THE EFFECT OF BODY WEIGHT ON THE DISPOSITION OF FLUNIXIN MEGLUMINE AND GENTAMICIN IN MINIATURE HORSES AND QUARTER HORSES

By

CRYSTAL LEE

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University of Saskatchewan

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Thesis Approved:

Lara Maxwell, PhD, DVM, Dipl. ACVCP

Thesis Adviser

Dianne McFarlane, PhD, DVM, Dipl. ACVIM, Dipl. ABVP

Todd Holbrook, DVM, Dipl. ACVIM, Dipl. ACVSMR

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Abstract: In most species, large variations in body size necessitate dose adjustments based on an allometric function of body weight. Despite the substantial disparity in body size between Miniature Horses and light-breed horses, there are no studies investigating appropriate dosing of any veterinary drug in Miniature Horses. The purpose of this study was primarily to develop a basis for pharmacologic scaling in the horse, and to determine the scaling exponents with which flunixin meglumine and gentamicin relate to body weight in the horse. A secondary purpose was to evaluate the current status of the therapeutic monitoring of gentamicin in the United States and Canada. To investigate pharmacologic scaling, a standard dose of flunixin meglumine was administered intravenously to eight Miniature Horses and eight Quarter Horses, and three-compartmental analysis was used to compare pharmacokinetic parameters between breed groups. The total body clearance of flunixin was 0.97±0.30 $mL \cdot min^{-1} \cdot kg^{-1}$ in Miniature Horses and 1.04±0.27 $mL \cdot min^{-1} \cdot kg^{-1}$ in Quarter Horses. Similarly, a standard dose of gentamicin was administered intravenously to eight Miniature Horses and eight Quarter Horses, and three-compartmental analysis was used to compare pharmacokinetic parameters between breeds. The total body clearance of gentamicin components C1a, C2, C1, and summed components was 0.68±0.15 $mL \cdot min^{-1} \cdot kg^{-1}$, 0.69±0.16 $mL \cdot min^{-1} \cdot kg^{-1}$, 0.72±0.17 $mL \cdot min^{-1} \cdot kg^{-1}$, and 0.71±0.16 $mL \cdot min^{-1} \cdot kg^{-1}$ respectively in Miniature Horses and 0.59±0.10 $mL \cdot min^{-1} \cdot kg^{-1}$, 0.61±0.09 $mL \cdot min^{-1} \cdot kg^{-1}$, 0.62±0.10 $mL \cdot min^{-1} \cdot kg^{-1}$, and 0.62±0.09 $mL \cdot min^{-1} \cdot kg^{-1}$ respectively in Quarter Horses. There were no significant differences between Miniature Horses and Quarter Horses in clinically significant pharmacokinetic parameters ($P>0.05$) for either flunixin meglumine or gentamicin. An email-based survey disseminated to each of the veterinary teaching hospitals in the United States and Canada revealed that 42% of respondents currently perform therapeutic drug monitoring of aminoglycosides, with an average of 3.9 samples annually. The majority of veterinary TDM is performed in equine medicine. The fact that TDM is performed infrequently in veterinary teaching hospitals is at odds with the importance of TDM for aminoglycosides as demonstrated in human medicine. In conclusion, both flunixin meglumine and gentamicin may be administered to Miniature Horses at the same dose rates used typically in light-breed horses, with similar recommendations for TDM of gentamicin.

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INTRODUCTION

When considerable variation in body weight exists among animals of the same or different species, physiological parameters such as metabolic rate, hepatic blood flow, glomerular filtration rate, and body surface area can all be described as a nonproportional, or allometric, function of body weight (Prothero, 1982; Prothero, 1984). Pharmacokinetic parameters rely on these physiological values, and therefore drug dosages also scale allometrically with body weight (Mordenti, 1986; Ritschel et al., 1992). The majority of descriptions of this phenomenon are interspecific, or between species, as the allometric relationships are used to make pharmacological dosage predictions across species and in humans from animal models (Mahmood et al., 2006; Huh et al., 2011). In general, smaller individuals have a higher surface area to volume ratio and a higher mass-specific metabolic rate. Therefore, faster drug clearance and shorter elimination half-life occur in smaller-sized individuals, necessitating higher drug doses on a body weight-normalized, or mg/kg basis, as compared to larger-sized individuals (Mordenti, 1986; Maxwell & Jacobson, 2008). When large variation exists in body weight among adults intraspecifically, or within a single species, allometric scaling within the species becomes important for the development of drug dosages (Maxwell & Jacobson, 2008; Martinez et al., 2009).

Within the equine species, significant variation in body weight exists. Between the smallest 100 kg Miniature Horse and the largest 1000 kg Percheron, there is a difference of about one order of magnitude. There have been no studies investigating a possible influence of allometry on pharmacokinetics within the equine species. Since pharmacokinetic studies are routinely performed in light-breed horses, current dosage recommendations for Miniature Horses may not be accurate if there is an allometric effect within the species. Miniature Horses exhibit unique medical predilections such as an increased propensity for fecalith obstructions (Haupt et al., 2008), tracheal collapse (Aleman et al., 2008), lateral patellar luxation, (Engelbert et al., 1993) and hypertriglyceridemia (Waitt & Cebra, 2009). Despite the increasing popularity of the Miniature Horse breed over the last thirty years that led to the aforementioned publications, to the author's knowledge there have been no studies that evaluate appropriate drug dosing in Miniature Horses.

There is a perception by both horse owners and veterinarians, based on anecdotal reports, that Miniature Horses are more likely to experience toxicity from nonsteroidal anti-inflammatory drugs (NSAIDs) than are light-breed horses (Mogg, 2012). Flunixin meglumine is one of the most common NSAIDs prescribed in equine practice, and potential toxicities associated with it include right dorsal colitis, oral and gastric ulceration, and nephrotoxicity (Black, 1986; McConnico et al., 2008; Videla & Andrews, 2009). It is therefore crucial to determine if dose recommendations for this drug in Miniature Horses are both efficacious and pose a minimal risk of toxicity.

Antibiotics are considered to have fairly straightforward scaling properties, because their efficacy is well correlated to their plasma concentration (Riviere et al., 1997). Specifically, gentamicin is an antibiotic that is eliminated unchanged through glomerular filtration, which depends directly on glomerular filtration rate and is likely to scale allometrically (Riviere et al., 1997). Gentamicin is a commonly used antibiotic in equine practice, and is potentially nephrotoxic. It is therefore both an important drug for which to improve dosage recommendations in Miniature Horses, and a

likely drug to enable the demonstration of allometry within the equine species. To improve the therapeutic efficacy and minimize the toxicity of aminoglycosides such as gentamicin, therapeutic drug monitoring (TDM) has become standard clinical practice in human medicine (Roberts et al., 2012). In general, commercially available immunoassays have been the most appropriate for routine TDM of aminoglycosides in clinical laboratories because they are relatively accurate, precise, rapid, and simple to use (Stead, 2000; Dasgupta, 2012). There is very little information available about the current use and availability of TDM for aminoglycoside therapy in veterinary medicine.

The purpose of the present study was primarily to develop a basis for pharmacokinetic scaling in the horse, and to determine the scaling exponents with which flunixin meglumine and gentamicin relate to body weight in the horse. A secondary purpose was to evaluate the current status of the therapeutic monitoring of gentamicin in the United States and Canada. If the disposition of flunixin meglumine and gentamicin were indeed nonproportional, or allometric, among horses of greatly different body weights, the administration of these drugs to Miniature Horses could be made safer and more effective by the determination of the appropriate dosage regimen for the breed.

CHAPTER II

EFFECT OF BODY WEIGHT ON THE PHARMACOKINETICS OF FLUNIXIN MEGLUMINE IN MINIATURE HORSES AND QUARTER HORSES

Introduction

The Miniature Horse breed has increased in popularity over the last thirty years. There have been multiple recent publications demonstrating the unique medical predilections of the breed, including an increased propensity for fecalith obstructions (Haupt et al., 2008), tracheal collapse (Aleman et al., 2008), lateral patellar luxation (Engelbert et al., 1993), and hypertriglyceridemia (Waitt & Cebra, 2009). Despite these developments, to the authors' knowledge there have been no studies evaluating appropriate drug dosing in Miniature Horses.

The large difference in body size between Miniature Horses and standard-sized horses suggests the need to take body size into account when dosing Miniature Horses. When there is considerable variation in body size among animals of the same (Maxwell & Jacobson, 2008; Martinez et al., 2009) or different species (Mahmood et al., 2006; Huh et al., 2011), pharmacokinetic parameters such as clearance and elimination half-life relate to body weight in a nonproportional manner (Mordenti, 1986; Ritschel et al., 1992). In general, smaller individuals have a higher surface area to volume ratio and a higher mass-specific metabolic rate. As a consequence, faster drug clearance and shorter elimination half-lives occur in smaller-sized individuals, necessitating higher drug doses on a body weight normalized, or mg/kg basis, as

compared to larger-sized individuals (Mordenti, 1986; Maxwell & Jacobson, 2008). This phenomenon is perhaps best recognized in the dosing of cytotoxic anticancer drugs, which is based on the nonproportional relationship between body surface area and body weight (Frazier & Price, 1998; Sparreboom, 2005; Loos et al., 2006). However, nonproportional drug disposition is also observed in numerous drug classes aside from anticancer drugs. Within the *Equus* genus, the clearance of phenylbutazone is much faster in miniature donkeys than is reported in standard donkeys (Matthews et al., 2001). Additionally, the clearance of phenolsulfonphthalein is faster in ponies than in light-breed horses (Hinchcliff et al., 1987). To the author's knowledge, there have been no studies comparing the disposition of any drug among the weight categories of the horse. If drug disposition is nonproportional among horses of greatly different body weights, then therapeutic regimens in horses could be made safer and more effective by better defining the relationship between body size and drug disposition.

Flunixin meglumine is a nonsteroidal anti-inflammatory drug used commonly in equine practice. In a recent survey of the American Association of Equine Practitioner member veterinarians, 91% of respondents prescribe it at least weekly (Hubbell et al., 2010). In addition to its use as a pain reliever, it is also used to inhibit the systemic effects of endotoxemia (Bryant et al., 2003). Potential toxicities associated with nonsteroidal anti-inflammatory drugs include right dorsal colitis, oral and gastric ulceration, and nephrotoxicity (Black, 1986; McConnico et al., 2008; Videla & Andrews, 2009). Because of these factors, it is important to ensure that dose recommendations for this drug are both efficacious and pose a minimal risk of toxicity.

The purpose of the present study was to determine whether Miniature Horses should receive a different dose rate of flunixin meglumine than that used typically in light-breed horses, and in so doing, form a basis for future studies addressing dose recommendations in the Miniature Horse. The study hypothesis was that the total body clearance of flunixin would be faster in Miniature Horses as compared to Quarter Horses.

