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Blood plasma concentrations of chlortetracycline achieved by administration of a mineral formulation to adult beef cows

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ABSTRACT

Objective: Our study objectives were to characterize steady-state plasma chlortetracycline (CTC) concentrations achieved by adult beef cows treated with a commercial, CTC-containing mineral supplement under 2 conditions, where intake was controlled and under conditions when intake was uncontrolled, as in a pasture setting typical of production practices for anaplasmosis control.

Materials and Methods: Thirty cows were allocated to 3 individually administered treatment groups: nonmedicated control (NM-F), medicated mineral administered via gelatin capsule (M-C), or medicated mineral fed in a small amount of grain (M-F). After a washout period, 15 of the original cows received an intended CTC dose of 1.1 mg·kg of BW^{-1}·d^{-1} administered via a mineral feeder in a pasture setting (M-P). Blood samples were collected at multiple time points, and plasma chlortetracycline concentrations were quantified.

Results and Discussion: Mean plasma chlortetracycline concentrations for M-C and M-F treatments were 20.2 and 19.3 ng/mL, respectively, with no differences in mean, minimum, or maximum plasma concentrations. Conversely, differences in both median and minimum plasma concentrations were detected when the dose was controlled (M-C or M-F) compared with when the dose was administered via mineral feeder in a pasture setting (M-P).

Implications and Applications: Future CTC studies can be designed using individual intake in grain because administration by gelatin capsule offers no advantage in dosage consistency. The assumption that mean consumption from a mineral feeder will achieve the same plasma CTC concentrations as individual or bunk-fed experiments should be further analyzed. The pharmacodynamic parameters associated with efficacy were not evaluated in this study and remain unknown.

Key words: *Anaplasma marginale*, anaplasmosis control, antimicrobial stewardship, pharmacokinetics, plasma concentration

INTRODUCTION

Due to concerns about extended administration of medically important antimicrobials, chlortetracycline (**CTC**) use in livestock has recently been scrutinized. Since January 2017 its legal use in the United States has shifted from an over-the-counter product to one requiring a veterinary feed directive (FDA, 2015a). The CTC label for anaplasmosis control lacks a defined duration. Revision of label claims to define duration is objective 1.1 in the FDA's report on their 5-yr plan for supporting antimicrobial stewardship in veterinary settings (FDA, 2018). From 2009 to 2016 drugs in the tetracycline class consistently constituted 70% by weight of all medically important antibiotics sold or distributed in the domestic United States for use in food-producing animals (FDA, 2016). This percentage is much greater than for any other single class of medically important antimicrobials. Although the potency of tetracyclines is relatively low compared with some other classes of drugs, this overwhelming percentage still indicates common and consistent use.

There are numerous efficacy studies examining CTC use for anaplasmosis control. Brock et al. (1957) demonstrated that an individually fed CTC dose of 1.1 mg·kg of BW^{-1}·d^{-1} prevented clinical disease in animals subsequently challenged with anaplasmosis. This experiment also demonstrated that treated animals did not become carriers of *Anaplasma marginale* as confirmed by blood-transfer inoculation of splenectomized calves. The use of CTC to eliminate the carrier stage of anaplasmosis was also explored by others (Pearson and Brock, 1953; Franklin et al., 1965; Franklin et al., 1967; Richey et al., 1976; Richey

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et al., 1977a,b). More recently, Reinbold et al. (2010b) used complement-ELISA and reverse-transcription PCR methods to document that a bunk-fed CTC dose of 4.4 mg·kg of $BW^{-1}·d^{-1}$ was sufficient to shift infected cattle to a negative test status; these researchers confirmed carrier elimination by inoculation of splenectomized calves.

The label dose for control of anaplasmosis is 1.1 mg·kg of $BW^{-1}·d^{-1}$ for hand-fed products (FDA, 1996c,b), and the target dose is 1.1 to 4.4 mg·kg of $BW^{-1}·d^{-1}$ for free-choice mineral products (FDA, 1996a, 2006). Because this was a supplemental approval, the pivotal studies for the mineral formulations reported mean consumption over 14-d periods rather than drug efficacy. The 1996 approval of a formulation of 6.61 g of CTC/kg evaluated 6 locations and reported that mean CTC intakes over 14-d periods ranged from 1.14 to 4.09 mg·kg of $BW^{-1}·d^{-1}$ (FDA, 1996a). For the 8.82 g/kg product approval in 2006, the mean CTC intakes from 4 locations ranged from 1.43 to 2.37 mg·kg of $BW^{-1}·d^{-1}$ (FDA, 2006).

A common use of CTC is to aid in control of active infection of anaplasmosis in adult cattle maintained on pasture. In this setting, CTC is generally administered as a component of a mineral supplement rather than bunk fed as a top dress or a component of a mixed diet. In the previously mentioned efficacy studies, CTC was either directly fed to individual animals or administered in a bunk-fed setting. These results were subsequently extrapolated to situations in which CTC was delivered via mineral, based on the assumption that if the average intake matched the targeted dose, efficacy would be achieved. To the authors' knowledge, no experiments have examined individual plasma concentrations of CTC when the drug was administered as part of a mineral supplement fed to adult cattle. This is a remarkable dearth of information because animal age, genotype, metabolic state, diet composition, and drug formulation may influence drug absorption and metabolism (Riviere, 2011); moreover, there is potential for individual variation in consumption when the dose is administered using a mineral feeder in a pasture setting regardless of whether the formulation is intended for free-choice or hand-fed administration.

