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PHARMACOKINETIC REPORT

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Pharmacokinetics of chloramphenicol base in horses and comparison to compounded formulations

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Abstract

Chloramphenicol is commonly used in horses; however, there are no studies evaluating the pharmacokinetics of veterinary canine-approved tablets. Studies using different formulations and earlier analytical techniques led to concerns over low bioavailability in horses. Safety concerns about human health have led many veterinarians to prescribe compounded formulations that are already in suspension or paste form. The objective of this study was to evaluate the pharmacokinetics of approved chloramphenicol tablets in horses, along with compounded preparations. The hypothesis was that chloramphenicol has low absorption and a short half-life in horses leading to low serum concentrations and that compounded preparations have lower relative bioavailability. Seven horses were administered chloramphenicol tablets (50 mg/kg orally). In a crossover design, they were administered two compounded preparations to compare all three formulations at the same dose (50 mg/kg). C_{max} was 5.25 ± 4.07 μg/ml at 4.89 hr, 4.96 ± 3.31 μg/ml at 4.14 hr, and 3.84 ± 2.96 μg/ml at 4.39 hr for the tablets, paste, and suspension, respectively. Elimination half-life was 2.65 ± 0.75, 3.47 ± 1.47, and 4.36 ± 4.54 hr for tablets, paste, and suspension, respectively. The $AUC_{0\rightarrow\infty}$ was 17.93 ± 7.69, 16.25 ± 1.85, and 14.00 ± 5.47 hr*μg/ml for the tablets, compounded paste, and compounded suspension, respectively. Relative bioavailability of compounded suspension and paste was 78.1% and 90.6%. C_{max} after administration of all formulations did not reach the recommended MIC target of 8 μg/ml set by the Clinical Laboratory Standards Institute (CLSI) for most bacteria. Multidose studies are warranted, but the low serum concentrations suggest that bacteria with MIC values lower than CLSI recommendations should be targeted in adult horses.

KEYWORDS
antibiotic, antimicrobial, equine, therapeutics

1 | INTRODUCTION

Chloramphenicol is a broad-spectrum antimicrobial commonly used in equine medicine to treat a variety of infections, including aerobic and anaerobic, and targeting both gram positive and negative bacteria. Chloramphenicol has several potential advantages for use in equine practice, including broad spectrum activity and a high degree of lipid penetrability. The injectable formulation of chloramphenicol is unavailable within the United States, leaving the oral route as the only option.

Patel and Magdesian contributed equally to this work.

Chloramphenicol is forbidden for use in food-producing animals and is no longer used systemically in humans. Potential adverse effects of chloramphenicol in humans include bone marrow suppression and rare life-threatening aplastic anemia, even from topical ophthalmic contact. These side effects have not been reported in horses, but warrant minimizing exposure of clients to chloramphenicol during administration and through minimizing its use.

Despite few publications on the pharmacokinetics of orally administered chloramphenicol in horses, it is commonly used in equine practice. Single-dose administration has demonstrated variable enteral absorption, with a reported bioavailability of 21%–40% (Gronwall, Brown, Merritt, & Stone, 1986; McElligott, Sommardahl, & Cox, 2017). Low and variable bioavailability, coupled with a very short half-life in horses, questions the validity of its use in equine practice at some of the published lower recommended doses (as low as 25–30 mg/kg q 6–8 hr) (Oh-Ishi, 1968; Sisodia, Kramer, Kramer, Gupta, Lerner, & Taksas, 1975).

Because of potential human exposure to chloramphenicol through the inhalational or cutaneous routes, crushing of tablets for administration to horses is not recommended. Out of a need for dosage formulations which can be administered safely, compounded preparations of chloramphenicol are commonly prescribed in equine practice so that owners do not have to directly handle the medication. Compounded formulations of other pharmaceuticals have been shown to have variable stability, potency, and efficacy, and whether this is true of compounded chloramphenicol remains to be studied (Merritt, Sanchez, Burrow, Church, & Ludzia, 2003; Stanley, Thomas, & Skinner, 2003). A recent report studying a university compounded formulation of chloramphenicol made from base powder in a water-soluble base showed a bioavailability of 28% in adult horses (McElligott et al., 2017). However, that study did not evaluate quality control of the product nor the pharmacokinetics of the commercially available veterinary tablets approved for dogs.

To the authors' knowledge, there are no reported studies evaluating the stability, potency, or relative bioavailability of compounded chloramphenicol preparations as compared to commercially available tablets approved for dogs. The use of compounded preparations without data in terms of drug concentration, stability, or relative bioavailability has risks of therapeutic failure and increased adverse effects.

