, while cattle randomly allocated to treatment 2 received treatment (MM) for the control of BRD with gamithromycin at the label dose of 6 mg/kg (2 mL/110 lb) subcutaneously in the neck. Additionally, all cattle received a modified live viral respiratory vaccine, clostridia vaccine, growth implant, injectable anthelmintic, and duplicate tags for study identification and were examined to ensure that no clinical signs of BRD were present on arrival. Once in their pens, cattle were fed a ration according to practices typical of the feedlot industry.

### *Clinical scoring and disease diagnosis*

Daily pen observations were performed by a veterinarian masked to treatment allocation. Clinical scoring was by exclusion, that is, only cattle scoring 1–4, as described in Table 1, were recorded on daily observation forms and brought to the chute for further evaluation. Cattle having a rectal temperature of  $\geq 104.0^\circ\text{F}$  ( $\geq 40.0^\circ\text{C}$ ) and a clinical score of  $\geq 1$  were diagnosed with BRD and included in this study. Cattle with a clinical score of  $\geq 1$  but not meeting the temperature requirements were returned to their home pen without treatment for further observation. Animals were clinically scored each day but were not eligible for treatment of BRD until the postcontrol

**Table 1.** Description of clinical scoring criteria used for daily clinical observations of feeder cattle to assist in diagnosis of bovine respiratory disease. Clinical scoring was performed by a veterinarian masked to study treatment allocation

Score	Description
0	No abnormal signs
1	Slower than pen mates but still perks up when approached; does not appear weak; actively follows movements with raised head
2	Stands with head lowered; perks up when approached but returns to depressed stance; moves slowly and falls to back of group; may display signs of weakness such as incoordination
3	Obviously very weak; difficulty in moving with group; raises head only when approached closely
4	Moribund, unable to rise

treatment moratorium had elapsed. Control animals were immediately eligible for treatment of BRD, and those receiving mass medication were eligible for treatment on study day 8 (7 day moratorium).

#### Sampling allocation and collection procedures

The day of enrollment (first diagnosis of BRD) was designated as Day 0 for each calf. Cattle meeting inclusion criteria for the study were randomly allocated to a sample collection scheme consisting of a collection of NPS, BAL, and plasma (Table 2). Allocation to sample collection procedures was performed in advance, in blocks of three, to ensure that there was an equal distribution across sampling time points in order to account for the fact that actual number of cases of naturally occurring BRD was unknown beforehand. Samples collected on Day 0 were collected just prior to treatment with gamithromycin. All treatments and procedures at the chute were performed by trained personnel not involved in clinical scoring of cattle. The veterinarian responsible for clinical scoring was not present for treatments and procedures performed at the chute and therefore remained masked throughout the duration of the study period. Deep nasopharyngeal swabs and an aliquot of BAL fluid were immediately sent for bacterial culture, while samples of plasma and BAL fluid were processed and frozen at  $<-70^{\circ}\text{C}$  for later analysis by HPLC-MS/MS.

The BAL procedure was used to collect PELF fluid from manually restrained, nonsedate cattle. The BAL tube (Bivonna, BAL-240) was introduced into the trachea via the nasal passage and advanced until wedged into a deep bronchus. Sterile saline (240 mL) was infused in 60 mL aliquots and aspirated immediately after each aliquot. Recovered BAL fluid was collected into a 250 mL centrifuge tube, mixed well, divided evenly among four 50-mL centrifuge tubes, and then placed on ice and centrifuged in the feedlot laboratory within 40 min of collection. One randomly selected cell pellet was resuspended in liquid Amies media and submitted to the KSVDL for bacterial isolation. The BAL tubes were cleaned by plasma sterilization between collection procedures to prevent cross-contamination.

Deep nasopharyngeal swabs were collected from both nares by a veterinarian trained in the procedure. Briefly, a double guarded sterile uterine swab was introduced through the nares

**Table 2.** Sampling scheme for bronchoalveolar lavage (BAL), deep nasopharyngeal swabs (NPS), and plasma in cattle diagnosed with bovine respiratory disease (BRD). The top row is hours after treatment, and 0 h is just prior to treatment with gamithromycin. The number of cattle enrolled in each collection scheme is represented in the first column (labeled N)

N	0 h	12 h	24 h	120 h
8	BAL/Plasma/ NPS	Plasma	Plasma	BAL/Plasma/NPS
9	Plasma/NPS	BAL/Plasma/ NPS	Plasma	BAL/Plasma/NPS
9	Plasma/NPS	Plasma	BAL/Plasma/ NPS	BAL/Plasma/NPS

into the nasal cavity and guided to the point where resistance was met in the area of the nasopharyngeal tonsillar tissue. At this point, the double guarded swab was retracted slightly, the interior sleeve portion containing the swab was pushed through the exterior guard, and the swab advanced from the sleeve and rotated to ensure a sufficient sample of the mucous and tonsillar secretions from the pharyngeal tissues. The swab was retracted into the guarded sleeve to prevent contamination while exiting the nares. The swabs were placed in liquid Amies media and transported on ice to the KSVDL for bacterial culture and isolation.