Materials and methods

Horses

Sixteen clinically healthy horses, consisting of Quarter Horse type horses (n = 8) and Miniature Horses (n = 8), were used in this study. Horses in each breed group were similar with respect to gender, age, and body condition score, but body weights were approximately five fold different between the two groups (Table 1). Horses were determined to be healthy by physical examination by a veterinarian, and a veterinarian assessed body condition score of each horse, using a nine point system (Henneke et al., 1983). Horses were housed in their usual environment or in individual stalls with free access to water and hay during the study. The study protocol was approved by the Oklahoma State University Animal Care and Use Committee and written informed consent was obtained for the four Quarter Horses and eight Miniature Horses that were privately owned. The remaining four Quarter Horses were maintained as part of a University owned teaching herd.

Table 1. Summary of demographic data for horses employed in the present study.

	Quarter Horses	Miniature Horses	P value
Age (yr)	7 (3-12)	6 (3-12)	0.63
Weight (kg)	489 (420-559)	100 (82-126)	<0.001
Body Condition Score	5.7 (5.3-6.3)	5.5 (4.7-6.7)	0.50
Gender			1.0
Gelding	4	4	
Mare	4	4	

Data are expressed as the mean, followed by the range.

Drug administration

A commercial formulation of flunixin meglumine (Flunixiject; Butler Schein Animal Health, Dublin, Ohio, USA).was administered as an intravenous bolus via an indwelling 14 gauge catheter placed in the right jugular vein using an aseptic technique. A dose of approximately 1.1

$mg \cdot kg^{-1}$ was calculated for each horse such that an accurate volume could be measured using standard syringes.

Blood collection

Baseline plasma samples were collected from all horses prior to drug administration. Following intravenous administration of flunixin meglumine, 6 mL blood samples were collected via a separate 14 gauge catheter previously placed in the opposite jugular vein at 3, 6, 10, 20, and 40 minutes, and at 1, 2, 3, 4, 6, 8, 10, 12 hours. Further sampling was performed by jugular venipuncture at 24 hours after the administration of flunixin. All samples were collected into heparinized blood collection tubes and placed immediately into an ice-water bath. Samples were then centrifuged within one hour of collection and plasma was separated and stored at -80 °C until assayed.

Flunixin assay

A novel assay utilizing high performance liquid chromatography (HPLC) with ultraviolet detection was developed for the sensitive and specific determination of plasma flunixin concentrations in equine plasma. The HPLC system consisted of a ProStar™ 210 pump, 410 autosampler and 285 nm ultraviolet detector (Varian Medical Systems, Palo Alto, California, USA). A reversed-phase column and guard column (Symmetry™ C18, 5 μm, 4.6 x 150 mm; Waters Corporation, Milford, Massachusetts, USA) were utilized at 30 °C for analyte separation. Mobile phase components were prepared fresh daily and consisted of 1.5% acetic acid and 10% acetonitrile in water (mobile phase A) and 1.5% acetic acid and 90% acetonitrile in water (mobile phase B). Stock solutions were prepared by adding flunixin (Sigma-Aldrich, St. Louis, Missouri, USA) or the internal standard, niflumic acid (Sigma-Aldrich, St. Louis, Missouri, USA), to methanol. Plasma calibrants were prepared at concentrations of 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10, 15, and 30 $\mu g \cdot mL^{-1}$ using flunixin stock solutions and heparinized plasma from

unmedicated horses. Calibrants and quality control samples were prepared by adding 950 μL of unmedicated equine heparinized plasma to 50 μL of the appropriate calibrant or quality control solution, followed by vortex mixing. Calibrant curves were constructed from the ratio of flunixin:niflumic acid and were weighted using the reciprocal of the flunixin concentration. Criteria for acceptance of each run included that a minimum of five calibrators back-calculated to within 15% of the nominal concentration and that the coefficient of determination was >0.99. All calibrators that met the acceptance criteria were included in the calibration curve associated with each run, and calibration samples always bracketed the experimentally determined flunixin concentrations. One milliliter of 2% phosphoric acid containing niflumic acid at a concentration of 4 $\mu g \cdot mL^{-1}$ was added to each 1 mL plasma sample and vortex mixed. Liquid-liquid extraction was performed via the addition of 10 mL of diethyl ether. End over end mixing for 15 minutes was followed by centrifuging for 10 minutes. The supernatant was aspirated and dried under nitrogen for 20 minutes at 30°C. The residue was dissolved in 200 μL of mobile phase and 50 μL were injected onto the column. The mobile phase consisted of 60% "A" and 40% "B" with isocratic flow at 1 $mL \cdot min^{-1}$. Flunixin eluted at approximately 10 minutes, and niflumic acid at approximately 20.5 minutes. Recovery estimates were performed in plasma using six replicates. Intraday accuracy and coefficient of variation estimates were performed in plasma using three replicates. Interday accuracy and coefficient of variation estimates were performed in plasma using three replicates on three separate days. The limit of quantification was estimated using six replicates and was defined as the lowest concentration associated with a tenfold signal:noise ratio. The limit of detection was estimated using three replicates and was defined as the concentration at which signal:noise was at least three fold.

Pharmacokinetic analysis

Plasma flunixin concentrations following intravenous administration of flunixin meglumine were analyzed compartmentally using Thermo Kinetica software version 5.0 (Thermo Fisher Scientific,

Philadelphia, Pennsylvania, USA). Intravenous data for each horse were fit to the following equation:

$$C = \sum_{i=1}^{n} A_i \cdot e^{-\alpha \cdot t}$$

Data were weighted by the reciprocal of the plasma flunixin concentration and were fit to standard compartmental models. The most appropriate model was selected using Aikaike's information criterion and the Schwarz criterion, and standard compartmental equations were then used to estimate the pharmacokinetic parameters for each horse. The mean and standard deviation for each group (Miniature Horses and Quarter Horses) was estimated for each pharmacokinetic parameter.

Allometric comparisons

The total body clearance for flunixin determined in Miniature Horses was compared to that predicted by standard allometry, as has been reported previously (Mordenti, 1986). The general form of the allometric equation used for scaling of pharmacokinetic parameters was:

$y = a \cdot BW^b$,

where y is flunixin clearance; BW is the body weight; a is the allometric coefficient, and b is the allometric exponent. The allometrically predicted flunixin clearance in Miniature Horses was calculated from the Quarter Horse clearance data by solving for the mass coefficient in the equation above and setting $b = 0.75$, as is frequently used in standard allometric calculations and is arguably a universal scaling exponent across species (Hu & Hayton, 2001). The values of $\log_{10}$ total body clearance were regressed against $\log_{10}$ body weight and compared to the curve predicted by standard allometry.

Statistical analysis

A Wilcoxon rank-sum test was used to analyze the difference in body condition score between the groups, whereas a two-sample t-test was used to test whether age differed between Quarter Horses and Miniature Horses. Two sample Student's t tests were used to test the difference in selected pharmacokinetic parameters: mass normalized clearance (Cl), elimination phase rate constant (γ), area under the plasma concentration versus time curve (AUC), apparent volume of distribution at steady state (Vd_{ss}), and volume of the central compartment (V_c).. Significance was set at $\alpha = 0.05$. Statistical calculations were performed using SigmaPlot software version 11.0 (Systat Software, Inc., Chicago, Illinois, USA).

Results

Assay

At fortified plasma flunixin concentrations of 0.075 $\mu g \cdot mL^{-1}$, 5 $\mu g \cdot mL^{-1}$, and 12.5 $\mu g \cdot mL^{-1}$, recovery of flunixin was 86 ± 5%, 86 ± 2%, and 85 ± 3%, respectively. At a plasma concentration of 4.0 $\mu g \cdot mL^{-1}$, recovery of niflumic acid was similar to that of flunixin at 83 ± 3%. Intraday accuracy of the assay at 0.075 $\mu g \cdot mL^{-1}$, 3.75 $\mu g \cdot mL^{-1}$ and 12.5 $\mu g \cdot mL^{-1}$ was 99%, 93%, and 92%, respectively. Intraday coefficient of variation at 0.075 $\mu g \cdot mL^{-1}$, 3.75 $\mu g \cdot mL^{-1}$, and 12.5 $\mu g \cdot mL^{-1}$ was 1%, 2%, and 2%, respectively. Interday accuracy at 0.075 $\mu g \cdot mL^{-1}$, 3.75 $\mu g \cdot mL^{-1}$ and 12.5 $\mu g \cdot mL^{-1}$ was 99%, 96%, and 91%, respectively. Interday coefficient of variation at 0.075 $\mu g \cdot mL^{-1}$, 3.75 $\mu g \cdot mL^{-1}$, and 12.5 $\mu g \cdot mL^{-1}$ was 3%, 2%, and 1%, respectively. The limit of quantification, 0.025 $\mu g \cdot mL^{-1}$, was associated with good accuracy and precision, with an accuracy of 93% and a coefficient of variation of 11%. The limit of detection was 0.00625 $\mu g \cdot mL^{-1}$. The

assay provided good separation of flunixin and the internal standard from endogenous plasma constituents, even at low plasma flunixin concentrations (Figure 1).

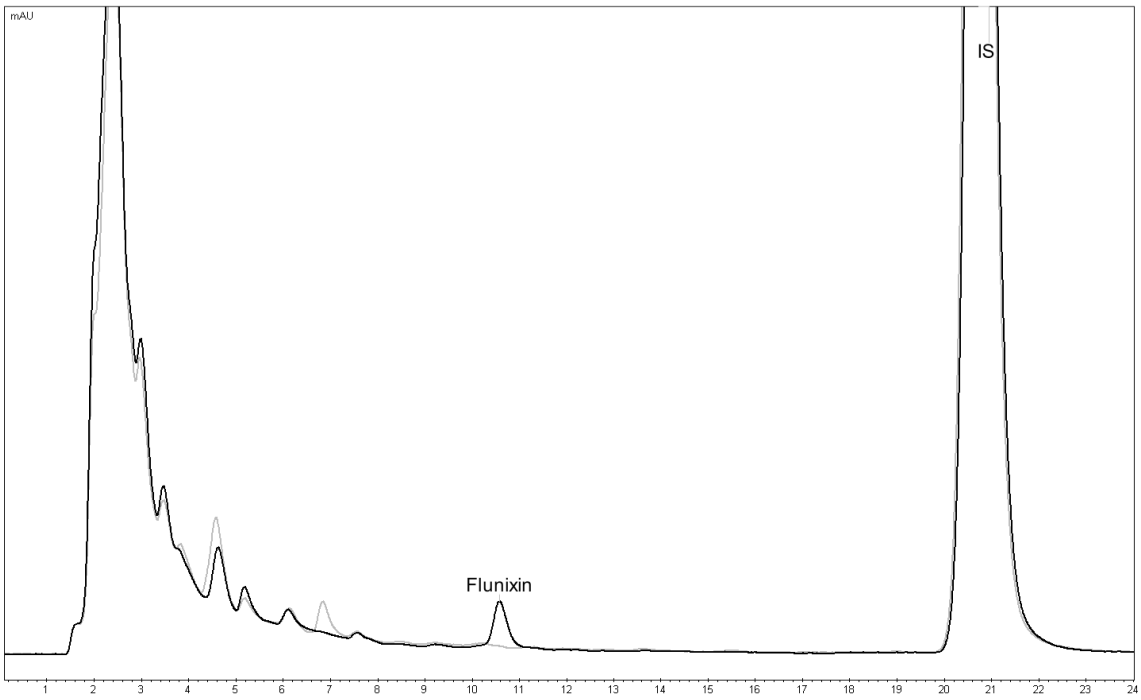


Figure 1: Chromatograms of unmedicated equine plasma (grey line) and equine plasma with an estimated flunixin concentration of 0.15 μg/mL (black line), sampled at 12 hours after administration of flunixin meglumine. Peaks: IS = internal standard.