The plasma concentrations and other population pharmacokinetic parameters have been established for CTC administration as a top-dressed, bunk-fed supplement at doses of 4.4, 11, or 22 mg·kg of $BW^{-1}·d^{-1}$ (Reinbold et al., 2010a). However, the product administered and mode of administration used by Reinbold et al. (2010a) is not what is typically used to control anaplasmosis in adult cows on pasture.

Basic concepts in pharmacodynamics have established that the drug must reach the target pathogen at an appropriate concentration for efficacy claims to be legitimate (Gabrielsson and Hjorth, 2012). As future research seeks to establish appropriate durations for CTC use, it is important to establish the target concentration needed for efficacy. Therefore, the objective of our experiment was to characterize steady-state plasma CTC concentrations achieved by adult beef cattle treated with a typical dose regimen of a mineral formulation of CTC when intake was controlled and to compare these with steady-state plasma CTC concentrations when the same cows were offered the same product in a pasture setting.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee reviewed and approved all animal handling and animal care practices used in our experiment. All animal procedures were conducted in accordance with the Guide for the Care and Use of Animals in Agricultural Research and Teaching (FASS, 2010).

Cattle and Husbandry

For phase 1 of the experiment, cows (n = 30) were housed in 2 adjacent, earth-floor pens and fed smooth bromegrass hay ad libitum. One pen contained 15 mature cows (initial BW range = 473 to 715 kg; age range = 3 to 8 yr) that were bred via AI approximately 30 d before phase 1 began. Cows were supplemented daily with 3 kg/head (as fed) of a 50:50 (wt/wt) mixture of dried distillers grain and ground corn. The adjacent pen contained 15 two-year-old cows that were nursing their first calves (initial BW range = 364 to 617 kg). These lactating cows were supplemented daily with the aforementioned dried distillers grain and ground corn mixture at a rate of 7.5 kg/head. For phase 2 of the experiment, 15 of the mature cows from phase 1 were housed on native grass pasture with free-choice water.

Experimental Design—Phase 1

Animals were blocked by pen and assigned randomly to 1 of 3 treatments (n = 10 cows per treatment) as defined in Table 1. Thirty cows were allocated to 3 individually administered treatments: nonmedicated control (**NM-F**), medicated mineral administered via gelatin capsule (**M-C**), or medicated mineral fed in a small amount of grain supplement (**M-F**). Each of the treatment groups received 1 of 2 mineral products: a nonmedicated mineral (**NM**) formulated for cattle on pasture (Purina Wind and Rain, Land O'Lakes Inc., Arden Hills, MN) or a medicated mineral (**M**) containing 6.61 g/kg CTC (Purina Wind and Rain—CTC Formula; 6.61 g/kg, Land O'Lakes Inc.) with all other ingredients identical to NM. The NM-F treatment group received NM fed individually. The M-C treatment group received M administered individually via gelatin capsule at a CTC dose of 1.1 mg·kg of $BW^{-1}·d^{-1}$. The M-F treatment group received M fed individually at a CTC dose of 1.1 mg·kg of $BW^{-1}·d^{-1}$.

Animals were weighed at 7-d intervals during treatment administration to ensure the targeted CTC dose was administered. All animals were handled once daily

Table 1. Treatment descriptions for phase 1 (M-C, M-F, NM-F) and phase 2 (M-P)

Treatment	Chlortetracycline daily dose (mg/kg)	No. of animals	Administration method	Formulation
Phase 1				
M-F[1]	1.1	10 (5 heifers, 5 mature cows)	Individually administered by mixing in 0.34 kg of grain supplement	Medicated mineral mix containing 6.61 g/kg (6,000 g/ton) chlortetracycline
M-C[2]	1.1	10 (5 heifers, 5 mature cows)	Individually administered by oral gelatin capsule	Medicated mineral mix containing 6.61 g/kg (6,000 g/ton) chlortetracycline
NM-F[3]	—	10 (5 heifers, 5 mature cows)	Individually administered by mixing in 0.34 kg of grain supplement	Nonmedicated mineral mix
Phase 2				
M-P[4]	1.1	15 (mature cows)	Ad libitum access to mineral feeder containing dose sufficient for treatment of group. Mineral was allocated to feeder daily.	Medicated mineral mix containing 6.61 g/kg (6,000 g/ton) chlortetracycline

[1]M-F = dosed at 1.1 mg·kg of BW^{-1} daily, administered via observed intake from an individual feed pan.

[2]M-C = dosed at 1.1 mg·kg of BW^{-1} daily, administered in a gelatin capsule by balling gun.

[3]NM-F = dosed with nonmedicated mineral, administered via observed intake from an individual feed pan in phase 1 and voluntary intake in phase 2.