The objective of this study was to characterize the pharmacokinetics of the veterinary-approved chloramphenicol formulation (canine tablets) in horses after single-dose administration, using modern analytical techniques. In addition, the relative bioavailability of two compounded preparations (paste and suspension) was compared to commercially available tablets. The hypotheses were as follows: (1) Chloramphenicol base achieves relatively low serum concentrations after oral administration to horses, and (2) compounded preparations of chloramphenicol have low relative bioavailability as compared to approved tablets.

2 | MATERIALS AND METHODS

The study was approved by the University of California Institutional Animal Care and Use Committee. Seven horses were studied, and they were determined to be healthy based on physical examination, CBC, and serum chemistry panel findings. They had a mean age of 9.1 ± 2.9 years and a weight of 520.3 ± 29.9 kg. Breeds consisted of six Thoroughbreds and one Quarter Horse, consisting of four mares and three geldings. Horses were housed in individual pens and fasted for 12 hr prior to administration of chloramphenicol. They were fed orchard grass hay and alfalfa 6 hr after the administration of chloramphenicol.

2.1 | Drug administration and sample preparation

Three different formulations of chloramphenicol were administered at a single dose of 50 mg/kg orally, in a three-way crossover design using the same horses. The three preparations of chloramphenicol were (1) base tablets approved for dogs (Viceton 1 g tablets, Bimeda Inc), (2) commercially available compounded paste made from chloramphenicol base, and (3) commercially available compounded suspension also made from chloramphenicol base. There was a minimum 14-day washout period between studies. The base tablets (1 gm tablets) were dissolved in two 60-cc catheter-tipped syringes (60-cc catheter-tipped syringe), with each syringe containing 45 ml of deionized water and 10 ml of Karo (Karo Light Corn Syrup, ACH Food Companies, Inc.) syrup, the latter added immediately prior to administration. The tablets were left in water for only as long as it took for them to completely dissolve, which was 5–5.5 hr. Chloramphenicol is stable in aqueous solutions at room temperature for over 24 hr (Ehrlich, Bartz, Smith, Joslyn, & Burkholder, 1947). Compounded preparations were also administered orally with the same type of oral dosing syringe. After swallowing the medication, the horses were administered 50 ml of deionized water orally to ensure complete administration of the dose. The compounded preparations used during the study were from two different lot numbers. Stability and potency were tested three times throughout the study period, over a period of 3 months.

Blood samples (approximately 5 ml) were collected through a 14-gauge catheter (i.v. catheter) placed in the jugular vein and transferred to red top vacuum-evacuated serum tubes. Blood samples were collected at the following time points: 0, 15, 30, and 45 min, and 1, 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, 16, 20, and 24 hr following drug administration.

For all three drug preparations, blood samples were stored at room temperature for one hour to allow for clotting and then centrifuged at 1,510 × *g* for 10 min. The serum was then transferred into cryovial tubes and stored at −80°C until analysis.

2.2 | Determination of serum chloramphenicol concentrations

The analytical methods described here were validated for horse serum. For analysis, chloramphenicol was prepared by dilution of 1 mg/ml stock solutions with acetonitrile (ACN; Burdick and Jackson) to concentrations of 0.01, 0.1, 1, and 10 ng/µl. Serum calibrators were prepared by dilution of the working standard solutions

with drug-free equine serum to concentrations ranging from 2 to 8,000 ng/ml. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality control samples (drug-free equine serum fortified with analyte at three concentrations within the standard curve) were included with each sample set as an additional check of accuracy.

Prior to analysis, 500 µl of serum was diluted with 200 µl of water containing 100 ng/ml of d5-chloramphenicol internal standard (Toronto Research Chemicals) and the samples were vortexed briefly to mix. Five milliliters of methyl tert-butyl ether (Fisher Scientific) was added to each serum sample, and the samples were mixed by rotation (Glas-Col) for 20 min at 40 revolutions per minute. After rotation, samples were centrifuged at 1,510 *g* for 5 min at 4°C and the top organic layer was transferred to a 12 × 75 mm glass tube (Fisher Scientific, Fair Lawn, NJ). Samples were dried under nitrogen and reconstituted in 120 µL of 5% ACN in water, both with 0.2% formic acid (Alfa Aesar). The injection volume was 30 µL into the liquid chromatography mass spectrometry system.