Blood was collected into 10-mL sodium heparin vacutainer tubes via jugular venipuncture, and tubes were centrifuged in the feedlot laboratory at 500 g for 15 min. Plasma was pipetted into duplicate cryovials and stored along with other cryovial samples of BAL fluid (duplicates of PELF fluid, resuspended cell pellets, and urea analysis samples from both PELF and plasma) at  $<-70^{\circ}\text{C}$  until analysis.

#### Treatment administration and case outcome determination

Cattle diagnosed with BRD were randomly assigned to a sample collection scheme, treated with gamithromycin according to label directions (6 mg/kg) s.c. in the neck, and returned to the home pen. Case outcome was determined until Day 9, post-treatment. A treatment failure was defined as the calf meeting study inclusion criteria as previously described, if the calf was recorded a clinical score 3 regardless of rectal temperature, or if a calf died from BRD. Those cattle not categorized as a treatment failure by Day 9 were therefore considered treatment successes. Therefore, comparisons within this manuscript are between cattle that were deemed a treatment success versus those that were deemed a treatment failure.

#### Gamithromycin concentration analysis

Concentrations of gamithromycin in PELF and plasma were determined by Merial personnel masked to treatment. Samples were analyzed by reversed-phase HPLC with detection via MS/MS transitions by methods previously described (Giguere *et al.*, 2011). The limit of detection and limit of quantitation were 5 and 10 ng/mL, respectively. The concentration of gamithromycin in PELF was estimated using the ratio of urea in BAL fluid to that as measured in serum as described previously (Rennard *et al.*, 1986).

#### Bacterial isolation and MIC determination

Nasopharyngeal swabs and PELF samples were plated directly onto trypticase soy + 5% blood, chocolate, and MacConkey agar plates and incubated in 5% CO<sub>2</sub> at 37 °C for 18–24 h. Up to 12 colonies displaying growth characteristics typical of *M. haemolytica* and *P. multocida* were isolated in pure culture. Identity was confirmed using MALDI-TOF<sup>®</sup> (Bruker Daltonics, Billerica, MA, USA) and frozen for later susceptibility testing at the USMARC in Clay Center, Nebraska.

Gamithromycin susceptibility of *M. haemolytica* and *P. multocida* isolates was performed at USMARC by broth microdilution with frozen panels from TREK Diagnostic Systems® (ThermoFisher Scientific Inc., Waltham, MA, USA) containing gamithromycin at concentrations ranging from 0.03 to 16 µg/mL. Bacterial suspensions were prepared and susceptibility plates inoculated as per CLSI guidelines (Clinical and Laboratory Standards Institute VET01-A4, 2013). In brief, isolates were cultured on chocolate agar and incubated with increased CO<sub>2</sub> at 37 °C for 18–20 h. Bacterial suspensions equivalent to 0.5 McFarland standard were made by suspending 3 to 5 isolated colonies from each plate, into 5 mL of demineralized water. Mueller–Hinton broth tubes were then inoculated with 140 µL of the resulting bacterial suspensions. A 12-channel pipette was used to dispense 50 µL of this suspension into each of 12 wells in the panel, such that each panel could be used to evaluate the susceptibility of 8 strains. Plates were sealed and incubated at 37 °C for 18–20 h at which time the plates were visually inspected and MIC values determined by noting the lowest concentration of antibiotic that completely inhibited growth. Determination of susceptible, intermediate, or resistant was based off of clinical breakpoints established for gamithromycin by CLSI (Clinical and Laboratory Standards Institute VET01-A4, 2013).

#### Pharmacokinetic modeling and pharmacodynamics

A user-defined, two compartment plus PELF compartment was built in Phoenix NLME® (Certara L.P., Cary, NC, USA). The model schematic can be seen in Fig. 1. The differential equation describing this model is as follows:

$$\begin{aligned} \frac{dA_1}{dt} &= -(A_1 * k_{10}) + (A_a * K_a) - (A_1 * k_{12} - A_2 * k_{21}) \\ &\quad - (A_1 * k_{13} - A_{\text{pelf}} * k_{31}) \\ \frac{dA_2}{dt} &= (A_1 * k_{12} - A_2 * k_{21}) \\ \frac{dA_{\text{pelf}}}{dt} &= (A_1 * k_{13} - A_{\text{pelf}} * k_{31}) \end{aligned}$$

where  $A_1$  is the amount in central compartment,  $k_{10}$  is the elimination rate constant from the central compartment,  $A_a$

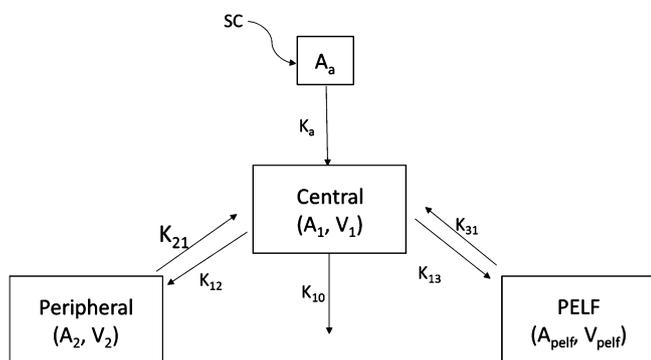


Fig. 1. Schematic representation of the final pharmacokinetic model for the two compartment plus PELF effect compartment for concentration of gamithromycin in feedlot cattle diagnosed with bovine respiratory disease.