[4]M-P = medicated mineral containing 6.61 g/kg chlortetracycline allocated to mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg of BW^{-1} daily for each animal in the group, voluntary intake.

for treatment administration. Animals assigned to NM-F and M-F treatments were individually penned at 0700 h daily and offered their respective supplements. Animals were observed by investigators until the supplement was completely consumed. Animals assigned to M-C were individually restrained in a squeeze chute at 0700 h daily, and medicated mineral was delivered orally via hard gelatin capsules (Torpac gelatin capsules, Fairfield, NJ) administered using a balling gun (Nebraska style balling gun, Miller Manufacturing, St. Paul, MN).

Animals were acclimated to diets and environmental conditions for 6 wk before treatment commenced. Initial blood samples were obtained via venipuncture on day zero of treatment to confirm no previous exposure to CTC. When multiple doses of CTC are administered at 24-h intervals, not all of the previous dose is eliminated at the time the next dose is given (elimination half-life = 16 h; Reinbold et al., 2010a). To avoid variation in plasma concentrations due to this accumulation effect, the time to steady-state concentration was calculated by multiplying the elimination half-life of CTC (elimination half-life = 16 h; Reinbold et al., 2010a) by 10 to arrive at the time point (160 h) at which plasma concentration would be at 99.9% of steady state (Gabrielsson and Hjorth, 2012). Therefore, to ensure that samples were taken when CTC concentrations had reached steady state, treatments were administered for 7 d (168 h) before commencing with blood collections.

The blood-sample collection schedule is shown in Table 2. Baseline blood samples were collected on d 0. Blood sampling for quantification of plasma CTC concentration occurred at 0700 h, just before dosing, on d 7, 14, and 21. Additionally on d 7 and 21, samples were also collected a 1900 h. Throughout the experiment, blood samples collected via venipuncture (approximately 10 mL) were collected into lithium-heparin tubes. The preferred venipuncture site was the coccygeal vein, but on days requiring multiple collections, the jugular vein was used as an alternative.

Experimental Design—Phase 2

After phase 1 was completed, CTC was removed from animal diets for 80 d. During this washout period, one cow was replaced because of a medical condition unrelated to treatment. After the completion of the washout period, the 15 mature cows from phase 1 were moved to a single native-grass pasture. All cows were first offered NM ad libitum from a single mineral feeder per product label for 14 d. Following this acclimation period, the final treatment (**M-P**) was administered by offering M in the same feeder for 21 d. Consistent with the *hand-fed* product label, only enough mineral supplement for the 15-cow group for 1 d at a common CTC dose of 1.1 mg·kg of BW^{-1} was allocated to the feeder daily.

Baseline blood samples were collected via coccygeal venipuncture on d 0, following acclimation to nonmedicated

Table 2. Blood collection schedule for phase 1 (M-C, M-F, NM-F) and phase 2 (M-P)[1]

Collection	Day	Time	Treatment	No. of animals sampled
Phase 1				
A	0	a.m.	NM-F[2]	30
B	7	a.m.	M-C,[3] M-F,[4] NM-F	30
C	7	p.m.	M-C, M-F, NM-F	30
D	10	a.m.	M-C, M-F, NM-F	30
E	14	a.m.	M-C, M-F, NM-F	30
F	14	p.m.	M-C, M-F, NM-F	30
Phase 2				
G	0	a.m.	NM-F	15
H	7	a.m.	M-P[5]	15
I	14	a.m.	M-P	15
J	21	a.m.	M-P	15

[1]Collections A and G were baseline samples to provide evidence that there was no pretreatment chlortetracycline exposure.

[2]NM-F = dosed with nonmedicated mineral, administered via observed intake from an individual feed pan in phase 1 and voluntary intake in phase 2.

[3]M-C = dosed at 1.1 mg·kg of BW^{-1} daily, administered in a gelatin capsule by balling gun.

[4]M-F = dosed at 1.1 mg·kg of BW^{-1} daily, administered via observed intake from an individual feed pan.

[5]M-P = medicated mineral containing 6.61 g/kg chlortetracycline allocated to mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg of BW^{-1} daily for each animal in the group, voluntary intake.

mineral. Blood sampling for quantification of plasma CTC concentration occurred once daily on d 7, 14, and 21, following introduction of medicated mineral (Table 2).

CTC Quantification

After collection, blood samples were immediately placed on ice. They were then transported and centrifuged at 2,750 × *g* at 4°C for 15 min. Plasma was then removed via pipette and stored at −62°C.

Complete methods for CTC quantification can be found in the Supplemental Material (https://doi.org/10.15232/aas.2019-01917). In summary, calibration standards, blank, quality control, and bovine plasma samples were extracted by solid-phase extraction using Oasis HLB Prime μElution 96-well plates (Waters Co., Milford, MA). Negative control, calibrants, quality control plasma, and bovine plasma sample were prepared on a 48-well, non-tissue culture treated plate (Corning Inc., Corning, NY). The 48-well plate was shaken gently at 350 revolutions per minute for 10 min at room temperature with the IKA MTS 2/4 Shaker (IKA Works Inc., Wilmington, NC) and then centrifuged at 1,370 × *g* for 30 min at 20°C (Thermo Scientific ST16 Benchtop Centrifuge, Waltham, MA).