The concentration of chloramphenicol was measured in serum by liquid chromatography tandem mass spectrometry (LC-MS/MS). Quantitative analysis of serum was performed on a TSQ Vantage triple quadrupole mass spectrometer (Thermo Scientific) coupled with a turbulent flow chromatography system (TFC TLX4 Thermo Scientific) having 1100 series liquid chromatography systems (Agilent Technologies) and operated in laminar flow mode. The system was operated using negative electrospray ionization (ESI(−)). The spray voltage was set at 2000 V; sheath gas and auxiliary gas were 40 and 20, respectively (arbitrary units); vaporizer temperature was 40°C; and the capillary temperature was 300°C. Product masses and collision energies were optimized by infusing the standards into the mass spectrometer. Chromatography employed an ACE 3 C18 10 cm × 2.1 mm column (Mac-Mod Analytical) and an isocratic flow of ACN in water, both with 0.2% formic acid, at a flow rate of 0.35 ml/min. The ACN concentration was held at 30% for 4.67 min.

Detection and quantification were conducted using selective reaction monitoring (SRM) of initial precursor ion for chloramphenicol (mass to charge ratio (m/z) 320.922) and the internal standard d5-chloramphenicol ((m/z) 325.939). The response for the product ions for chloramphenicol (m/z 151.1) and the internal standard d5-chloramphenicol (m/z 261.2) were plotted and peaks at the proper retention time integrated using Quanbrowser software (Thermo Scientific). Quanbrowser software was used to generate calibration curves and quantitate analytes in all samples by linear regression analysis. A weighting factor of 1/X was used for all calibration curves.

The tablets and formulations were stored in light-proof containers supplied by the manufacturers/pharmacy at 71–73°F (22–23°C) in a laboratory and out of direct sunlight. The chloramphenicol concentrations within the compounded preparations were tested for potency and stability. In addition, the compounded preparations were serially tested at 1.5 and 4 months to evaluate for stability during the course of the study. Prior to testing, aliquots of each dosing solution (n = 3 replicates/formulation) were dissolved and/or diluted sequentially four times in acetone using volumetric flasks. A calibration curve, ranging from 10 to 50 µg/ml, and quality control samples were prepared, and the LC-MS/MS method described above used to determine the potency of the formulations.

2.3 | Pharmacokinetic analysis

Pharmacokinetic analysis was performed on serum chloramphenicol concentrations using noncompartmental analysis and a commercially available software program (Phoenix Winnonlin Version 6.2). Maximum serum concentrations (C_{max}) and time to maximal serum concentration (T_{max}) were obtained directly from the serum concentration data. Calculated pharmacokinetic parameters using noncompartmental models included the terminal-phase rate constant (λz), terminal-phase half-life (λz HL), the area under the curve from time 0 to infinity ($AUC_{0\rightarrow\infty}$), the area under the curve from time zero to the last measurable serum concentration ($AUC_{(last)}$), and the extrapolated percentage of the area under the curve (AUC %). The λz HL was calculated using the $t_{1/2} = 0.693/\lambda z$ equation, and the AUC was calculated using the log-linear trapezoidal method. Relative bioavailability was calculated for the compounded formulations using the formula: $(AUC_{0\rightarrow\infty})_{\text{compounded preparation}}/(AUC_{0\rightarrow\infty})_{\text{tablets.}}$

2.4 | Statistics

Descriptive statistics were employed to describe the pharmacokinetic variables.

3 | RESULTS

3.1 | Results for accuracy and precision

The response for chloramphenicol was linear and gave correlation coefficients of 0.99 or better. The interday, intraday, analyst to analyst precision and accuracy of the assay were determined by assaying quality control samples in replicates (n = 6). Accuracy was reported as percent nominal concentration, and precision was reported as percent relative standard deviation. Precision and accuracy values for serum concentration determination assays are reported in Table 1. The technique was optimized to provide a limit of quantitation of 2 ng/ml and a limit of detection of approximately 0.5 ng/ml for chloramphenicol. The liquid–liquid extraction recovery was 96% for chloramphenicol. Precision and accuracy values for determination of concentrations in the compounded chloramphenicol formulations were determined as described above for serum and were ±15% of nominal concentrations.

3.2 | Concentrations of chloramphenicol in compounded formulations

Over the six-month period of the study, the compounded paste varied as much as 103.7%–115.8% of the intended label concentration, whereas the suspension ranged from 93.0% to 117.8% of the intended label concentration.