is the amount at the site of the s.c. injection,  $K_a$  is the absorption rate constant from the site of the injection,  $k_{12}$  is the rate constant for the central to the peripheral compartment,  $A_2$  is the amount in the peripheral compartment,  $k_{21}$  is the rate constant for the peripheral to the central compartment,  $k_{13}$  is the rate constant for the central to the PELF compartment,  $A_{\text{pelf}}$  is the amount in the PELF compartment, and  $k_{31}$  is the rate constant for the PELF to the central compartment.

Data collected from published (Huang *et al.*, 2010; Giguere *et al.*, 2011) and unpublished (personal communication with coauthor RKT) PK trials of gamithromycin in cattle were used to generate initial estimates to develop the model. These data consisted of samples of plasma and PELF concentrations after administration of the label dosage of gamithromycin in healthy beef calves. When specific data were not available in the manuscript, data were extracted using an online tool, WebPlotDigitizer (<http://arohatgi.info/WebPlotDigitizer/>).

Next, the sparse data from this trial were integrated into the model using a nonlinear mixed effects approach, without the inclusion of covariates, to predict parameter values for each animal in our study. Parameter values were calculated for each individual animal within this study by use of the typical value and the individual ETA [e.g.,  $V = tvV^* \exp(nV)$ ]. Individual ETA values for each parameter of the PK model can be found online in supplemental materials. One hundred simulations were conducted, predicting complete plasma and PELF time–concentration curves for each animal in this study. Average simulated data were analyzed by NCA in order to compare the results of this study to those in the literature and to calculate PK/PD indices for plasma ( $AUC_{24}/MIC$ ,  $AUC_{\infty}/MIC$ ,  $C_{\text{MAX}}/MIC$ ) and PELF ( $AUC_{24}/MIC$ ,  $AUC_{\infty}/MIC$ ,  $C_{\text{MAX}}/MIC$ ,  $T > MIC$ ). Clearance and  $V$  were calculated from extrapolated graphical data contained in each manuscript as follows and for the calves in this study:  $Cl = \text{Dose}/AUC_{\text{inf}}$  and  $V = Cl/Kel$ .

The PK/PD indices were calculated from  $AUC_{24}$ ,  $AUC_{\infty}$ , and  $C_{\text{MAX}}$  of the simulated plasma and PELF time–concentration profiles of each individual animal and the MIC of the sample collected from that animal.  $T > MIC$  was calculated using predicted PELF data only because plasma concentrations did not reach levels above the MICs in this study. The MIC used for the calculation was from the isolate with the highest MIC collected from that calf at time 0 only (i.e., prior to therapeutic treatment) but could be from either BAL or NPS. Time above MIC was not calculated for plasma as the plasma concentrations did not reach that of the lowest MIC dilution tested.

#### Statistical analysis

A generalized linear mixed model fit by maximum-likelihood regression was built in STATA (Stata/SE 12.1 for Windows; StataCorp LP, College Station, TX, USA) to compare morbidity between MM and CON using pen as a random clustering effect. Initial covariates included in the model, but ultimately excluded due to lack of significance, were trailer compartment and state of animal origination.

Statistical comparisons of PK and PD parameters between treatment outcome (success and failure) were performed with the Kruskal–Wallis test for nonparametric data using SAS<sup>®</sup> software (Version 9.3; SAS Institute Inc., Cary, NC, USA). The level of significance was set at  $P \leq 0.05$  using a two-tailed test. Both compartmental and NCA PK parameters were compared statistically as this report focuses on both types of modeling.

## RESULTS

### Morbidity outcome

Descriptive morbidity, mortality, and treatment failure data can be found in Table 3. Treatment for control of BRD with gamithromycin resulted in a numerically lower morbidity but a numerically higher relapse rate (therapeutic failure); however, these differences were not statistically significant ( $P = 0.25$ ). Total morbidity throughout the 28 days of the trial was much lower than anticipated, and the power of the study may therefore have been insufficient to detect significant differences in morbidity between sham-injected cattle and those treated for control of BRD with gamithromycin.