Chromatographic separations were performed using an Acquity Ultra Performance Liquid Chromatography system including a heated column compartment, a binary pump, and autosampler (Waters Co.). The ultraperformance liquid chromatography column used was an Acquity C18 column Waters HSS T3, 1.8 μm, 2.1 × 50 mm, heated to 50°C. The detection and quantification of oxytetracycline and CTC were performed using a Xevo TQ-S triple quadrupole mass spectrometer equipped with an electrospray ionization source (Waters Co.). Data acquisition and data quantification were performed using the Waters MassLynx and TargetLynx software 4.1, respectively. The lower limit of quantitation of CTC was determined to be 1 ng/mL according to the FDA analytical validation guidelines (FDA, 2015b).

Statistical Analysis

All data handling and statistical testing were performed using the software R Studio Version 1.1.456 (User interface for R statistical software, R Core Team, 2019; R Foundation for Statistical Computing, Vienna, Austria). Within R studio, the following packages were used: tidyverse (Hadley Wickham, 2017; R package version 1.2.1; https://CRAN.R-project.org/package=tidyverse), ggplot2 (H. Wickham, Springer-Verlag, New York, 2016), and lme4 (Bates et al., 2015).

A linear, mixed model was created for phase 1 (M-C, M-F, NM-F) to explore the effects of potential confounders (animal age and sampling time) and sources of random error (cow and collection). This model was created using the lmer function from the lme4 package in R.

Table 3. Mixed linear model parameters where *y* = phase 1 (M-C, M-F, NM-F) plasma concentrations

Model variable	Item	Estimate (ng/mL)	SE (ng/mL)	*P*-value	Variance	SD
Fixed effect						
Treatment group	M-C[2]	19.7	1.5	<0.01		
	M-F[3]	18.8	1.5			
	NM-F[4] (intercept)	−1.1	1.5			
Animal age	Young	1.1	1.2	0.39		
a.m. vs. p.m.	p.m.	2.6	1.4	0.07		
Random effect						
Cow	Intercept				6.4	2.5
Collection	Intercept				3.3	1.8

[1]This model was generated to explore the effects of possible cofactors (e.g., time of day and animal age) and to describe the amount of variability associated with known confounders (e.g., cow and collection).

[2]M-C = dosed at 1.1 mg·kg of BW^{-1} daily, administered in a gelatin capsule by balling gun.

[3]M-F = dosed at 1.1 mg·kg of BW^{-1} daily, administered via observed intake from an individual feed pan.

[4]NM-F = dosed with nonmedicated mineral, administered via observed intake from an individual feed pan.

To fully describe these data, complete descriptive statistics for each sample collected were summarized by treatment, showing the mean CTC concentration, SD, median, absolute range, interquartile range, and mean absolute deviation values for each treatment group. Additionally, the parameter values of mean, median, minimum, and maximum plasma concentrations were calculated for individual cows. These are presented as scatter-and-box plots. A *t*-test was used to compare parameter values of mean, median, and minimum between M-C and M-F treatments.

Further analyses were performed for the subset of animals that were dosed with 1.1 mg of CTC·kg of $BW^{-1}\cdot d^{-1}$ during phase 1 (M-C, M-F) and were also present during phase 2 (M-P). After the replacement of one of the original cows, this left 9 individuals for which the animal level median, minimum, and maximum plasma CTC concentra-

Table 4. Summary of chlortetracycline plasma concentrations (ng/mL) by treatment[1]

Treatment	Mean	SD	Median	Minimum	Maximum	Interquartile range	Median absolute deviation
M-C[2]	20	8	19	5	46	11	9
M-F[3]	19	7	20	6	35	8	6
NM-F[4]	BLQ	BLQ	BLQ	BLQ	2	BLQ	BLQ
M-P[5]	NC	NC	6	BLQ	51	20	9

[1]The mean, SD, median, minimum, and maximum were calculated at the level of treatment group for all samples collected. BLQ = multiple values below limit of quantification, 1 ng/mL; NC = not calculated due to the skewed distribution of values for this treatment or BLQ values.

[2]M-C = dosed at 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$, administered in a gelatin capsule via balling gun.

[3]M-F = dosed at 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$, administered via observed intake from an individual feed pan.

[4]NM-F = dosed with nonmedicated mineral, administered via observed intake from an individual feed pan in phase 1 and voluntary intake in phase 2.

[5]M-P = mineral containing 6.61 g/kg chlortetracycline allocated to a mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$ chlortetracycline for each animal in a group, voluntary intake.

tions could be compared between phases 1 and 2. Due to differences in distributions between phases, a Wilcoxon signed rank test was used to compare the known dose administered in phase 1 (M-C and M-F) with the intended dose administered in phase 2 (M-P). Comparisons were made between individual-animal median, minimum, and maximum plasma CTC concentrations.

RESULTS AND DISCUSSION

Linear Mixed Model

Estimates for model parameters for phase 1 (M-C, M-F, NM-F) are presented in Table 3. Treatment was the only significant predictor of plasma CTC concentration ($P < 0.01$; Table 3). Animal age (young vs. older cows) and collection time of day (a.m. vs. p.m.) were not significant at $\alpha = 0.05$ ($P = 0.39$). Blood collection time (a.m. vs. p.m.) tended ($P = 0.07$) to influence plasma CTC concentration (estimate = 2.6 ng/mL; Table 3).