TABLE 1 Accuracy and precision values for LC-MS/MS analysis of chloramphenicol in equine serum

Concentration (ng/mL)	Intraday accuracy (% nominal concentration)	Intraday precision (% relative *SD*)	Interday accuracy (% nominal concentration)	Interday precision (% relative *SD*)
40	95.0	2.0	97.0	3.0
350	98.0	2.0	98.0	3.0
1,500	98.0	3.0	98.0	3.0

3.3 | Pharmacokinetics

Serum concentrations of chloramphenicol over time after base tablet administration are depicted in Figure 1. The concentrations over time after compounded paste and suspension preparations are shown in Figures 2 and 3, respectively. The serum concentrations after administration of all three formulations are presented in Figure 4. Results for the noncompartmental pharmacokinetic variables for the chloramphenicol tablets are listed in Table 2. They are listed in Tables 3 and 4 for compounded paste and suspension, respectively. Following oral administration of chloramphenicol base tablets (50 mg/kg), the mean C_{max} was 5.25 μg/ml (±4.07) at 4.89 hr after chloramphenicol administration. The C_{max} and T_{max} for the compounded suspension and paste were 3.84 μg/ml (±2.97) at 4.39 h and 4.96 μg/ml (±3.31) at 4.14 hr after chloramphenicol administration, respectively. The relative bioavailabilities of the compounded suspension and paste, relative to the commercially available tablets, were 78.1% and 90.6%, respectively.

4 | DISCUSSION

Chloramphenicol base tablets and compounded suspension and paste preparations were evaluated in this study. To the authors' knowledge, this is the first study to evaluate the pharmacokinetics of the only veterinary-approved form of chloramphenicol (canine tablets), as well as of commercially available compounded chloramphenicol formulations, in horses. It is also the first to evaluate the stability and potency of compounded chloramphenicol formulations.

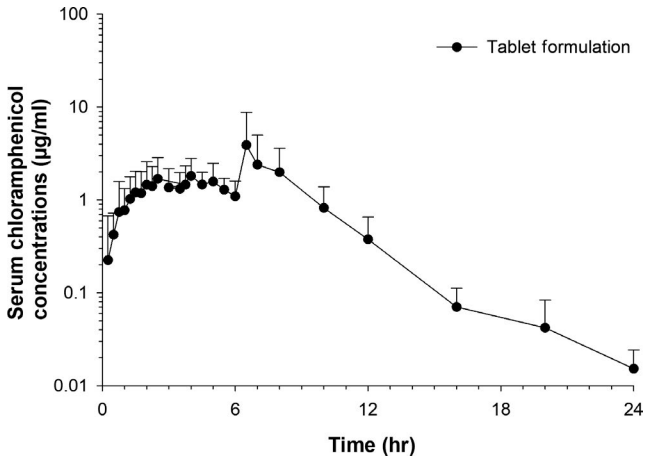

FIGURE 1 Serum chloramphenicol concentrations over time after a single dose of base tablets (50 mg/kg)

The maximum serum concentrations of chloramphenicol in the horses of this study varied from a mean of 3.84 for suspension to 5.25 μg/ml for the tablets. The difference may have reflected more rapid dissolution of the tablets or vehicle components in the compounded preparation that may have reduced or delayed absorption. The time to maximum concentration was similar for all three formulations; however, there was marked interindividual variability in T_{max}. Similarly, C_{max} had a high degree of interindividual variability within a formulation.

The mean C_{max} values after administration of all formulations of chloramphenicol did not reach the recommended MIC target of 8 μg/ml, set as the susceptibility breakpoint for most human and veterinary bacterial pathogens by the Clinical Laboratory Standards Institute (CLSI, 2018). While this is a single-dose study, and multiple-dose studies are required to determine the steady-state concentrations of chloramphenicol, this suggests that bacteria considered susceptible to chloramphenicol in other species, based on MIC values of 8 μg/ml, should not be considered susceptible in horse after oral administration of the dose used in this study. Based on the results of this study, it may be more appropriate to target bacteria with MIC values of ≤1–2 μg/ml, as concentrations at the 4-hr mark were approximately 1.8 μg/ml. As chloramphenicol is a time-dependent antimicrobial, serum concentrations optimally should be above the MIC of the offending organism for ≥50% of the dosing interval. The four-hour mark represents half of the dosing interval when chloramphenicol is administered every 8 hr, as is often done in equine practice; however, a multidose study, taken to steady state, is optimally required to further evaluate this estimation. In a previous study evaluating five repeated doses of chloramphenicol in mares, the bioavailability of chloramphenicol was lower after the fifth dose as compared to the first and resulted in lower serum concentrations (Gronwall et al., 1986). These findings are unexpected because chloramphenicol is a cytochrome P450 enzyme inhibitor and would be expected to slow its own metabolism, resulting in higher serum concentrations (Gerken & Sams, 1985; Park, Kim, & Kim, 2003). However, multiday studies of chloramphenicol have not been performed. These are warranted, in order to evaluate whether the findings of Gronwall et al are repeatable, and whether multiday dosing would result in even lower serum concentrations than were observed in this single dose study, or whether the serum concentrations would rise due to hepatic enzyme inhibition. In addition, the Gronwall study evaluated a compounded preparation containing 40% propylene glycol, and the authors speculated that this may have been responsible for the reduced bioavailability over time due to gut inflammation (Gronwall et al., 1986).