### PK model development

Model development was guided by goodness-of-fit plots within the modeling software. The results of the final model can be

**Table 3.** Summary comparison of morbidity, mortality, and treatment failure rates of bovine respiratory disease among feedlot cattle allocated to either sham injection or mass medication with gamithromycin at 6 mg/kg. Treatment for BRD was also with gamithromycin at the time of BRD diagnosis by a veterinarian

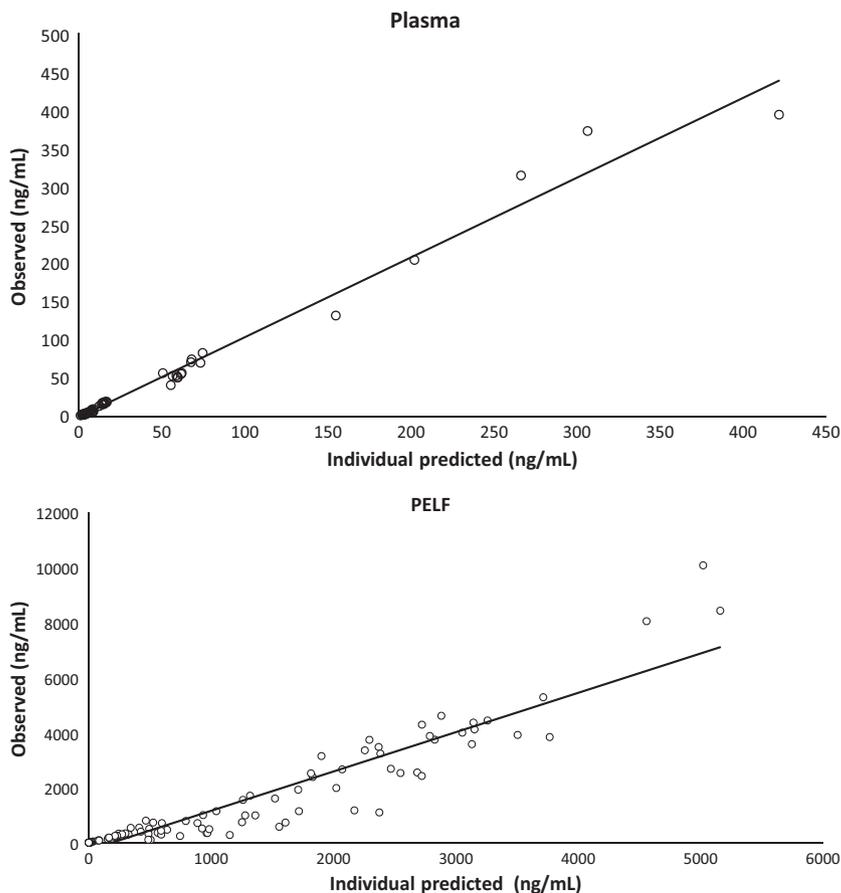
	Control (%)	Mass medicated (%)	Total (%)
Morbidity from BRD	17/90 (18.9)	9/90 (10.0)	26/180 (14.4)
Mortality from BRD	0	0	0
BRD treatment failure	2/17 (11.8)	2/9 (22.2)	4/26 (15.4)

The numerical differences were not statistically significant (% = percentage of subjects).

seen in Fig. 2. There is an excellent fit of predicted data to actual data in plasma and PELF at lower concentrations. The model slightly under-predicts PELF at higher concentrations, but this is likely due to difficulty in accurately predicting the  $K_a$  due to the paucity of data in this area of the curve.

### Pharmacokinetics of study animals

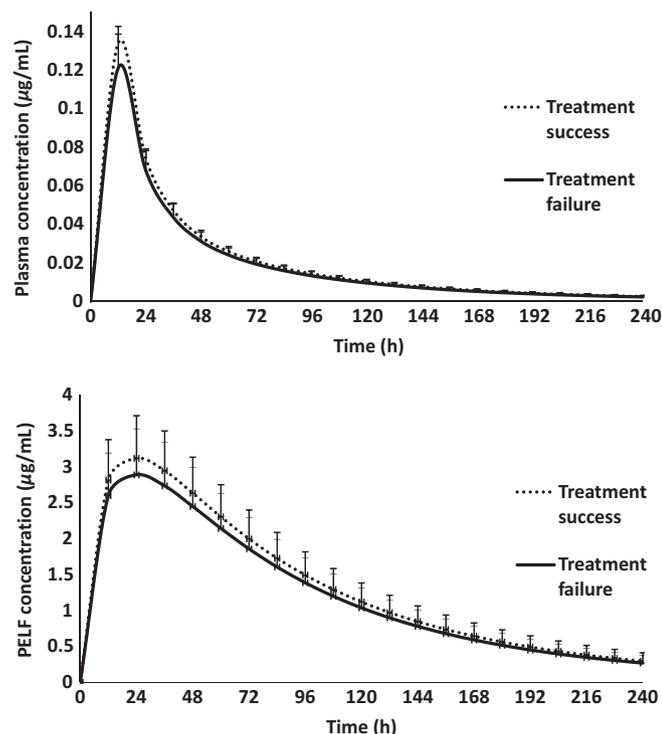
The estimated typical value of the compartmental model parameters for the population (regardless of treatment outcome) are summarized in Table 4. Note that the volume parameters ( $V$  and  $V_{PELF}$ ) are not weight normalized because



**Fig. 2.** Goodness-of-fit plots for model predicted data versus actual observed data from two previously published studies (Huang *et al.*, 2010; Giguere *et al.*, 2011) and data obtained from personal communication with one of the co-authors (RKT).