The small effect of collection time might have been due to the difference in sample collection time relative to trough concentrations. The morning sample was collected at the steady-state trough just before dose administration. However, the relationship of the evening sample to a steady-state peak is not clear. The elimination half-life of CTC was estimated to be 16 h using a one-compartment, open model with first-order absorption and elimination, whereas it was estimated to be 35 h using a noncompartmental analysis (Reinbold et al., 2010a). The same study calculated time to maximum concentration to be 38 h when the dose regimen was 2.2 mg/kg of BW administered every 12 h. Data for time to maximum concentration are not available for a once-daily dose of 1.1 mg/kg of BW.

Individual-animal and blood-collection events were modeled as sources of random error in plasma CTC concentrations (Table 3). The variation attributed to cow was 6.4 ng/mL, with a SD of 2.5 ng/mL. Variability between individual animals cannot be avoided. Weekly animal BW were measured in our experiment. The authors assume that variability in plasma CTC concentration that occurs between animals on the same oral CTC regimen would likely be greater under field conditions because of less accurate BW estimations.

Plasma Concentrations

Plasma concentrations are summarized by treatment in Table 4 and depicted as a box-and-scatter plot by treatment in Figure 1. The distributions of the plasma CTC concentrations differed between phases 1 and 2. Administration by M-C or M-F at 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$ resulted in reasonably normal distributions of plasma CTC concentrations, whereas administration by mineral feeder (M-P) resulted in a right-skewed distribution. This skewed distribution can be observed in the large number of points at or below the quantification limit in the box plot for the M-P treatment in Figure 1.

Dose Linearity

Despite the differences in animal age, product formulation, and dosing method, plasma CTC concentrations observed in phase 1 (M-C, M-F) of this experiment were consistent with steady-state mean concentrations reported by Reinbold et al. (2010a). Mean plasma CTC concentrations detected in phase 1 of our experiment (dose = 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$) were combined with mean plasma CTC concentrations when CTC was administration as part of a bunk-fed supplement formulation (dose = 4.4, 11, or 22 mg·kg of $BW^{-1}\cdot d^{-1}$; Reinbold et al., 2010a). Simple linear regression was performed, resulting in an R^2 value of 0.99. This was interpreted to suggest that the bioavailability of CTC in the mineral formulation was not drastically affected by mineral salts. The plasma concentrations we observed were consistent with nonmineral formulations that were used in previous efficacy experiments, provided that the method of administration achieved consistent intake.

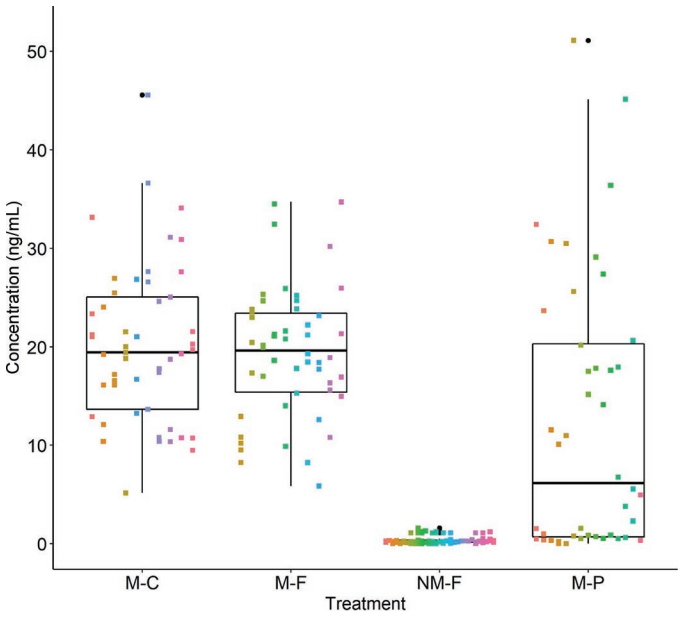

Figure 1. Scatter-and-box plots of plasma chlortetracycline concentrations of individual samples measured at a steady state. For box plots, the dark center line represents the median, outside edges of the box represent the interquartile range, and whiskers represent the range. Outliers are depicted as black dots. Each vertically associated column of points represents the concentration results for an individual animal. M-C = dosed with chlortetracycline at 1.1 mg·kg of BW^{-1} daily, administered in a gelatin capsule by balling gun; M-F = dosed with chlortetracycline at 1.1 mg·kg of BW^{-1} daily, administered via observed intake from an individual feed pan; M-P = medicated mineral containing 6.61 g/kg chlortetracycline allocated to mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg of BW^{-1} daily for each animal in the group, voluntary intake; NM-F = dosed with nonmedicated mineral, administered via observed intake from an individual feed pan in phase 1 and voluntary intake in phase 2. Limit of quantification is 1 ng/mL.