Quantification of flunixin

All horses tolerated the administration of a single dose of flunixin meglumine well, with no adverse effects noted for the duration of the study. In addition to sampling times described above, additional sampling was performed at 36 hours in the first three Quarter Horses and the first three Miniature Horses studied, but flunixin could not be detected in any of these six samples. Thereafter, collection of the 36 hour post-administration sample was discontinued. Flunixin concentrations rapidly declined after intravenous administration, followed by a slower distribution phase, and then an extended elimination phase; flunixin could be quantified for at

least 12 hours in all horses and for 24 hours in 5/8 Quarter Horses and 4/8 Miniature Horses (Figure 2).

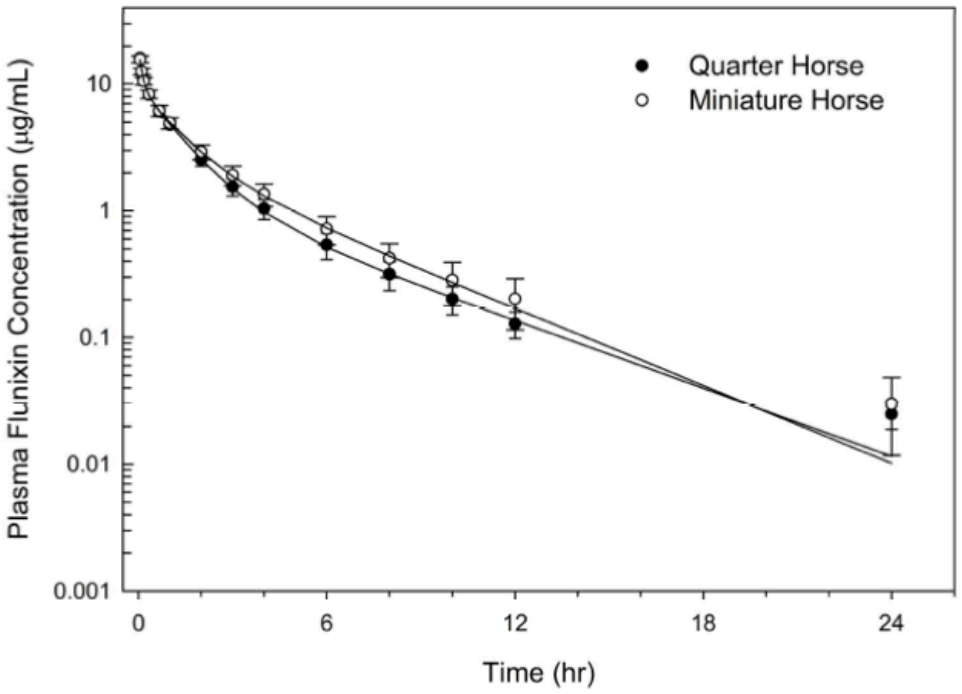


Figure 2: Mean (± s.e.m.) plasma concentrations of flunixin after i.v. administration of flunixin meglumine at a dose rate of 1.1 mg/kg to eight Miniature Horses and eight Quarter Horses. Flunixin concentrations were similar between the two groups throughout the sampling period.

Pharmacokinetics

The most appropriate compartmental model for pharmacokinetic analysis was determined to be a three compartment model in all horses (Table 2). There were no significant differences between groups in clearance ($P = 0.66$), elimination phase rate constant ($P = 0.44$), AUC ($P = 0.51$), Vd_{ss} ($P = 0.89$), or the V_c ($P = 0.49$). In addition, the mass specific clearance of flunixin was not related to body weight (Figure 3a).

Table 2. Comparison of pharmacokinetic parameters for flunixin after i.v. administration to Quarter Horses and Miniature Horses.

Parameter	Quarter Horses	Miniature Horses
Dose (mg·kg bwt^{-1})	1.10 ± 0.02 (1.07-1.14)	1.11 ± 0.02 (1.08-1.13)
C_0 (μg·mL^{-1})	19.8 ± 2.7 (17.3-25.2)	19.1 ± 3.4 (14.7-23.1)
A (μg·mL^{-1})	9.9 ± 2.1 (7.4-13.5)	9.7 ± 1.9 (7.5-12.2)
B (μg·mL^{-1})	8.3 ± 0.8 (7.2-9.5)	6.6 ± 1.1 (4.7-7.7)
C (μg·mL^{-1})	1.6 ± 1.1 (0.5-3.5)	2.8 ± 2.3 (0.4-8.2)
$t_{1/2}\alpha$ (h)	0.07 ± 0.01 (0.05-0.08)	0.08 ± 0.02 (0.05-0.10)
$t_{1/2}\beta$ (h)	0.81 ± 0.18 (0.57-1.11)	0.79 ± 0.21 (0.53-1.13)
$t_{1/2}\gamma$ (h)	3.38 ± 1.14 (2.06-6.03)	2.96 ± 1.00 (2.14-5.98)
$t_{1/2}k_{10}$ (h)	0.62 ± 0.16 (0.47-1.17)	0.70 ± 0.22 (0.47-1.45)
k_{10} (hr^{-1})	1.1 ± 0.3 (0.6-1.5)	1.0 ± 0.3 (0.5-1.5)
k_{12} (hr^{-1})	3.9 ± 0.7 (3.3-5.5)	3.5 ± 1.1 (2.7-6.2)
k_{21} (hr^{-1})	5.3 ± 0.6 (4.6-6.6)	4.7 ± 1.2 (3.6-6.9)
k_{13} (hr^{-1})	0.3 ± 0.1 (0.2-0.5)	0.3 ± 0.1 (0.2-0.4)
k_{31} (hr^{-1})	0.3 ± 0.1 (0.1-0.6)	0.4 ± 0.2 (0.1-0.9)
V_c (L·kg bwt^{-1})	0.056 ± 0.007 (0.042-0.063)	0.059 ± 0.011 (0.048-0.074)
$V_{d(ss)}$ (L·kg bwt^{-1})	0.157 ± 0.022 (0.119-0.183)	0.159 ± 0.039 (0.095-0.216)
$V_{d(area)}$ (L·kg bwt^{-1})	0.324 ± 0.104 (0.182-0.507)	0.279 ± 0.149 (0.111-0.621)
Cl (mL·min^{-1}·kg bwt^{-1})	1.04 ± 0.27 (0.60-1.48)	0.97 ± 0.30 (0.47-1.20)
AUC (μg·h·mL^{-1})	18.8 ± 5.5 (12.4-30.8)	21.3 ± 9.2 (14.0-39.6)
MRT (hr)	2.7 ± 0.7 (1.8-3.7)	3.0 ± 1.3 (1.7-5.9)

Values are expressed as mean or *harmonic mean ± s.d. (range)[37]. Dose = dose administered; C_0 = serum drug concentration at time 0; A = coefficient of rapid distribution phase; B = coefficient of slow distribution phase; C = coefficient of elimination phase; $t_{1/2}\alpha$ = rapid distributional half-life; $t_{1/2}\beta$= slow distributional half-life; $t_{1/2}\gamma$ = terminal elimination phase half-life; $t_{1/2}k_{10}$ = elimination half-life; k_{10} = first-order rate constant for elimination from the central compartment; other intercompartmental rate constants follow similar nomenclature; V_c = apparent volume of the central compartment; $V_{d(ss)}$ = apparent volume of distribution at steady state; $V_{d(area)}$ = apparent

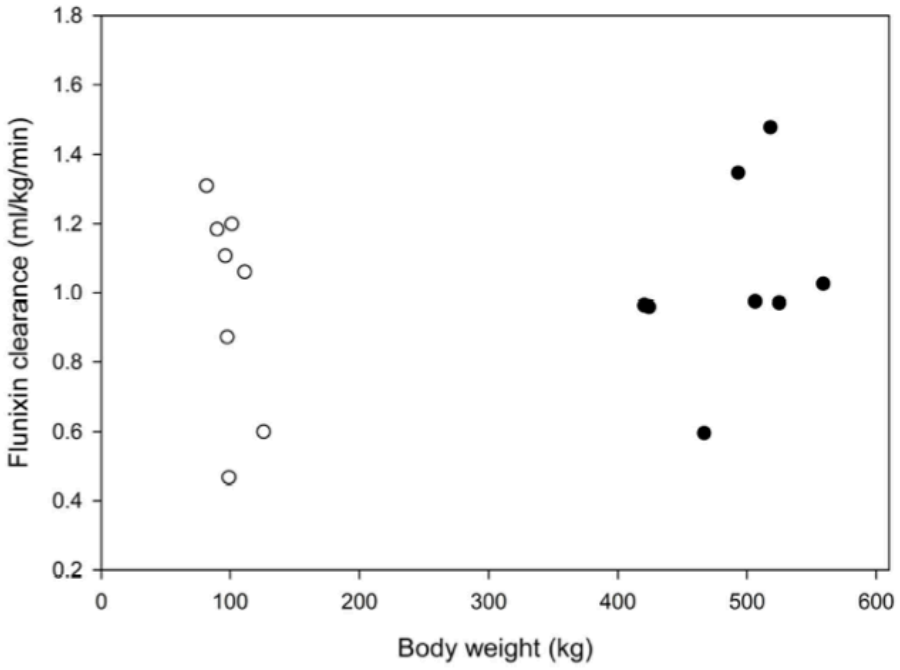
volume of distribution by area; Cl = total body clearance; AUC = Area under the plasma concentration versus time curve, extrapolated to infinity; MRT = mean residence time

Allometric comparison

The regression curve relating measured total body clearance to body weight was associated with a mass exponent of approximately unity:

$$CL = 0.046 \cdot BW^{1.04},\ R^2 = 0.88$$

The 95% confidence interval (0.82, 1.26) for the calculated mass exponent did not contain the ¾ exponent predicted by the principles of standard allometry (Figure 3b). If the clearance of flunixin in Miniature Horses had varied allometrically from that of Quarter Horses by the ¾ mass exponent, then the predicted flunixin clearance in the Miniature Horses would have been 1.56 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$, more than 50% greater than the measured clearance of 0.97 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$. The statistical power (β) of the present study to detect such a difference was 0.95, with s.d. = 0.27 and $\alpha = 0.05$. The other pharmacokinetic parameters tested, including the elimination rate, AUC, Vd_{ss} and V_c, were similarly unrelated to body weight (elimination rate and AUC) or were directly proportional to body weight (Vd_{ss}, V_c; data not shown).