**Table 4.** Population pharmacokinetic parameter estimates following administration of gamithromycin administered at an average dose of 6 mg/kg subcutaneously for the treatment of acute bovine respiratory disease

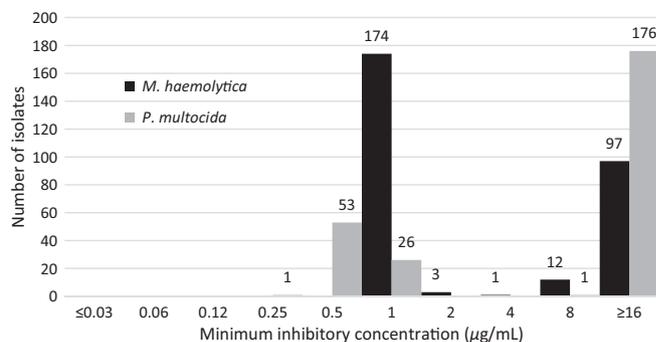
	Units	Estimates
$V$	L	183
$k_{10}$	1/h	1.84
$K_a$	1/h	8.83
$k_{12}$	1/h	7.09
$k_{21}$	1/h	0.26
$k_{13}$	1/h	0.98
$k_{31}$	1/h	0.02
$V_{PELF}$	L	201



**Fig. 3.** Full simulated time–concentration curves for plasma and PELF. Curve comparisons are by treatment outcome, and error bars represent standard error. Statistical comparison yielded no significant differences between outcomes in maximum concentration (plasma  $P = 0.12$ , PELF  $P = 0.22$ ).

weight was not a significant covariate in the nonlinear mixed effects model.

The simulated full time–concentration curves for the treatment successes and failures can be found in Fig. 3. The  $Cl$ ,  $V$ , rate constants,  $C_{MAX}$ ,  $AUC_{0-t}$ ,  $AUC_{24}$ ,  $AUC_{0-\infty}$ , and  $MRT$  calculated from these curves were compared between treatment successes and treatment failures, and no statistical differences were observed.  $C_{MAX}$  was, however, numerically higher in the treatment success group. Variability was high, and this may or may not be a true difference (plasma  $P = 0.12$ , PELF  $P = 0.22$ ).



**Fig. 4.** Frequency distribution of clinical isolates of *Mannheimia haemolytica* ( $n = 287$ ) and *Pasteurella multocida* ( $n = 257$ ) MIC to gamithromycin cultured from bronchoalveolar and nasopharyngeal samples from cattle diagnosed with bovine respiratory disease. Samples were collected at time 0, 12, 24, and 120 h post-treatment. Numbers above bars represent isolate number at that respective MIC dilution.

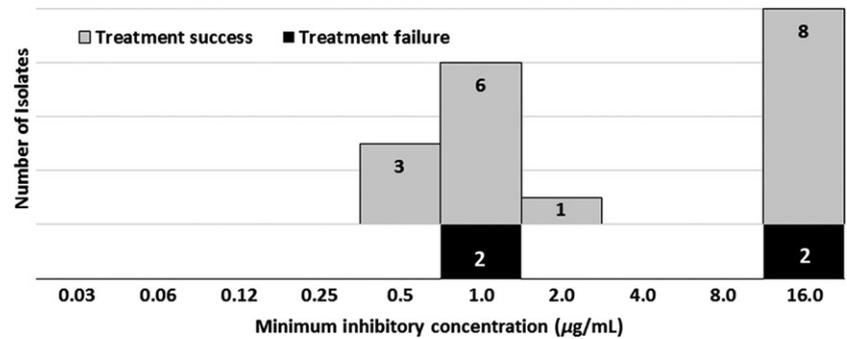
#### MIC and pharmacodynamics

The MIC distribution for the clinical (BAL and NPS) isolates of *M. haemolytica* ( $n = 287$ ) and *P. multocida* ( $n = 257$ ) from this trial can be seen in Fig. 4. Isolates of *M. haemolytica* in the susceptible category represent 60% of the isolate population, and those in the resistant category encompass 36% as compared to 31% and 68% for *P. multocida*, respectively. The isolates tested were collected over all time points (0, 12, 24, and 120 h post-treatment) from both CON and MM cattle and therefore represent isolates not yet exposed to therapeutic drug as well as isolates exposed to gamithromycin in the later sample collections. Additionally, nine of the 26 cattle diagnosed with BRD and subsequently sampled were from the treatment group receiving mass medication with gamithromycin on arrival and would have therefore had previous exposure to gamithromycin.

The bivariate histogram in Fig. 5 shows the MIC distribution for both *M. haemolytica* and *P. multocida* at time 0 by treatment outcome. This graphic suggests that the sample size was not equivalent across outcome groups, but the MIC comparisons within outcome group are quite similar. As such, there were just 22 cattle in the success group and 4 in the failure group with the plots representing a single isolate per calf for those yielding an isolate (4 calves did not yield an isolate at time 0). Due to small sample size and confounding of arrival treatment (CON and MM) within therapeutic treatment outcome, it was not possible to statistically compare the MIC by outcome. However, it can be visually appreciated that there is a symmetry in each group with the treatment success group having 10 susceptible isolates and 8 resistant isolates. The treatment failures represent cattle yielding 2 susceptible and 2 resistant isolates, prior to treatment.