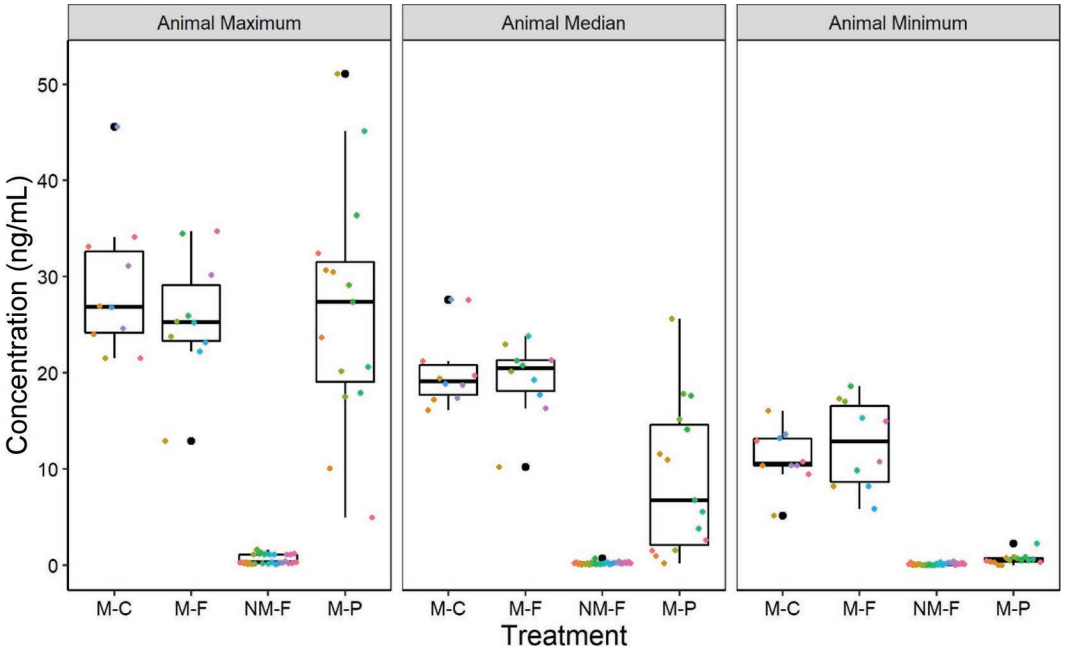

Figure 2. Scatter-and-box plots of selected parameters describing plasma chlortetracycline concentrations summarized at the individual cow level. The mean was not calculated because of the skewed distribution of plasma concentrations in the M-P treatment group. M-C = dosed with chlortetracycline at 1.1 mg·kg of BW^{-1} daily, administered in a gelatin capsule by balling gun; M-F = dosed with chlortetracycline at 1.1 mg·kg of BW^{-1} daily, administered via observed intake from an individual feed pan; M-P = medicated mineral containing 6.61 g/kg chlortetracycline allocated to mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg of BW^{-1} daily for each animal in the group, voluntary intake; NM-F = dosed with nonmedicated mineral, administered via observed intake from an individual feed pan in phase 1 and voluntary intake in phase 2. Limit of quantification is 1 ng/mL. Each point represents the median, minimum, or maximum concentration of an individual animal for all sample collections. For each box plot, the bottom of the box represents the junction of first and second quartile, the top of the box represents the junction of third and fourth quartile, the center line in each box represents the median or junction of second and third quartiles. The tips of the whiskers represent the range, and bold black dots beyond the whiskers represent outliers of more than 3 SD from the mean.

Parameters of Interest

Recognizing that the individual animal is the target of anaplasmosis treatment, plasma concentrations of CTC were summarized within animal and treatment according several different parameters: maximum, mean, median, and minimum. For example, if cow A were allocated to the M-C treatment, the 5 samples collected during phase 1 were used to calculate a mean for this cow. The additional parameters of median, minimum, and maximum were also

Table 5. Results of *t*-test performed for various within-cow descriptive parameters comparing treatments of M-C and M-F[1]

	t-test			
Parameter	***P*-value**	**Estimate M-C[1]**	**Estimate M-F[2]**	**95% CI[4]**
Animal mean	0.66	20	19	−3.2, 5.0
Animal median	0.58	20	19	−2.8, 4.8
Animal minimum	0.43	11	12	−5.0, 2.2
Animal maximum	0.32	28	25	−3.3, 9.6

[1]Treatment groups did not significantly differ for any of the calculated parameters. n = 9.

[2]M-C = dosed at 1.1 mg·kg of $BW^{-1}·d^{-1}$, administered in a gelatin capsule via balling gun.

[3]M-F = dosed at 1.1 mg·kg of $BW^{-1}·d^{-1}$, administered via observed intake from an individual feed pan.

[4]Identical parameter comparisons would have a mean difference of zero. This is the CI around zero for the difference between the 2 treatment groups.

calculated for each individual animal within treatment group. These values are shown in Figure 2 with each dot representing a parameter value of an individual animal within treatment group. Mean values were not calculated for M-P treatment group due to the right skewed distribution of plasma concentrations in this treatment group.

Because there was normal distribution of M-C and M-F treatments, a *t*-test was used to compare each of these parameters, and the results are shown in Table 5. No differences were detected between the maximum, mean, median, or minimum cow-level parameters between M-C and M-F.