Flunixin clearance (ml/kg/min)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
100
200
300
400
500
600
Body weight (kg)

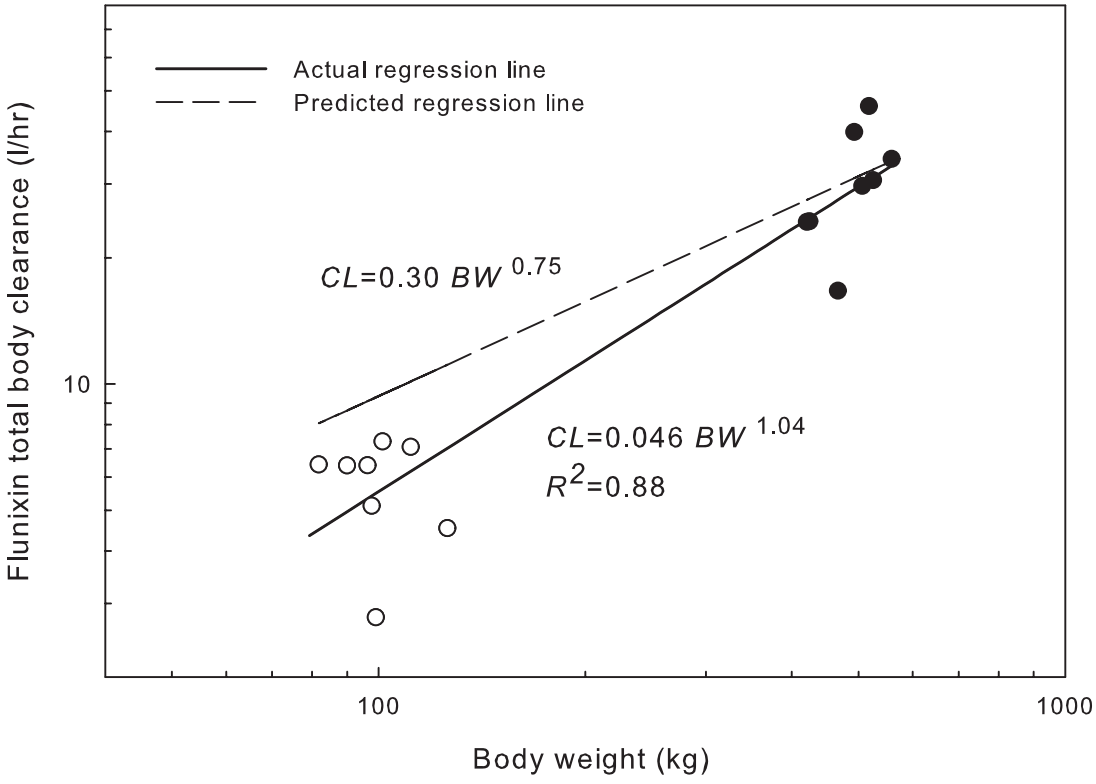

Actual regression line
Predicted regression line
CL=0.30 BW 0.75
CL=0.046 BW 1.04
R2=0.88
Flunixin total body clearance (l/hr)
10
100
1000
Body weight (kg)

Figure 3: Relationship between flunixin clearance and body weight.. a) Mass specific clearance of flunixin versus body weight in Miniature Horses and Quarter Horses, demonstrating that total body clearance was similar between the two groups when normalized to body weight. b) Total body clearance of flunixin versus body weight in Miniature Horses and Quarter Horses, showing that total body clearance varied proportionally with body weight. The regression line calculated from the data (solid line) is compared to that predicted from standard allometry (dashed line).

Discussion

The novel flunixin assay employed in the present study allowed the sensitive and specific determination of flunixin concentrations in equine plasma. The sensitivity of this method was improved as compared with previously reported HPLC methods, with the limit of detection of 0.00625 $\mu g \cdot mL^{-1}$ lower than previous reports of 0.05 $\mu g \cdot mL^{-1}$ (Semrad et al., 1985; Higgins et al., 1987) and the limit of quantification of 0.025 $\mu g \cdot mL^{-1}$ lower than previous reports of 0.05 $\mu g \cdot mL^{-1}$ (Soma et al., 1988). Although the two compartment model describing flunixin pharmacokinetics in horses predominates in the literature (Chay et al., 1982; Semrad et al., 1985; Lees et al., 1987; Soma et al., 1988), the sensitivity of the present study allowed for the first time the reliable quantification of flunixin for up to 24 hours after administration. The longer detection time demonstrated the presence of a third compartment, which was confirmed by the Akaike information criterion and the Schwarz criterion in all horses. The stated accuracy, precision, and recovery of the novel assay were also well within acceptable limits. As this was the first description of this specific method for analysis of flunixin in equine plasma, the method was deemed robust and feasible for future pharmacokinetic studies.

The pharmacokinetic parameters calculated in the present study for light-breed horses were similar when compared to those in previous studies (Lees et al., 1987; Coakley et al., 1999). Specifically, the calculated mass specific clearance of flunixin in light-breed horses of 1.04 ± 0.27 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$ was very similar to the clearance of 1.1 ± 0.2 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$ (Coakley et al., 1999) reported previously in light-breed horses given the same dose of flunixin. Low

variability in the kinetics of flunixin was observed among horses in the present study, demonstrating that flunixin pharmacokinetics are generally predictable, even within these two disparate breeds of horses.

An acknowledged shortcoming of this study was the lack of any samples taken between the 12 hour and 24 hour time points. The study was designed in this fashion to encourage the participation of privately owned horses. The inclusion of 16 hour and 20 hour post-administration samples would have improved the definition of the third compartment. To investigate the possibility that sampling times affected the calculation of the key parameters under investigation, the pharmacokinetic analysis was repeated on the plasma flunixin versus time data truncated at the 12 hour time point, when all horses had quantifiable plasma flunixin concentrations. The elimination rate constant was not substantially affected by this truncated analysis, and the significance of the comparisons did not change. For example, the terminal phase elimination rates in Quarter Horses and Miniature Horses with inclusion of quantifiable 24 hour time points were 0.20 hr^{-1} and 0.23 hr^{-1}, respectively. When the data were truncated at 12 hours, the terminal phase elimination rates in Quarter Horses and Miniature Horses were 0.25 hr^{-1} and 0.24 hr^{-1}, respectively. Therefore, the selected sampling time points did not appear to play an important role in the outcome of the study.

Physiological parameters such as metabolic rate, hepatic blood flow, glomerular filtration rate, and body surface area can all be described as a nonproportional, or allometric, function of body weight when there is a large amount of size variation (Prothero, 1982; Prothero, 1984). Because of this allometric relationship, pharmacokinetic parameters and therefore drug dosages also scale allometrically with body weight (Mordenti, 1986; Ritschel et al., 1992). This is described most extensively between species, or interspecifically (Mahmood et al., 2006; Huh et al., 2011). However, intraspecific allometric scaling, or allometry within a single species, becomes

important when there is considerable variation in body weight among adults within the species (Maxwell & Jacobson, 2008; Martinez et al., 2009).

The large variation in body size within the equine species led the authors to hypothesize that flunixin would follow an allometric relationship in the horse, as has been reported for phenylbutazone clearance in mininature donkeys as compared to full-sized donkeys (Matthews et al., 2001). However, the results of the present study in Miniature Horses did not demonstrate a breed related effect on the disposition of flunixin. Statistical comparisons between breed groups were performed on those pharmacokinetic parameters used commonly in allometric calculations and deemed clinically relevant to dose calculations (Cox et al., 2004; Dinev, 2008; Gebru et al., 2011). These key pharmacokinetic parameters for flunixin showed no significant differences between Miniature Horses and Quarter Horses. Furthermore, the present data did not support a non-proportional, or allometric, relationship between flunixin disposition and body weight in the horse.

For species in which standard allometry is followed, drug clearance is proportional to body weight raised to the ¾ power on a double $\log_{10}$ plot (Figure 3b). The range in equine body weights utilized in the present study was sufficient to detect an allometric effect on flunixin clearance, if the horses had indeed followed standard allometry as we initially hypothesized. It was also possible that flunixin clearance followed a scaling factor other than the ¾ power, such as the 2/3 power commonly utilized in body surface area calculations (Gouma et al., 2012). Therefore, flunixin clearance was also plotted against body weight on a double $\log_{10}$ plot to examine the possibility of a nonproportional relationship. However, the mass exponent of that comparison was very similar to unity, further demonstrating the absence of any allometric effect. Other pharmacokinetic parameters (elimination rate, AUC, and volumes of distribution) that were subject to allometric effects in previous studies failed to vary non-proportionally with body

weight in the present study in horses. Therefore, the present data do not support adjustment of the dose rate of flunixin meglumine in Miniature Horses for pharmacokinetic reasons.

Although the efficacy and toxicity of flunixin have not been compared between breeds, the pharmacokinetic/pharmacodynamic relationship of flunixin has been well described in the horse (Toutain et al., 1994; Landoni & Lees, 1995; Lees et al., 2004). An intravenous dose of 1 mg/kg was predicted to have near maximal effects for two to ten hours after administration on the pharmacodynamic endpoints of local skin temperature, stride length, rest angle flexion, maximal carpal flexion and circumference of the inflamed joint after induction of carpal osteoarthritis (Toutain et al., 1994). The majority of these effects were explained by drug concentration (Lees et al., 2004). Because the efficacy and toxicity of flunixin are dependent on its pharmacokinetic interactions, dosage recommendations for this drug in Miniature Horses can justifiably rely on the consistency of pharmacokinetic parameters without specifically comparing the pharmacodynamics of the drug between breeds.

While the present results do not support a need to adjust the dose rate for flunixin administration to Miniature Horses, drugs that are subject to different routes of elimination might be subject to size effects on drug disposition. Flunixin is a highly protein bound drug that is metabolized in the liver but is not avidly extracted, characteristics that may be associated with poor correlation between body weight and drug clearance when compared across species (Riviere et al., 1997). Indeed, a compilation of interspecific allometric data from forty-four different drugs across multiple veterinary species reported that the elimination half-life of flunixin did not correlate with body weight, with a coefficient of determination of 0.40 (Riviere et al., 1997). In contrast, the same study reported that elimination half-life was significantly correlated with body weight in drugs cleared via glomerular filtration such as carbenicillin, tetracycline, cephapirin, apramycin, chlortetracycline, gentamicin, and ampicillin (Riviere et al., 1997). Future studies with these

drugs may be more likely to show allometric scaling within the equine species, and it is possible that they would require dose adjustments for use in Miniature Horses.