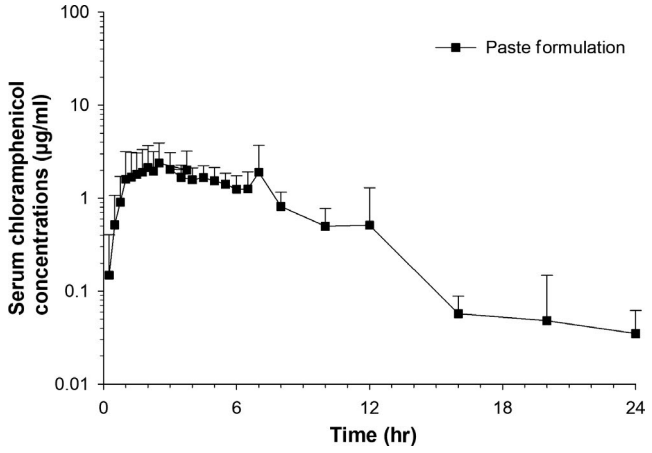

FIGURE 2 Serum chloramphenicol concentrations over time after a single dose of compounded paste (50 mg/kg)

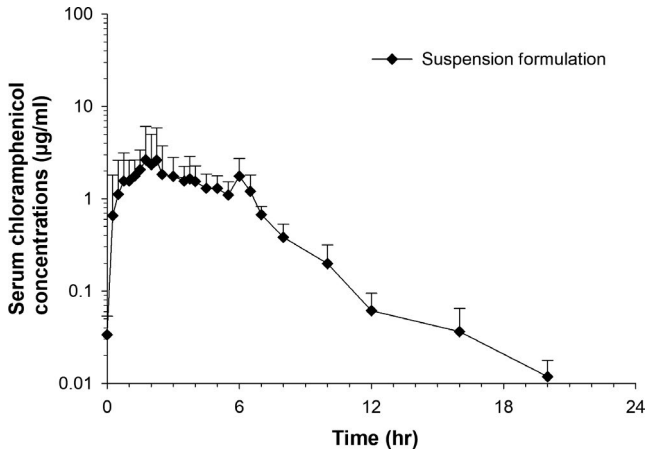

FIGURE 3 Serum chloramphenicol concentrations over time after a single dose of compounded suspension (50 mg/kg)

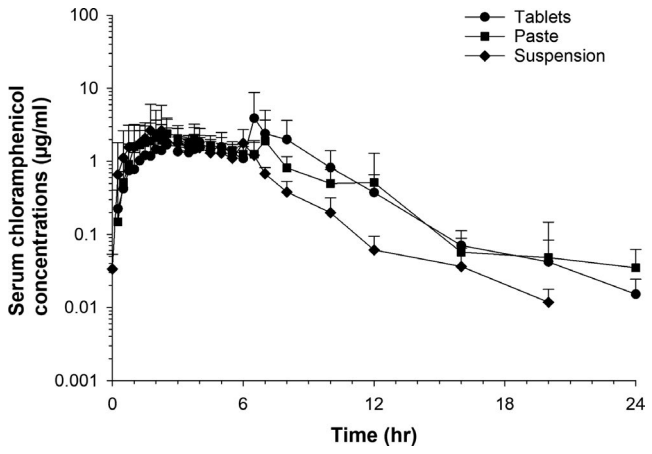

FIGURE 4 Comparison of serum chloramphenicol concentrations over time after 50 mg/kg of base tablets, compounded paste, and compounded suspension

Equine bacterial isolates that have reported MIC values ≤2 µg/ml for chloramphenicol include *Streptococcus equi ss zooepidemicus*, *Streptococcus equi ss equi*, and *Actinobacillus equuli* (Adamson, Wilson,