As shown in Figure 2, the animal maximum CTC concentrations differed only slightly between treatments, but the median and minimum concentrations appeared lower for M-P than for M-C and M-F treatments. To explore this apparent difference between phase 1 and 2 (M-C, M-F vs. M-P), analyses were limited only to those animals that received a known dose of CTC in phase 1 (M-C or M-F) and were still present for phase 2 (Table 6, Figure 3). This eliminated the potential of individual cow effects confounding the comparison. The exposure of the group to a single mineral feeder in a single pasture resulted in no true replication of this treatment. Thus, these data should be interpreted carefully, serving primarily as pilot data for future studies. Differences in CTC dosing methods in phase 1 (M-C or M-F) and phase 2 (M-P) of this experiment were tested using the Wilcoxon sum rank test (Table 7). The minimum detected CTC plasma concentrations differed ($P < 0.01$) between the 2 experimental phases (M-C and M-F vs. M-P). At 90% confidence, the median CTC concentration also differed ($P = 0.05$); however, the detected CTC maximum showed no evidence of a difference ($P = 1.0$).

Implications for Regimen Optimization

The most important pharmacodynamic parameters for effective control of anaplasmosis with CTC are unknown. Parameters such as time above a minimum concentration, maximum concentration, or total area under the plasma concentration curve could be relevant; however, their appropriate application for anaplasmosis control remains undocumented. These results may have implications for the establishment of target plasma concentrations of CTC for anaplasmosis control. Due to the complexity of culturing *A. marginale* in vitro, the use of standard methods such as pairing pharmacodynamic targets with mean inhibitory concentrations to help establish dose regimens and target plasma drug concentrations are not currently practical.

Clinical trials will likely be necessary to establish appropriate target plasma concentrations for control of active anaplasmosis infections, as well as elimination of carrier states. For example, it is possible that for the control of active infection, the median plasma concentration might be best correlated with positive clinical outcome, but for elimination of carriers, the minimum concentration might be the parameter that is most important. As regimens are optimized for antimicrobial stewardship, care should be taken to ensure that the method of administration applicable to production systems matches the experimental delivery method used to evaluate efficacy.

Limitations

The variation observed in the M-P treatment group warrants further study. Consumption of mineral from a mineral feeder in a pasture setting is known to be variable and may be influenced by factors such as mineral-feeder location, season of the year, and pasture size (Greene,

Table 6. Median, maximum, and minimum plasma chlortetracycline concentrations (ng/mL) were calculated for each cow receiving chlortetracycline during phase 1 and phase 2

	Animal maximum		Animal median		Animal minimum	
Item	Phase 1[1]	Phase 2[2]	Phase 1	Phase 2	Phase 1	Phase 2
Animal A	33.1	32.4	21.2	1.5	12.9	0.5
Animal B	24.0	30.7	16.1	11.6	10.4	0.3
Animal C	26.9	10.1	17.2	0.2	16.1	<0.1
Animal D	12.9	30.5	10.2	11.0	8.2	<0.1
Animal E	21.5	51.1	19.4	25.6	5.2	0.8
Animal F	25.3	17.5	20.1	15.2	17.0	0.8
Animal G	34.5	36.4	21.2	17.6	18.6	0.9
Animal H	25.9	17.9	20.8	6.8	9.9	0.5
Animal I	21.5	4.9	19.7	2.6	9.5	0.3

[1]Phase 1 refers to phase 1 of the experiment, where cattle were treated with 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$ chlortetracycline individually administered by gelatin capsule or observed intake.

[2]Phase 2 refers to phase 2 of the experiment, where cattle were treated with 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$ chlortetracycline administered by allocation of chlortetracycline product to a pasture mineral feeder in a sufficient amount to treat the group for 1 d at the specified dose.