There is a current perception by both horse owners and veterinarians that Miniature Horses are more likely to experience toxicity from nonsteroidal anti-inflammatory drugs (NSAIDs) than are light-breed horses (Mogg, 2012). Such evidence is primarily anecdotal, and likely represents overdosage of NSAIDs in Miniature Horses when their smaller body weight is not taken into account, a situation observed previously in a case referred to this institution (Lyndi Gilliam, personal communication). The absence of objective studies investigating the pharmacokinetics of any veterinary drug in Miniature Horses has required the veterinary practitioner to use only subjective information to support therapeutic decisions in this unique breed. Although it remains imperative to adjust drug doses to body weight when administering therapeutics to Miniature Horses, the results of the present study allow practitioners to more confidently administer flunixin meglumine to Miniature Horses using typical equine dosing regimens.

CHAPTER III

EFFECT OF BODY WEIGHT ON THE PHARMACOKINETICS OF GENTAMICIN IN MINIATURE HORSES AND QUARTER HORSES

Introduction:

Aminoglycosides are commonly administered in veterinary medicine due to their activity against many gram-negative bacteria. Gentamicin use is especially common in equine practice. In a survey of Diplomates of the American College of Veterinary Surgeons performing equine surgery at veterinary teaching hospitals in the United States, gentamicin was used routinely by 84% of respondents along with potassium penicillin as a preoperative drug for colic surgery (Traub-Dargatz et al., 2002). Although it is frequently used, the potential nephrotoxicity of gentamicin is a concern (van der Harst et al., 2005). The current recommendation for gentamicin administration is once daily dosing, allowing higher therapeutic efficacy with no increase in nephrotoxicity due to the saturable nature of uptake by the renal tubular cells (Magdesian et al., 1998; Turnidge, 2003). More recent studies have looked at using therapeutic drug monitoring to refine the dosing regimens for gentamicin, and it remains a current topic of research within the field of equine medicine (Read et al., 2011).

Therapeutic drug monitoring (TDM) can be used to combine maximal antimicrobial efficacy with minimal toxicity, and this has become standard clinical practice in human medicine (Roberts

et al., 2012). A mathematical model taking both efficacy and toxicity into account showed that optimal aminoglycoside dosing requires a sophisticated system of TDM (Croes et al., 2012). With a once daily dosing regimen, it is recommended that trough concentration should be monitored to ensure levels below 2 μg/mL (Dasgupta, 2012). In general, commercially available immunoassays have been the most appropriate for routine TDM of aminoglycosides in clinical laboratories (Stead, 2000; Dasgupta, 2012). However, most immunoassay methods measure total gentamicin concentration in serum or plasma but do not measure the individual components within the gentamicin complex (Dasgupta, 2012). There is minimal information available about the current use of TDM for aminoglycosides in veterinary medicine, but a recent change in the availability of commonly used clinical equipment may have affected the routine monitoring of aminoglycosides in veterinary patients.

When considering appropriate dose regimens for gentamicin within the equine species, the impact of allometry may be important to take into account. The concept of allometry is based on nonproportional changes in physiological parameters such as glomerular filtration rate, oxygen consumption, heart and respiratory rate, cardiac output, and basal metabolic rate with body weight when considered over a wide range in species (Prothero, 1982; Prothero, 1984). Because pharmacokinetic parameters and corresponding drug dosages are dependent upon physiologic functions, they may similarly vary in a nonproportional, or allometric, manner (Mordenti, 1986; Ritschel et al., 1992). Aminoglycosides in particular have been reported to scale allometrically between species (Riviere et al., 1997). The elimination of aminoglycosides such as gentamicin through glomerular filtration depends directly on glomerular filtration rate, which clearly scales allometrically across species (Kirkwood & Merriam, 1990; Riviere et al., 1997).

When there is considerable variation in body weight among adults within a species, intraspecific allometric scaling, or allometry within a single species, becomes important (Maxwell & Jacobson, 2008; Martinez et al., 2009). The large difference in size between Miniature Horses and

standard-sized horses suggests the need to consider body size when developing dosage regimens for Miniature Horses. To date, there has only been one study investigating appropriate drug dosing in Miniature Horses (Lee & Maxwell, in progress). Although this study did not support a need to adjust the dose rate for flunixin administration to Miniature Horses beyond proportional adjustment for body weight, flunixin is a highly protein bound drug that is metabolized in the liver but is not avidly extracted, characteristics that make it less likely than gentamicin to follow an allometric relationship (Riviere et al., 2007). If the disposition of gentamicin is indeed nonproportional among horses of greatly differing body weights, then the administration of this drug could be made safer and more effective by better definition of the allometric effect of body size on drug disposition.

The objectives of the present study were to evaluate the current status of the therapeutic drug monitoring of aminoglycosides in veterinary patients in the United States and Canada and to determine whether gentamicin disposition varies allometrically with body weight in Miniature Horses as compared to Quarter Horses..

Materials and methods:

Therapeutic drug monitoring survey

A survey on current practices in aminoglycoside TDM at North American veterinary teaching hospitals was sent electronically to list serves of the American College of Veterinary Pharmacology and Therapeutics and to the American Association of Veterinary Pharmacology and Therapeutics (Appendix A). The first question of the survey elicited information regarding which veterinary teaching hospital they worked for, and whether that hospital currently performs TDM of aminoglycosides. If the hospital was not currently performing TDM, the survey was terminated. The second part of the survey pertained to which assay and instrument was utilized

for TDM, whether the assay was performed in-house or off site, approximately how many samples were analyzed per year, and approximately what proportion of samples were from small animal patients as compared to large animal patients. The survey was subsequently approved by the Association of American Veterinary Medical Colleges and disseminated to pharmacologists, equine clinicians, or the administration of each veterinary teaching hospital in the United States and Canada, if no response had been received from the electronic survey.

Horses

Sixteen clinically healthy horses, consisting of Quarter Horse type horses (n = 8) and Miniature Horses (n = 8), were used in this study. Horses in each breed group were similar with respect to gender, age, and body condition score, but body weights were approximately five fold different between the two groups (Table 3). Horses were determined to be healthy by physical examination by a veterinarian, and a veterinarian assessed body condition score of each horse, using a nine point system (Henneke et al., 1983). Horses were housed in their usual environment or in individual stalls with free access to water and hay during the study. The study protocol was approved by the Oklahoma State University Animal Care and Use Committee and written informed consent was obtained for the five Quarter Horses and eight Miniature Horses that were privately owned. The remaining three Quarter Horses were maintained as part of a University owned teaching herd.

Table 3. Summary of demographic data for horses employed in the gentamicin study.

	Quarter Horses	Miniature Horses	P value
Age (yr)	9 (4-14)	7 (3-14)	0.50
Weight (kg)	476 (420-527)	109 (70-141)	<0.001
Body Condition Score	5.3 (5.0-5.5)	5.6 (4.7-6.8)	0.25
Gender			1.0
Gelding	4	4	
Mare	4	4	

Data are expressed as the mean, followed by the range.

Drug administration

A commercial formulation of gentamicin (Gentamicin sulfate; Sparhawk Laboratories, Lenexa, Kansas, USA) was administered as an intravenous bolus via an indwelling 14 gauge catheter placed using aseptic technique in the right jugular vein. A dose of approximately 6.6 $mg \cdot kg^{-1}$ of gentamicin was calculated for each horse and rounded to the nearest syringe increment for accurate calculation of the administered dose.

Blood collection

Baseline plasma samples were collected from all horses prior to drug administration. Following bolus intravenous administration of gentamicin, 10 mL blood samples were collected via a separate 14 gauge catheter previously placed in the left jugular vein at 3, 6, 10, 20, and 40 minutes, and at 1, 1.5, 2.5, 4, 6, 8, 10, 12 hours. Further sampling was performed by jugular venipuncture at 24 hours after the administration of gentamicin. All samples were collected into heparinized blood collection tubes and placed immediately into an ice-water bath. Samples were then centrifuged within one hour of collection and plasma was separated and stored at -80 °C until assayed.

Gentamicin assay

A previously described high performance liquid chromatography (HPLC) assay was modified for the sensitive and specific determination of plasma gentamicin concentrations in equine plasma (Isoherranen and Soback 2000). The HPLC system consisted of a ProStar™ 210 pump, 410 autosampler and ultraviolet detector (Varian Medical Systems, Palo Alto, California, USA). A reversed-phase column and guard column (Symmetry™ C18, 5 μm, 4.6 x 150 mm; Waters Corporation, Milford, Massachusetts, USA) were utilized at 25 °C for analyte separation. Mobile phase components were 8 mM Tris at pH = 7 (mobile phase A) and 50% acetonitrile and 50%

methanol (mobile phase B). The mobile phase consisted of 78% "A" and 22% "B" with gradient flow at 1.2 $mL \cdot min^{-1}$. Stock solutions were prepared by adding gentamicin components C1, C1a, or C2 (Toku-E, Bellingham, Washington, USA) or the internal standard, tobramycin (Sigma-Aldrich, St. Louis, Missouri, USA), to water. Plasma calibrants were prepared at concentrations of 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 $\mu g \cdot mL^{-1}$ using gentamicin component stock solutions and heparinized plasma from unmedicated horses. Calibrants and quality control samples were prepared by adding 970 μL of unmedicated equine heparinized plasma to 30 μL of the appropriate calibrant or quality control solution, followed by vortex mixing. Calibrant curves were constructed from the ratio of gentamicin:tobramycin for each component and were weighted using the reciprocal of the gentamicin concentration. Criteria for acceptance of each run included that a minimum of five calibrators back-calculated to within 15% of the nominal concentration and that the coefficient of determination was >0.99. Ten micrograms of tobramycin in 25 μL of water were added to each 1 mL calibrator, quality control, or experimental sample and vortex mixed. For protein precipitation and alkalization, 500 μL of 170mM Tris at pH = 12 and 2,000 μL acetonitrile were added. After mixing and centrifugation, derivitization was performed by adding 200 μL of 1-fluoro-2,4-dinitrobenzene/ acetonitrile (20:80, v/v) to the supernatant and heating in a 80°C water bath for 60 minutes. Samples were then applied to a 500 mg C8 solid phase extraction cartridge (Bond-Elut, Agilent Technologies, Santa Clara, California). The cartridges were first conditioned with methanol and water. After application of the derivitized sample mixed with 1 mL 40% acetonitrile, cartridges were washed with 1 mL 10% methanol in 10 mM Tris at pH = 10 and eluted with 2 mL acetonitrile. The eluent was dried under nitrogen for 50 minutes at 40°C. The residue was dissolved in 500 μL of mobile phase and 50 μL were injected onto the column. Tobramycin eluted at approximately 5 minutes, gentamicin C1a at approximately 9.5 minutes, gentamicin C2 at approximately 11 minutes, and gentamicin C1 at approximately 12.5 minutes. Recovery estimates were performed in plasma using three replicates.