The results of the PK/PD index calculations can be found in Table 5 and represent a comparison between treatment successes and failures by pathogen. Although the standard error in the failure group is relatively large and likely reflective of the small sample size, comparing the means between outcomes indicate that in all cases, more active ingredient is present for

**Fig. 5.** Bivariate histogram of MIC distribution of *Mannheimia haemolytica* and *Pasteurella multocida* cultured from bronchoalveolar and nasopharyngeal samples just prior to treatment (time 0) for bovine respiratory disease in 26 head of cattle (4 calves did not yield an isolate at time 0).



**Table 5.** Pharmacokinetic/pharmacodynamic indices for cattle treated with gamithromycin for acute bovine respiratory disease [mean ( $\pm$ SE)]. Parameter comparisons are by pathogen (cultured at time 0) and treatment outcome within either plasma or the effect compartment, PELF. Values are calculated as free unbound drug using 26% protein binding from a previous study (Huang *et al.*, 2010)

Parameter	<i>M. haemolytica</i>		<i>P. multocida</i>		
	Success ( $\pm$ SE)	Failure ( $\pm$ SE)	Success ( $\pm$ SE)	Failure ( $\pm$ SE)	
Plasma	AUC <sub>24</sub> /MIC	1.32 ( $\pm$ 0.1) <sup>‡</sup>	0.67 ( $\pm$ 0.59) <sup>‡</sup>	1.24 ( $\pm$ 0.41)	0.72 ( $\pm$ 0.42)
	AUC <sub>∞</sub> /MIC	3.49 ( $\pm$ 0.4) <sup>‡</sup>	1.78 ( $\pm$ 1.55) <sup>‡</sup>	3.21 ( $\pm$ 1.07)	1.91 ( $\pm$ 1.12)
	C <sub>MAX</sub> /MIC	0.09 ( $\pm$ 0.01) <sup>‡</sup>	0.04 ( $\pm$ 0.037) <sup>‡</sup>	0.08 ( $\pm$ 0.03)	0.04 ( $\pm$ 0.03)
PELF	AUC <sub>24</sub> /MIC	31.5 ( $\pm$ 4.4)	24.6 ( $\pm$ 22.4)	31.0 ( $\pm$ 9.8) <sup>*</sup>	22.4 ( $\pm$ 11.7) <sup>*</sup>
	AUC <sub>∞</sub> /MIC	205 ( $\pm$ 29)	164 ( $\pm$ 150)	209 ( $\pm$ 67)	148 ( $\pm$ 77)
	C <sub>MAX</sub> /MIC	1.86 ( $\pm$ 0.26)	1.45 ( $\pm$ 1.32)	1.83 ( $\pm$ 0.58) <sup>†</sup>	1.32 ( $\pm$ 0.69) <sup>†</sup>
	T>MIC	77 ( $\pm$ 10)	44 ( $\pm$ 44)	56 ( $\pm$ 19)	44 ( $\pm$ 25)

\* $P = 0.04$ ; <sup>†</sup> $P = 0.07$ ; <sup>‡</sup> $P = 0.10$ .

a longer duration in the treatment success group. Statistical differences between treatment successes and failures were noted in AUC<sub>24</sub>/MIC ( $P = 0.04$ ) for *P. multocida* in PELF. Additionally, an association ( $P = 0.10$ ) was observed with higher AUC<sub>24</sub>/MIC, AUC<sub>∞</sub>/MIC, and C<sub>MAX</sub>/MIC for treatment successes as compared to failures in plasma, and AUC<sub>24</sub>/MIC ( $P = 0.07$ ) in PELF for *M. haemolytica*. Half of the failures (2/4) and 41% (7/17) of the cattle in the success group were from the MM group that received gamithromycin on arrival to the feedyard. Those cattle from the MM group accounted for 80% (8/10) of the isolates displaying an MIC of  $\geq 16$  µg/mL (at time 0). The isolate yielding the highest MIC, as cultured prior to treatment for BRD, is reported by treatment group (CON, MM) in Table 6.

Comparison of the PK parameters resulting from this study compared to those of previously published studies can be found in Table 7. The calves in this study demonstrated an increased Cl and V in plasma as compared to the healthy calves in the two previous reports. The MRT of both plasma and PELF are quite similar to those reported previously by Giguere *et al.* Both the C<sub>MAX</sub> and AUC<sub>0-∞</sub> reported herein are quite different, especially in plasma, from the previously published reports of gamithromycin PK in healthy cattle (Huang *et al.*, 2010; Giguere *et al.*, 2011).