TABLE 2 Noncompartmental pharmacokinetic variables for chloramphenicol base tablets following a single oral dose of 50 mg/kg

Parameter	Units	Horse							Mean	*SD*
		1	2	3	4	5	6	7		
λz	1/hr	0.24	0.32	0.35	0.26	0.34	0.17	0.26	0.28	0.07
λz Half-life	hr	2.94	2.16	1.96	2.63	2.05	4.13	2.69	2.65	0.75
T_{max}	hr	8.00	6.50	0.75	4.00	6.50	6.50	2.00	4.89	2.70
C_{max}	µg/ml	4.85	4.05	2.57	2.96	4.39	14.31	3.61	5.25	4.07
$AUC_{(last)}$	h*µg/ml	17.35	13.68	17.48	13.13	17.53	29.68	11.62	17.21	6.00
$AUC_{(all)}$	h*µg/ml	17.35	13.68	17.48	13.13	17.53	29.68	11.62	17.21	6.00
$AUC_{0\rightarrow\infty}$ (obs)	h*µg/ml	17.44	13.72	17.51	13.17	17.55	29.88	11.65	17.27	6.06
AUC ∞ D obs	h*kg*ng ml^{-1} mg^{-1}	348.83	274.40	350.25	263.41	351.05	597.5	232.94	345.48	121.13
AUC% (Extrap obs)	%	0.54	0.32	0.19	0.31	0.14	0.65	0.26	0.34	0.19
Cl *F* (obs)	ml hr^{-1} kg^{-1}	2866.75	3644.34	2855.06	3796.42	2848.62	1674.00	4292.89	3139.67	856.88
$MRT_{0\rightarrow\infty}$ (obs)	hr	7.67	7.47	6.85	4.86	5.65	7.69	3.82	6.29	1.54

Abbreviation: Cl *F* (obs), observed clearance relative to bioavailability.

TABLE 3 Noncompartmental pharmacokinetic variables for chloramphenicol compounded paste after a single oral dose of 50 mg/kg

		Horse								
Parameter	**Units**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**	***SD***
λz	1/hr	0.15	0.11	0.30	0.26	0.27	0.24	0.24	0.22	0.07
λz Half-life	hr	4.73	6.28	2.28	2.67	2.57	2.93	2.85	3.47	1.47
T_{max}	hr	7.00	7.00	1.00	2.50	2.50	7.00	2.00	4.14	2.72
C_{max}	μg/ml	2.44	12.18	4.37	4.60	3.76	2.69	4.70	4.96	3.31
$AUC_{(last)}$	h*μg/ml	18.02	15.45	17.52	14.62	15.66	15.33	14.91	15.93	1.31
$AUC_{(all)}$	h*μg/ml	18.02	15.45	17.52	14.62	15.66	15.33	14.91	15.93	1.31
$AUC_{o\to\infty}$ (obs)	h*μg/ml	19.88	15.68	17.54	14.64	15.71	15.36	14.94	16.25	1.85
AUC ∞ D obs	$h*kg*ng\ ml^{-1}\ mg^{-1}$	397.69	313.62	350.76	292.81	314.16	307.25	298.80	325.01	37.04
AUC% (Extrap obs)	%	9.37	1.50	0.08	0.15	0.31	0.19	0.19	1.69	3.43
Cl *F* (obs)	$ml\ hr^{-1}\ kg^{-1}$	2514.55	3188.54	2850.92	3415.20	3183.05	3254.70	3346.76	3107.67	317.09
$MRT_{o\to\infty}$ (obs)	hr	10.70	7.68	4.84	4.10	4.92	6.46	3.72	6.06	2.46

Abbreviation: Cl *F* (obs), observed clearance relative to bioavailability.

TABLE 4 Noncompartmental pharmacokinetic variables for chloramphenicol compounded suspension after a single oral dose of 50mg/kg