Intraday and interday accuracy and coefficient of variation were estimated using quality control samples in plasma using three replicates. The limit of quantification was estimated using three replicates and was defined as the lowest concentration associated with a tenfold signal:noise ratio. The limit of detection was estimated using three replicates and was defined as the concentration at which signal:noise was at least three fold.

Component analysis of injectable formulation

A component analysis was determined by calculating the ratios of the three peak areas representing gentamicin C1a, C2, and C1 in the HPLC chromatograms. The component analysis was confirmed by proton NMR spectrum of the commercial injectable formulation of gentamicin (Gentamicin sulfate; Sparhawk Laboratories, Lenexa, Kansas, USA) from the same manufacturing lot that was administered to the horses. A 1 mg/mL in 99% D2O gentamicin solution was made from the injectable solution, and 5 μL of a DSS solution in D2O (3mM) was added, giving a final concentration of 0.03 mM of DSS. The proton NMR spectrum using a one-pulse sequence with water presaturation and 1024 scans was taken of the 1 mg/mL solution at 600 MHz. Peak assignments were made using the purpurosamine anomeric protons that are close to 6 ppm, and resonances were based on previous assignments (Deubner et al., 2003). The component analysis was compared to the requirements for the manufacturing of gentamicin published by the US Pharmacopoeia (Vydrin et al., 2003). This analysis was used to determine the dose of each gentamicin component that was administered to each horse and was used for pharmacokinetic determinations for each component.

Pharmacokinetic analysis

Plasma gentamicin component concentrations following the intravenous administration of gentamicin were analyzed compartmentally using Thermo Kinetica™ software version 5.0

(Thermo Fisher Scientific, Philadelphia, Pennsylvania, USA). Intravenous data for each horse were fit to the following equation:

$$C = \sum_{i=1}^{n} A_i \cdot e^{-\alpha \cdot t}$$

Data were weighted by the reciprocal of the plasma gentamicin concentration and were fit to standard compartmental models. The most appropriate model was selected using Aikaike's information criterion and the Schwarz criterion, and standard compartmental equations were then used to estimate the pharmacokinetic parameters for each horse. The mean and standard deviation for each group (Miniature Horses and Quarter Horses) was estimated for each pharmacokinetic parameter.

Allometric comparisons

The total body clearance for the three gentamicin components determined in Miniature Horses was compared to that predicted by standard allometry, as has been reported previously (Mordenti, 1986). The general form of the allometric equation used for scaling of pharmacokinetic parameters was:

$y = a \cdot BW^b$,

where y is gentamicin clearance; BW is the body weight; *a* is the allometric coefficient, and *b* is the allometric exponent. The allometrically predicted gentamicin clearance in Miniature Horses was calculated from the Quarter Horse clearance data by solving for the mass coefficient in the equation above and setting $b = 0.75$, as is frequently used in standard allometric calculations and is arguably a universal scaling exponent across species (Hu & Hayton, 2001). The values of $\log_{10}$ total body clearance were regressed against $\log_{10}$ body weight and compared to the curve predicted by standard allometry.

Statistical analysis

A Wilcoxon rank-sum test was used to analyze the difference in body condition score between the groups, whereas a two-sample t-test was used to test whether age differed between Quarter Horses and Miniature Horses. Two sample Student's t tests were used to test the difference in selected pharmacokinetic parameters: mass normalized clearance (Cl), elimination phase rate constant (γ), area under the plasma concentration versus time curve (AUC), apparent volume of distribution at steady state (Vd_{ss}), and volume of the central compartment (V_c). Significance was set at $\alpha = 0.05$. Statistical calculations were performed using SigmaPlot™ software version 11.0 (Systat Software, Inc., Chicago, Illinois, USA).

Results:

Therapeutic drug monitoring survey

The email-based survey was sent to representatives of each of the 33 veterinary teaching hospitals in the United States and Canada. Twenty-six (79%) of the institutions contacted responded to the questionnaire (Table 4). Less than one-half of the 26 respondents indicated that TDM of aminoglycosides is performed currently by their hospital, and a smaller minority (15 %) of the respondents indicated that TDM is performed in house at their hospital. The majority of TDM performed by respondents is for equine patients and uses an antibody-based assay (For complete results, see Appendix B).

Table 4. Summary of survey data for therapeutic drug monitoring survey.

	Frequency (Percentage)	**Annual samples performed (average #)**
Response rate	26/33 (79%)	
TDM performed	11/26 (42%)	3.9
TDM performed in house	4/26 (15%)	5.8

HPLC Assay

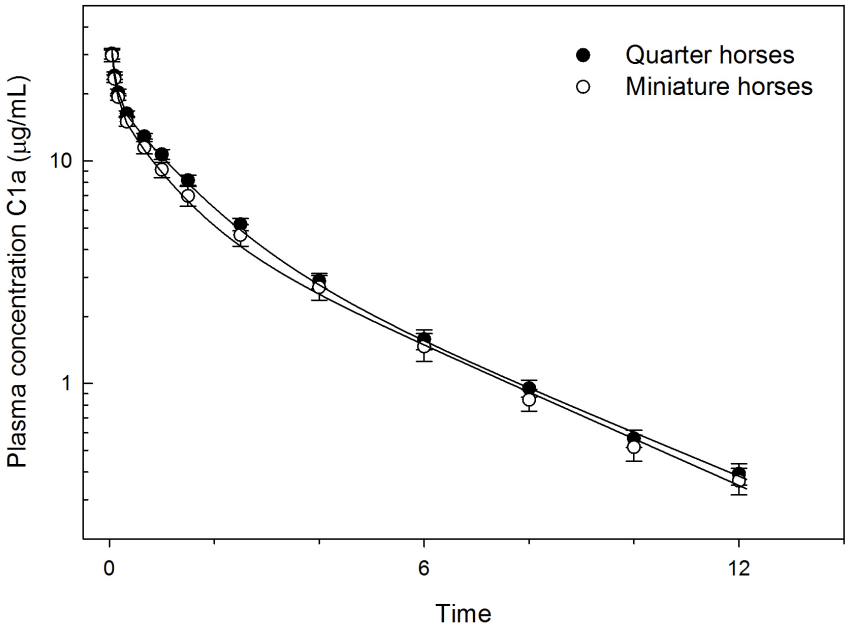
At fortified plasma gentamicin component concentrations of 1.5 μg·mL^{-1}, 3.5 μg·mL^{-1}, and 17.5 μg·mL^{-1}, recovery of gentamicin was 55 ± 8%, 57 ± 14%, and 54 ± 13%, respectively, for gentamicin component C1a, 56 ± 10%, 57 ± 19%, and 56 ± 14%, respectively, for gentamicin component C2, and 63 ± 12%, 66 ± 12%, and 58 ± 14%, respectively, for gentamicin component C1. At a plasma concentration of 10 μg·mL^{-1}, recovery of tobramycin was 68 ± 17%. Intraday accuracy of the assay at 1.5 μg·mL^{-1}, 3.5 μg·mL^{-1} and 17.5 μg·mL^{-1} was 94%, 98%, and 100%, respectively, for gentamicin component C1a, 97%, 98%, and 99%, respectively, for gentamicin component C2, and 100%, 98%, and 98%, respectively, for gentamicin component C1. Intraday coefficient of variation at 1.5 μg·mL^{-1}, 3.5 μg·mL^{-1}, and 17.5 μg·mL^{-1} was 1%, 3%, and 2%, respectively, for gentamicin component C1a, 2%, 1%, and 2%, respectively, for gentamicin component C2, and 1%, 1%, and 3%, respectively, for gentamicin component C1. Interday accuracy at 1.5 μg·mL^{-1}, 3.5 μg·mL^{-1} and 17.5 μg·mL^{-1} was 97%, 98%, and 98%, respectively, for gentamicin component C1a, 99%, 98%, and 98%, respectively, for gentamicin component C2, and 96%, 97%, and 97%, respectively, for gentamicin component C1. Interday coefficient of variation at 1.5 μg·mL^{-1}, 3.5 μg·mL^{-1}, and 17.5 μg·mL^{-1} was 1%, 4%, and 3%, respectively, for gentamicin component C1a, 1%, 3%, and 3%, respectively, for gentamicin component C2, and 2%, 4%, and 4%, respectively, for gentamicin component C1. The limit of quantification was 0.2 μg·mL^{-1} for gentamicin components C1a and C2 and was 0.1 μg·mL^{-1} for gentamicin component C1. The LOQ of the three components was associated with good accuracy and precision, with an accuracy of 95%, 94%, and 94%, for C1a, C2, and C1 respectively, and a coefficient of variation of 5%, 4%, and 8%, respectively. The limit of detection was 0.05 μg·mL^{-1} for each gentamicin component.

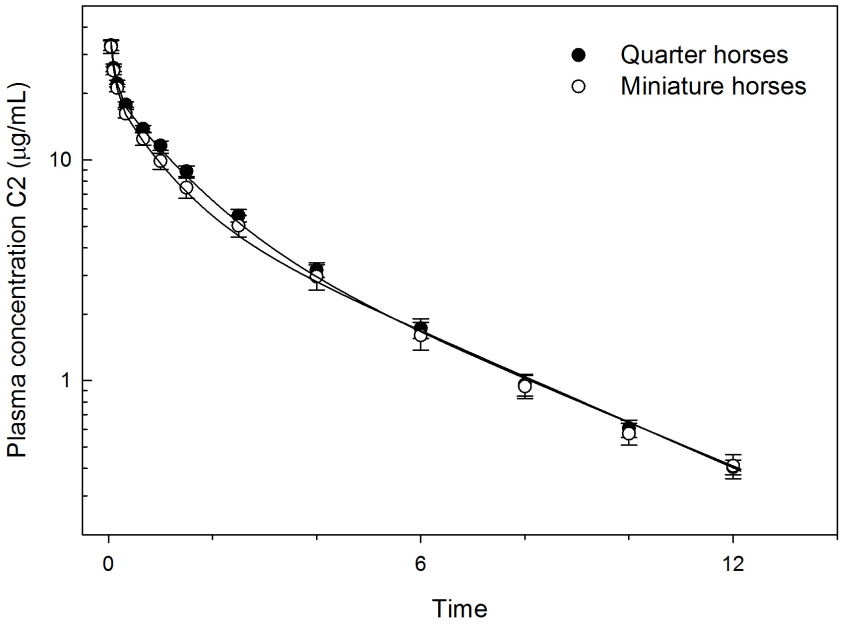
Component analysis of injectable formulation

Proton NMR determined that the injectable gentamicin used in the study was composed of 22% component C1a, 30% component C2, 32% component C1, and 16% component C2a. Ratio calculations of peak area from the HPLC chromatograms in the study determined that the percentages of the three components detected were 24% C1a, 26% C2, and 50% C1.