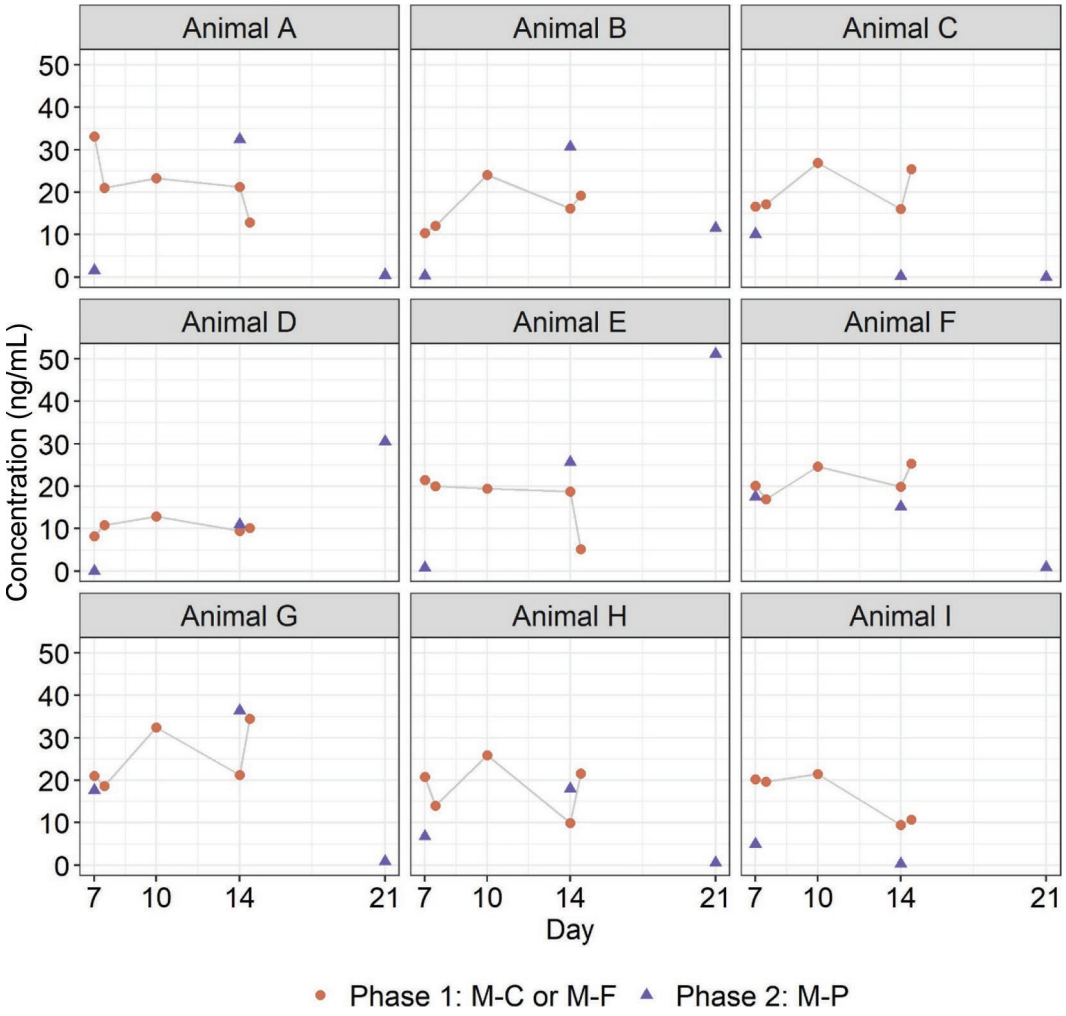

Figure 3. Plasma concentrations of chlortetracycline for phase 1 (M-C, M-F) and phase 2 (M-P). M-C = dosed with chlortetracycline at 1.1 mg·kg of BW^{-1} daily, administered in a gelatin capsule by balling gun; M-F = dosed with chlortetracycline at 1.1 mg·kg of BW^{-1} daily, administered via observed intake from an individual feed pan; M-P = medicated mineral containing 6.61 g/kg chlortetracycline allocated to mineral feeder daily in an amount sufficient to achieve 1.1 mg·kg of BW^{-1} daily for each animal in the group, voluntary intake. Each graph represents an individual cow identified by tag number. Cows that received nonmedicated mineral in phase 1 are not shown. The line between points for phase 1 is included because the dosing method used was consistent and samples were taken at steady state. Therefore, it is reasonable to assume that the plasma concentration between points remains close to the line. The line is intentionally excluded from phase 2 points because the potential for variation in intake reduces the expectation that the values between time points would lie near a line drawn between points.

2000); however, every attempt was made to minimize such factors in our experimental approach. Subsequent experiments should examine distributions of plasma CTC concentrations to describe variation associated with multiple pastures, groups of cattle, mineral feeders, and product formulations.

The label of the mineral product used in our experiment required that the amount of mineral offered to cattle be allocated to the feeder each day, as performed for this trial. In a typical production setting, it is a common practice to fill the feeder with an amount of an appropriate mineral product sufficient for the group for multiple days or weeks. Therefore, consumption in a production setting where a free-choice mineral formulation is used may vary somewhat from this trial.

APPLICATIONS

Administration of a CTC-containing mineral supplement resulted in similar plasma concentrations of CTC across animal subjects when intake was encouraged by adding the supplement to a small amount of individually fed ration (M-F) or when the supplement was dosed using a gelatin capsule (M-C). The authors conclude that direct gelatin-capsule dosing of CTC-containing products offered no advantage in consistency over feeding the same product mixed with feed. The authors also conclude that dose linearity is maintained with a mineral formulation when compared with bunk-administered supplements, implying similar bioavailability in spite of differences in formulation. Conversely, there was an indication that voluntary

Table 7. Significance test for a difference between plasma chlortetracycline concentrations achieved in phase 1 versus phase 2[1] within individual animal

Parameter	Wilcoxon signed rank, V	*P*-value	Pseudo median[2]	95% CI
Animal median	39	0.05	8.8	−0.6, 16.9
Animal maximum	23	1	0.6	−14.4, 12.3
Animal minimum	45	<0.01	11.3	8.2, 16.1

[1]Phase 1 refers to phase 1 of the experiment, where cattle were treated with 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$ chlortetracycline individually administered by gelatin capsule or observed intake. Phase 2 refers to phase 2 of the experiment, where cattle were treated with 1.1 mg·kg of $BW^{-1}\cdot d^{-1}$ chlortetracycline administered by allocation of chlortetracycline product to a pasture mineral feeder in a sufficient amount to treat the group for 1 d at the specified dose.

[2]Identical comparisons would have a pseudo median difference of zero.

intake from a pasture mineral feeder should be further studied. The clinical significance of differences in plasma CTC concentrations between phase 1 (M-C or M-F) and phase 2 (M-P) of this experiment for maintaining adequate anaplasmosis control are unknown but should be further explored as they could result in inadequate protection from disease in some circumstances.

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