		Horse								
Parameter	**Units**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**	***SD***
λz	1/hr	0.24	0.05	0.28	0.23	0.31	0.31	0.23	0.23	0.09
λz Half-life	hr	2.90	14.62	2.51	2.96	2.27	2.23	3.06	4.36	4.54
T_{max}	hr	6.50	6.50	4.50	3.50	2.00	0.75	7.00	4.39	2.43
C_{max}	μg/ml	2.81	3.15	2.14	1.82	10.27	4.42	2.29	3.84	2.96
$AUC_{(last)}$	h*μg/ml	13.42	12.73	12.31	10.21	26.00	12.55	10.08	13.90	5.48
$AUC_{(all)}$	h*μg/ml	13.42	12.73	12.31	10.21	26.00	12.55	10.08	13.90	5.48
$AUC_{o\to\infty}$ (obs)	h*μg/ml	13.50	13.17	12.34	10.21	26.03	12.56	10.13	14.00	5.47
AUC ∞ D obs	$h*kg*ng\ ml^{-1}\ mg^{-1}$	269.90	263.34	246.73	205.32	520.59	251.21	202.63	279.96	153.83
AUC% (Extrap obs)	%	0.55	0.33	0.20	0.55	0.12	0.10	0.48	0.76	1.14
Cl *F* (obs)	$ml\ hr^{-1}\ kg^{-1}$	3705.07	3797.37	4053.08	4870.36	1920.90	3980.76	4935.22	3894.68	1000.70
$MRT_{o\to\infty}$ (obs)	hr	7.11	6.74	5.21	5.96	3.98	3.58	6.65	5.61	1.40

Abbreviation: Cl *F* (obs), observed clearance relative to bioavailability.

Hirsh, Baggot, & Martin, 1985; Ensink, Klingeren, Houwers, Klein, & Vulto, 1993), although the MIC values were most commonly 2 μg/ml. However, many isolates of other *Actinobacillus* species have been reported to have higher MIC values (>8 μg/ml) and should be considered resistant without susceptibility testing results (Adamson et al., 1985). Though usually susceptible in vivo, *Streptococcus equi ss equi* usually forms thick-capsuled abscesses, and it is unknown whether adequate concentrations of chloramphenicol can be achieved inside of abscesses when serum concentrations are approximately 2 μg/ml. A number of anaerobes are susceptible to low concentrations of chloramphenicol as well, including *Porphryomonas spp* (MIC = 0.38–0.5 μg/ml, *n* = 2 isolates) *and Prevotella spp* (MIC = 0.38–1.5 μg/ml, *n* = 8), *and most Peptostreptococcus spp* (MIC = 0.38–2 μg/ml *n* = 14, with one isolate having an MIC of 4 μg/ml) and *Fusobacterium spp*. isolates (0.064–2 μg/ml *n* = 12, with one isolate having an MIC of 8 μg/ml), and they would be considered susceptible to attainable concentrations in horses (Lawhon, Taylor, & Fajt, 2013). A report of the antimicrobial susceptibility of two *Lawsonia intracellularis* isolates demonstrated intracellular MIC values for chloramphenicol ≤2 μg/mL, and this suggests that chloramphenicol could be used to treat this infection (Pereira et al., 2019). Chloramphenicol should not be used to treat infections with Gram-negative enteric bacteria, including *Enterobacter*, *E coli*, *Klebsiella pneumoniae* and *Salmonella* spp., as well as *Pseudomonas aeruginosa*, based on their high MIC values (Adamson et al., 1985; Burrows, Morton, & Fales, 1993). Staphylococci should also be considered resistant, unless individual isolates are tested for susceptibility and found to have low MIC values (Adamson et al., 1985).

The mean C_{max} of the base tablets in our study was 5.25 μg/ml, with a range of 2.57–14.31, which is toward the lower end of a wide range of reported C_{max} values (3.6-46 μg/ml) in previous studies (English & Withy, 1959; Oh-Ishi, 1968; Sisodia et al., 1975). The wide variation in C_{max} is therefore consistent with other studies and could be attributed to high interindividual differences in absorption or metabolism/elimination of chloramphenicol. Previous studies in horses have used varying dosages of chloramphenicol, and it is often unclear which salt form of the drug was administered. One study used an oral dose of 20 mg/kg and found a C_{max} of 3.6 μg/ml at T_{max} 1 hr (English & Withy, 1959). Another study used an oral dose of 30 mg/kg of chloramphenicol palmitate and found a C_{max} of 4.9 μg/ml at T_{max} 1 hr after administration (Sisodia et al., 1975).

It is of note that a small secondary peak in chloramphenicol serum concentrations was noted in some of the horses at the 6- to 7-hr mark for the tablets (4/7 of the horses) and both compounded preparations (3/7 horses with the paste, 2/7 with the suspension). Secondary peaks can occur due to enterohepatic recycling as well as variations in gastric emptying, small intestinal transit, or site-specific absorption. In the case of chloramphenicol in our study, the small secondary peak is likely a result of reduced gastric emptying and small intestinal motility from withholding feed, with a secondary peak shortly after feeding was reinstituted at 6 hr postdosing. This is supported by the fact that a study evaluating compounded chloramphenicol in nonfasted horses did not demonstrate such a secondary peak (McElligott et al., 2017).