Quantification of gentamicin

All horses tolerated the administration of a single dose of gentamicin well, with no adverse effects noted for the duration of the study. Gentamicin concentrations were rapidly distributed after intravenous administration, followed by a slower distributive phase and an elimination phase. Gentamicin components C1a, C2, and C1 could be quantified for at least 12 hours in all horses and gentamicin component C1 could be quantified for 24 hours in 7/8 Quarter Horses and 8/8 Miniature Horses (Figure 4).


Quarter horses
Miniature horses
Plasma concentration C1a (µg/mL)
10
1
0
6
12
Time


Quarter horses
Miniature horses
Plasma concentration C2 (µg/mL)
10
1
0
6
12
Time

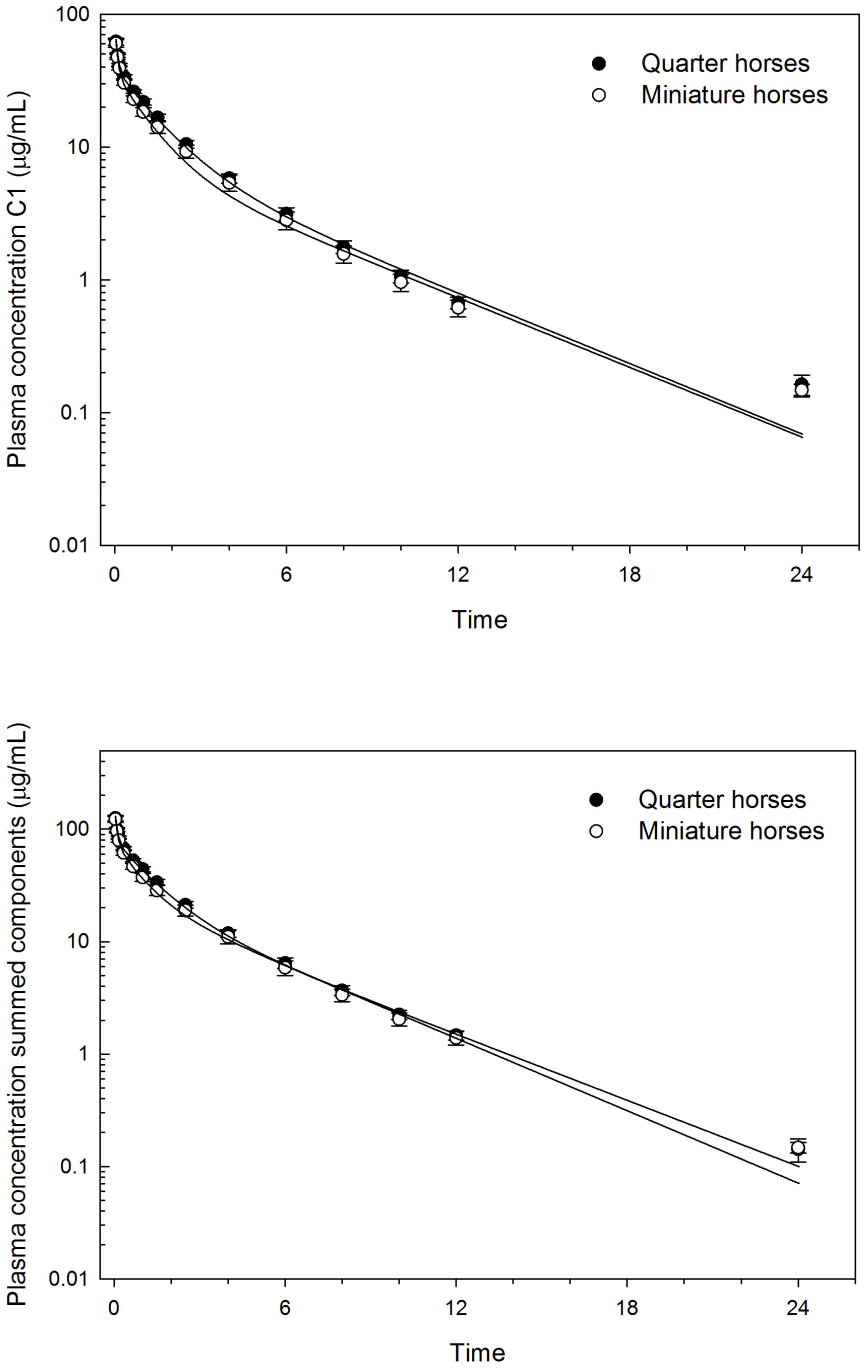


Figure 4: Mean (± s.e.m.) plasma concentrations of gentamicin components a) C1a b) C2 c) C1 and d) summed gentamicin components C1a, C2, and C1 after i.v. administration of gentamicin at a dose rate of 6.6 mg/kg to eight Miniature Horses and eight Quarter Horses. Gentamicin component concentrations were similar between the two groups throughout the sampling period.

Pharmacokinetics

The most appropriate compartmental model for pharmacokinetic analysis was determined to be a three compartment model (Table 5). There were no significant differences in the pharmacokinetic parameters of gentamicin components C1a, C2, C1, or the summed gentamicin components between breed groups in total body clearance (P = 0.18, 0.23, 0.20, 0.20 respectively), elimination phase rate constant (P = 0.37, 0.51, 0.96, 0.37 respectively), AUC (P = 0.19, 0.26, 0.21, 0.21 respectively), or the V_c (P = 0.99, 0.93, 0.98, 0.93 respectively). In addition, the mass specific clearance of gentamicin was not related to body weight (Figure 5a).

Table 5a. Comparison of pharmacokinetic parameters after i.v. administration of gentamicin to Quarter Horses and Miniature Horses. a) gentamicin component C1a

Parameter	Quarter Horses	Miniature Horses
Dose (mg·kg bwt^{-1})	1.58 ± 0.01 (1.57-1.59)	1.57 ± 0.01 (1.55-1.59)
C_0 (μg·mL^{-1})	43.1 ± 14.5 (30.6-70.9)	41.7 ± 10.2 (31.2-58.2)
A (μg·mL^{-1})	23.5 ± 14.0 (10.5-51.9)	22.8 ± 7.6 (16.5-37.5)
B (μg·mL^{-1})	14.0 ± 3.1 (8.8-18.4)	12.6 ± 2.2 (9.7-15.4)
C (μg·mL^{-1})	5.5 ± 2.9 (0.6-10.0)	6.3 ± 3.3 (3.2-12.7)
$t_{1/2}\alpha$ (h)*	0.06 ± 0.02 (0.03-0.09)	0.06 ± 0.02 (0.04-0.08)
$t_{1/2}\beta$ (h)*	0.82 ± 0.18 (0.54-1.66)	0.60 ± 0.33 (0.30-1.14)
$t_{1/2}\gamma$ (h)*	3.27 ± 0.38 (2.52-20.14)	2.87 ± 0.62 (2.22-3.87)
$t_{1/2}k_{10}$ (h)*	0.74 ± 0.15 (0.49-0.96)	0.64 ± 0.22 (0.43-0.96)
k_{10} (hr^{-1})	0.94 ± 0.23 (0.75-1.41)	1.08 ± 0.34 (0.72-1.61)
k_{12} (hr^{-1})	0.40 ± 0.25 (0.22-0.96)	0.72 ± 0.61 (0.23-1.95)
k_{21} (hr^{-1})	0.40 ± 0.20 (0.05-0.68)	0.54 ± 0.31 (0.29-1.10)
k_{13} (hr^{-1})	5.89 ± 4.46 (2.20-15.63)	5.42 ± 1.92 (3.31-8.07)
k_{31} (hr^{-1})	5.88 ± 0.85 (4.58-6.49)	6.16 ± 1.85 (4.45-9.32)

V_c (L·kg bwt^{-1})	0.040 ± 0.010 (0.022-0.052)	0.040 ± 0.009 (0.027-0.050)
$V_{d(ss)}$ (L·kg bwt^{-1})	0.123 ± 0.046 (0.096-0.236)	0.120 ± 0.020 (0.093-0.149)
$V_{d(area)}$ (L·kg bwt^{-1})	0.231 ± 0.208 (0.125-0.742)	0.175 ± 0.047 (0.106-0.236)
Cl (mL·min^{-1}·kg bwt^{-1})	0.59 ± 0.10 (0.43-0.74)	0.68 ± 0.15 (0.52-0.99)
AUC (μg·h·mL^{-1})	45.6 ± 8.3 (35.3-62.4)	40.0 ± 8.1 (26.8-51.1)
MRT (hr)	3.7 ± 2.2 (2.7-9.2)	3.0 ± 0.5 (2.4-3.7)

Table 5b. Comparison of pharmacokinetic parameters after i.v. administration of gentamicin to Quarter Horses and Miniature Horses. b) gentamicin component C2

Parameter	**Quarter Horses**	**Miniature Horses**
Dose (mg·kg bwt^{-1})	1.75 ± 0.01 (1.73-1.76)	1.74 ± 0.02 (1.72-1.76)
C_0 (μg·mL^{-1})	46.9 ± 16.2 (33.3-77.2)	45.9 ± 11.6 (32.8-63.6)
A (μg·mL^{-1})	25.8 ± 15.5 (11.9-56.7)	25.2 ± 8.7 (16.8-41.1)
B (μg·mL^{-1})	15.1 ± 3.6 (9.4-20.0)	13.8 ± 3.1 (8.5-17.6)
C (μg·mL^{-1})	6.0 ± 3.6 (0.6-11.5)	7.0 ± 4.2 (3.0-14.2)
$t_{1/2}\alpha$ (h)*	0.06 ± 0.02 (0.03-0.09)	0.05 ± 0.01 (0.04-0.10)
$t_{1/2}\beta$ (h)*	0.80 ± 0.20 (0.46-1.70)	0.58 ± 0.29 (0.31-1.38)
$t_{1/2}\gamma$ (h)*	3.22 ± 0.46 (2.38-13.69)	2.92 ± 0.72 (2.23-5.04)
$t_{1/2}k_{10}$ (h)*	0.72 ± 0.16 (0.47-0.95)	0.63 ± 0.24 (0.42-1.03)
k_{10} (hr^{-1})	0.96 ± 0.26 (0.73-1.46)	1.09 ± 0.36 (0.67-1.63)
k_{12} (hr^{-1})	0.41 ± 0.34 (0.20-1.20)	0.76 ± 0.61 (0.33-2.04)
k_{21} (hr^{-1})	0.42 ± 0.23 (0.06-0.75)	0.55 ± 0.31 (0.20-1.11)
k_{13} (hr^{-1})	5.82 ± 4.54 (2.40-15.66)	5.51 ± 1.84 (2.91-8.10)
k_{31} (hr^{-1})	5.77 ± 1.02 (4.34-7.51)	6.27 ± 1.72 (3.53-9.19)
V_c (L·kg bwt^{-1})	0.040 ± 0.012 (0.023-0.053)	0.040 ± 0.009 (0.027-0.053)
$V_{d(ss)}$ (L·kg bwt^{-1})	0.114 ± 0.017 (0.098-0.153)	0.124 ± 0.021 (0.094-0.152)