## DISCUSSION

Trials involving sparsely sampled data, such as this one, represent difficulties in parameter estimation. Mixed effects models

help to overcome these challenges by partitioning sources of variability in hierarchical statistical models, thereby allowing a reduction in the variance of the estimated population parameters. These models also have the advantage of allowing quality analysis from fewer samples, thereby sparing expense and the stress from additional animal handling. However, quality prior information on the parameters is a requirement to inform these models (Dodds *et al.*, 2005; Riviere, 2011; Mould & Upton, 2012). It was fortunate to have had access to rich data to externally validate the model. This allowed a comparison of the simulated data in this study to the results of two previously published reports to confirm the models' accuracy.

Using only two samples of PELF per animal, the mixed effect model utilized in this study allowed for the estimation of the gamithromycin PK/PD values achieved in cattle diagnosed with BRD. The resulting simulated individual animal profiles were used to run a NCA in order to compare our model output to previous publication results. The PK parameter results obtained from that analysis are comparable to the values obtained previously (Huang *et al.*, 2010; Giguere *et al.*, 2011) as shown in Table 7. Considering the differences in the physiological status of the study participants under investigation (healthy cattle in previous publications versus cattle diagnosed with BRD in this report) and the fact that this model under-predicts PELF, the resulting comparable PK parameters from this study demonstrates that our two compartment plus PELF effect compartment model was acceptable. However, the lower drug exposure found in the morbid animals in this study as compared to those utilizing healthy animals is noteworthy.

**Table 6.** Minimum inhibitory concentration ( $\mu\text{g}/\text{mL}$ ) comparison of time 0 isolates from both PELF and NPS isolates by treatment group. The single isolate with the highest MIC is reported for each calf (four calves yielded no isolates at time 0)

ID	<i>M. haemolytica</i>	<i>P. multocida</i>
CON		
102	–	–
104	$\geq 16.0$	–
106	–	$\geq 16.0$
116	–	0.5
118	–	0.5
126	–	0.5
174	1.0	–
175	1.0	0.5
222*	1.0	1.0
224	–	–
225	1.0	1.0
229	1.0	–
236*	–	1.0
240	–	–
241	1.0	–
243	1.0	1.0
245	–	–
MM		
150	1.0	–
195*	–	$\geq 16.0$
204	1.0	$\geq 16.0$
206	1.0	$\geq 16.0$
209	–	$\geq 16.0$
212*	–	$\geq 16.0$
213	–	$\geq 16.0$
218	–	$\geq 16.0$

\*Treatment failure.

– = culture yielded no isolate.

Table 4 displays the estimated typical values of the compartmental model parameters. It should be noted that the  $V_{\text{PELF}}$  is much larger than its actual physical volume in cattle because the role of this parameter in the model is a virtual compartment in which relatively low concentrations were observed for the administered dose.

Gamithromycin has been reported to be a low protein binding drug (26%) in the serum of healthy animals (Huang *et al.*, 2010). Protein binding was not an objective of this study and therefore was not evaluated. Variation in protein binding is expected to have minimal effect for drugs that display low pro-

tein binding. However, it is possible that alterations in the protein binding of the morbid animals in this study did have some effect on the PK differences we observed. Future studies focusing on determining the differences between healthy and diseased animals needs to be considered to determine whether this was simply an effect of the modeling/sampling strategy utilized in this study or a difference truly exists.

Much discussion is available in the literature surrounding the selection of the proper pharmacodynamic index to determine the optimal dosing of the macrolide class of antimicrobials. Contemporary thought on the newer 'longer acting' injectable macrolides in veterinary medicine is that the most important index is AUC/MIC. However, intense debate remains whether this should be measured and reported for plasma (Toutain, 2009; Papich, 2014), at the site of infection (Amsden, 2001; Evans, 2005) or both (Rodvold *et al.*, 2011). Although not statistically significant, the marginally significant association between plasma PKPD indices and treatment outcome ( $P = 0.10$ ) would seem to substantiate the claims of using plasma drug concentrations. However, we did observe a significant association between PELF AUC<sub>24</sub>/MIC and treatment outcome ( $P = 0.04$ ) with *P. multocida*, suggesting that both plasma and PELF are correlated with treatment outcome. This finding is not surprising given the fact that the drug in the PELF is derived from and thus correlated with the drug in the plasma. However, completely ignoring the PELF compartment and confining interpretation to plasma alone could be misleading, especially when considering drugs with very extensive tissue distribution such as gamithromycin and other macrolide class antibiotics.

Prior to the initiation of the study, gamithromycin did not have clinical breakpoints determined by the CLSI. However, since the conclusion of the live phase of this study, gamithromycin breakpoints have been reported for *M. haemolytica*, *P. multocida*, and *H. somni* at  $\leq 4.0$ , 8.0, and  $\geq 16.0$   $\mu\text{g}/\text{mL}$  for susceptible, intermediate, and resistant, respectively. The biphasic population of bacteria cultured from cattle displaying signs of BRD in this report fit those breakpoints. Conversely, in this study, there seemed to be little association between the *in vitro*-determined MIC and treatment outcome, especially for *P. multocida* (Table 7). Several authors have noted differences between the MIC determined *in vitro* and the MIC determined in the more physiologically relevant matrix, serum (Evans, 2005; Mitchell *et al.*, 2012, 2013). It remains a possibility that