The elimination half-life of chloramphenicol in this study was short, with a mean of 2.65 hr ± 0.75 (range, 1.96–4.13 hr). The short half-life of chloramphenicol in horses contributes to the overall low serum concentrations relative to those in humans, as a consequence of rapid elimination. The elimination half-life determined in this study is similar to that previously reported for adult horses after oral administration (1–3 hr) (Brook & Paris, 1956; Divers et al., 1982; McElligott et al., 2017; Oh-Ishi, 1968; Sisodia et al., 1975).

When comparing our results to the study by Oishi with the same administered dose (50 mg/kg), peak serum concentrations of 46 μg/ml at 1 hr and 37 μg/ml at 2 hr following oral administration of chloramphenicol base and palmitate salt, respectively, were reported (Oh-Ishi, 1968). However, it is important to note that that study employed colorimetry to determine results and modern analytical techniques can provide more accurate results. A recent study using HPLC to measure serum chloramphenicol after administration of a compounded formulation of chloramphenicol base reported a C_{max} of 3.46 ± 1.43 μg/ml (range, 0.81–5.45 μg/ml) and T_{max} of 0.79 ± 0.64 hr (0.25–2 hr), which more closely mirror the results of our study, although they were somewhat lower in value (McElligott et al., 2017). The decreased serum concentrations in that study by McElligott et al may have been due to the fact that horses were not withheld from feed prior to chloramphenicol in that study, or due to reduced bioavailability associated with the particular compounded formulation utilized in that study. Elimination half-life (2.85 ± 1.32 hr), mean residence time (5.82 ± 4.23 hr), and AUC (13.44 ± 5.26 hr*μg/ml) in that study using a compounded preparation were similar to those of our study using the same dose.

The relative bioavailability of the compounded paste formulation used in our study was within 10% of the commercial chloramphenicol base tablets, whereas it was somewhat lower for the suspension. The compounded preparations were fairly stable over time and were 93.0%–117.8% of the intended label concentration. Despite these favorable findings, caution should always be used in prescription of compounded preparations made from bulk, for medicolegal reasons. In general, the bioavailability of oral chloramphenicol should be regarded as poor. A recent study with a compounded formulation had an absolute oral bioavailability of 28 ± 10% (McElligott et al., 2017). The wide standard deviation reflects the marked interindividual variation in bioavailability of orally administered chloramphenicol.

It should be noted that the use of chloramphenicol tablets approved for dogs in horses constitutes extra-label drug use and should meet the conditions as outlined in the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994, as there is no approved formulation for horses. Limitations of the present study include the single-dose protocol used. In addition, the relatively small number of horses studied could have affected the results, especially in light of the interindividual variation in pharmacokinetic parameters among horses. Absolute bioavailability could not be calculated, due to unavailability of injectable chloramphenicol from either commercial sources or compounding pharmacies.

5 | CONCLUSIONS

Oral chloramphenicol base tablets and compounded preparations, after a single 50 mg/kg dose, produce serum concentrations in adult horses that are below desired concentrations in humans and those that would be conducive to the susceptibility break point of 8 µg/ml recommended by CLSI for most bacteria. The relative bioavailabilities of a compounded suspension and paste, relative to commercially available tablets, were 78.1% and 90.6%, respectively. Based on the results of this study, chloramphenicol tablets or compounded formulations can be used to treat infections in adult horses when the offending bacteria have lower MIC values (≤2 µg/mL). Because of the low MIC target, the use of chloramphenicol in adult horses should be directed by culture and susceptibility results. Generally, this will target *Streptococcus equi ss zooepidemicus*, *Actinobacillus equuli*, and selected anaerobes. Further studies are needed to evaluate the pharmacokinetics of oral chloramphenicol in horses at steady state and administered over several days to evaluate for alterations in serum pharmacokinetics over time.

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AUTHOR CONTRIBUTIONS

TP, KE, KGM, and JE conducted study; TP, KGM, KE, and JE collected data; TP, KGM, and KE interpreted the results; TP, KGM, and HK authored portions of manuscript; KGM and KE provided idea for study, and KGM edited the manuscript; JE involved in sample processing; HK conducted PK analysis on software; and TP, KGM, KE, JE, and HK reviewed the manuscript. HK also performed drug analysis.

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