	Huang <i>et al.</i>	Giguere <i>et al.</i>		Current study	
	Plasma	Plasma	PELF	Plasma	PELF
No. animals	32	30	30	26	26
$C_{\text{MAX}}$ ( $\mu\text{g}/\text{mL}$ )	0.27	0.43	4.61	0.13 ( $\pm 0.003$ )	3.04 ( $\pm 0.09$ )
AUC <sub>0–∞</sub> ( $\mu\text{g}\cdot\text{h}/\text{mL}$ )	8.28	7.95	348	5.4 ( $\pm 0.13$ )	340 ( $\pm 12$ )
Cl ( $\text{mL}/\text{h}/\text{kg}$ )	654*	830*	–	1140 ( $\pm 27$ )	–
V (L/kg)	56.9*	94.2*	–	97.4 ( $\pm 2.4$ )	–
MRT (h)	41.3*	43.1	71.1	52.8 ( $\pm 0.18$ )	79.0 ( $\pm 0.29$ )

$\pm$ SE where available; \*Calculated from extrapolation of graphical data in manuscript.

**Table 7.** Comparison of this study models simulated output of pharmacokinetic parameters to previously published work by noncompartmental analysis. The label dose of 6 mg/kg subcutaneously was administered in each study. Values of  $C_{\text{MAX}}$  and AUC<sub>0–∞</sub> are reported as total drug as neither publication corrected for protein binding

the lack of association, in this study, between reported MIC and treatment outcome could be due to a similar phenomenon.

Several cattle yielded multiple isolates of *M. haemolytica* and *P. multocida* from either NPS or BAL, and selection of isolate MIC was performed by choosing the isolate with the highest MIC at time 0 (Table 6). Therefore, PD indices reported in this study are likely to represent worst-case scenarios as the results of this trial are also confounded by on arrival treatment allocation. Giguere *et al.* reported MIC<sub>90</sub> values of 0.5 and 1.0 µg/mL for *M. haemolytica* and *P. multocida* in 2011 (Giguere *et al.*, 2011). Using those MICs would have certainly resulted in much different PD indices.

Reported herein were the AUC/MIC ratios for both PELF and plasma, as well as the other standard PKPD indices (C<sub>MAX</sub>/MIC, T>MIC). Although minimal statistical differences were observed, our findings indicate that for both *M. haemolytica* and *P. multocida*, a longer drug exposure was more closely related to a successful treatment outcome. While some small differences in exposure were observed, it is unlikely that these differences substantially contributed to the difference in clinical outcome. However, it is important to keep in mind that due to small sample size, there was a large amount of variability in the data which could contribute to the lack of statistical significance.

Additionally, given the small numerical difference between success and failure PD indices, it seems that there are likely many factors beyond PK, PD, and MICs that contribute to the success of a treatment regimen. For example, the immunological status of the animal and the environmental conditions that the animal is subjected to undoubtedly also play a role in disease outcome. It has been shown that some macrolides have anti-inflammatory and immunomodulatory effects in addition to antimicrobial activity. Tulathromycin, a semi-synthetic macrolide of the subclass triamillide, has been extensively researched in this area (Fischer *et al.*, 2011, 2013, 2014; Er & Yazar, 2012; Duquette *et al.*, 2015). Azithromycin, a macrolide of the same subclass as gamithromycin, has recently been shown to exert anti-inflammatory properties on lung epithelial cells in humans (Kitsiouli *et al.*, 2015). While data specific to gamithromycin are currently lacking in this area, it is possible that anti-inflammatory activity similar to that of azithromycin and tulathromycin could have facilitated a 'self-cure' in this study. This should be further considered, especially considering the high treatment success rate observed in this study given the isolation of many resistant organisms as shown in Table 6.

There appears to be an over-representation of resistant *P. multocida* isolates in the MM treatment group. This is likely explained by the fact that gamithromycin was utilized for both mass medication and treatment in this study, a practice that is not common in the field. The reason for the lack of resistant *M. haemolytica* in the MM group is not clear and deserves further attention in future studies.

Another layer of complexity must also be appreciated; there remains a possibility that bacteria other than *M. haemolytica* and *P. multocida* are responsible wholly, or in part, for the treatment failures. While *H. somni* was isolated (data not

shown), it was present very infrequently. It is possible that another resistant pathogen is contributing to the clinical signs associated with the BRD cases in this study.

This report is, to the authors' knowledge, the first of its kind in the veterinary literature, to perform such a large PK/PD examining drug concentrations in PELF within a group of cattle experiencing naturally occurring BRD. Although challenged by sample size limitations of ultimately diseased animals, a compartmental PK model was developed to which the sparse clinical data from this trial were successfully fit. Therefore, complete concentration–time profiles were simulated for the central and PELF effect compartment for each animal in order to determine PK/PD indices for *M. haemolytica* and *P. multocida* unique to each animal in this study. The findings from this study indicate that PK variability in cattle diagnosed with BRD seems at least as important as the MIC of *M. haemolytica* or *P. multocida*. Additionally, further consideration should be paid to other possible bacterial pathogens in association with BRD.

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