$V_{d(area)}$ (L·kg bwt^{-1})	0.211 ± 0.144 (0.123-0.559)	0.184 ± 0.053 (0.107-0.240)
Cl (mL·min^{-1}·kg bwt^{-1})	0.61 ± 0.09 (0.47-0.76)	0.69 ± 0.16 (0.52-1.01)
AUC (μg·h·mL^{-1})	48.6 ± 7.6 (38.0-62.3)	43.7 ± 9.1 (29.1-56.0)
MRT (hr)	3.2 ± 0.9 (2.6-5.4)	3.0 ± 0.5 (2.4-4.1)

Table 5c. Comparison of pharmacokinetic parameters after i.v. administration of gentamicin to Quarter Horses and Miniature Horses. c) gentamicin component C1

Parameter	**Quarter Horses**	**Miniature Horses**
Dose (mg·kg bwt^{-1})	3.30 ± 0.02 (3.27-3.33)	3.29 ± 0.03 (3.25-3.32)
C_0 (μg·mL^{-1})	86.9 ± 30.3 (58.2-145.8)	86.6 ± 30.6 (62.1-148.2)
A (μg·mL^{-1})	48.1 ± 28.6 (20.5-107.0)	50.1 ± 22.8 (30.5-93.5)
B (μg·mL^{-1})	29.8 ± 5.5 (19.3-37.4)	28.4 ± 5.6 (21.5-37.7)
C (μg·mL^{-1})	9.0 ± 5.8 (1.3-18.0)	8.2 ± 6.0 (0.7-17.2)
$t_{1/2}\alpha$ (h)*	0.06 ± 0.02 (0.03-0.13)	0.06 ± 0.02 (0.02-0.09)
$t_{1/2}\beta$ (h)*	0.91 ± 0.31 (0.56-1.69)	0.73 ± 0.37 (0.29-1.88)
$t_{1/2}\gamma$ (h)*	3.42 ± 0.90 (2.57-9.63)	3.44 ± 1.44 (2.20-9.59)
$t_{1/2}k_{10}$ (h)*	0.72 ± 0.21 (0.51-1.04)	0.61 ± 0.26 (0.34-1.02)
k_{10} (hr^{-1})	0.96 ± 0.26 (0.67-1.35)	1.14 ± 0.49 (0.68-1.35)
k_{12} (hr^{-1})	0.32 ± 0.26 (0.13-0.90)	0.61 ± 0.82 (0.06-2.54)
k_{21} (hr^{-1})	0.34 ± 0.17 (0.08-0.60)	0.37 ± 0.26 (0.08-0.92)
k_{13} (hr^{-1})	5.68 ± 4.41 (1.37-15.38)	5.84 ± 4.24 (3.00-15.67)
k_{31} (hr^{-1})	5.47 ± 1.43 (3.03-7.08)	5.64 ± 2.94 (3.31-12.63)
V_c (L·kg bwt^{-1})	0.041 ± 0.012 (0.023-0.057)	0.041 ± 0.011 (0.022-0.052)
$V_{d(ss)}$ (L·kg bwt^{-1})	0.112 ± 0.011 (0.094-0.124)	0.127 ± 0.012 (0.100-0.156)
$V_{d(area)}$ (L·kg bwt^{-1})	0.209 ± 0.089 (0.134-0.403)	0.246 ± 0.105 (0.129-0.459)
Cl (mL·min^{-1}·kg bwt^{-1})	0.62 ± 0.10 (0.48-0.79)	0.72 ± 0.17 (0.53-1.06)

AUC ($\mu g \cdot h \cdot mL^{-1}$)	90.1 ± 14.2 (68.6-114.6)	79.8 ± 17.3 (52.0-103.2)
MRT (hr)	3.1 ± 0.6 (2.3-4.2)	3.0 ± 0.6 (2.1-3.7)

Table 5d. Comparison of pharmacokinetic parameters after i.v. administration of gentamicin to Quarter Horses and Miniature Horses. d) gentamicin components C1a, C2, and C1 summed

Parameter	Quarter Horses	Miniature Horses
Dose ($mg \cdot kg\ bwt^{-1}$)	6.62 ± 0.04 (6.57-6.68)	6.60 ± 0.06 (6.52-6.67)
C_0 ($\mu g \cdot mL^{-1}$)	176.7 ± 60.3 (122.7-292.2)	174.7 ± 50.0 (127.4-259.3)
A ($\mu g \cdot mL^{-1}$)	96.5 ± 58.0 (41.0-214.0)	96.5 ± 37.1 (64.9-155.7)
B ($\mu g \cdot mL^{-1}$)	57.8 ± 11.8 (37.4-73.3)	51.3 ± 13.6 (41.0-72.1)
C ($\mu g \cdot mL^{-1}$)	22.4 ± 10.9 (4.9-39.9)	26.8 ± 15.1 (12.2-54.2)
$t_{1/2}\alpha$ (h)*	0.06 ± 0.02 (0.03-0.10)	0.05 ± 0.02 (0.03-0.09)
$t_{1/2}\beta$ (h)*	0.84 ± 0.20 (0.53-1.64)	0.58 ± 0.30 (0.29-1.11)
$t_{1/2}\gamma$ (h)*	3.07 ± 0.13 (2.51-5.82)	2.80 ± 0.72 (2.21-3.53)
$t_{1/2}k_{10}$ (h)*	0.72 ± 0.19 (0.50-0.97)	0.62 ± 0.22 (0.39-0.99)
k_{10} (hr^{-1})	0.97 ± 0.26 (0.71-1.39)	1.12 ± 0.40 (0.70-1.76)
k_{12} (hr^{-1})	0.36 ± 0.28 (0.14-0.99)	0.75 ± 0.69 (0.23-2.27)
k_{21} (hr^{-1})	0.40 ± 0.16 (0.14-0.64)	0.57 ± 0.33 (0.28-1.21)
k_{13} (hr^{-1})	5.74 ± 4.39 (1.91-15.39)	5.65 ± 2.76 (3.29-11.49)
k_{31} (hr^{-1})	5.74 ± 0.87 (4.36-7.01)	6.23 ± 2.23 (4.40-11.35)
V_c ($L \cdot kg\ bwt^{-1}$)	0.041 ± 0.011 (0.023-0.054)	0.040 ± 0.010 (0.026-0.051)
$V_{d(ss)}$ ($L \cdot kg\ bwt^{-1}$)	0.107 ± 0.008 (0.095-0.148)	0.120 ± 0.019 (0.095-0.148)
$V_{d(area)}$ ($L \cdot kg\ bwt^{-1}$)	0.171 ± 0.039 (0.128-0.250)	0.177 ± 0.039 (0.107-0.238)
Cl ($mL \cdot min^{-1} \cdot kg\ bwt^{-1}$)	0.62 ± 0.09 (0.50-0.77)	0.71 ± 0.16 (0.53-1.03)
AUC ($\mu g \cdot h \cdot mL^{-1}$)	182.0 ± 26.2 (141.9-224.3)	162.4 ± 33.7 (108.0-210.0)
MRT (hr)	2.9 ± 0.3 (2.5-3.4)	2.9 ± 0.4 (2.3-3.5)

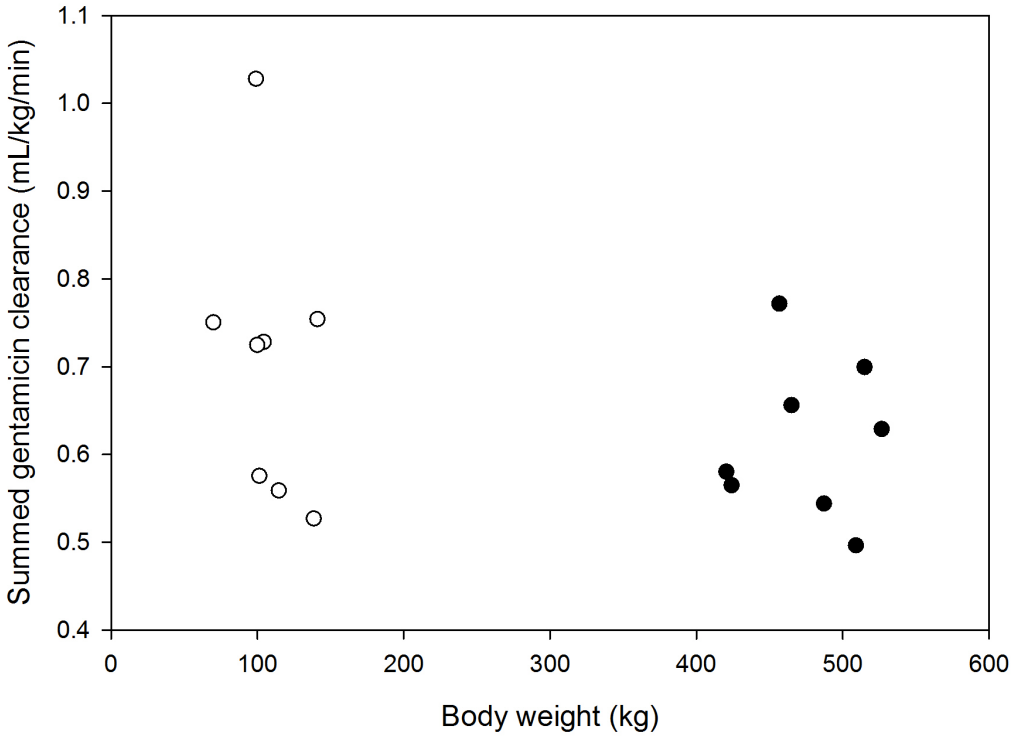
Values are expressed as mean or *harmonic mean ± s.d. (range)[37]. Dose = dose administered; C_0 = serum drug concentration at time 0; A = coefficient of rapid distribution phase; B = coefficient of slow distribution phase; C = coefficient of elimination phase; $t_{1/2}\alpha$ = rapid distributional half-life; $t_{1/2}\beta$= slow distributional half-life; $t_{1/2}\gamma$ = terminal elimination phase half-life; $t_{1/2}k_{10}$ = elimination half-life; k_{10} = first-order rate constant for elimination from the central compartment; other intercompartmental rate constants follow similar nomenclature; V_c = apparent volume of the central compartment; $V_{d(ss)}$ = apparent volume of distribution at steady state; $V_{d(area)}$ = apparent volume of distribution by area; Cl = total body clearance; AUC = Area under the plasma concentration versus time curve, extrapolated to infinity; MRT = mean residence time

Allometric comparison

The regression curve relating measured total body clearance of summed gentamicin to body weight (Figure 5b) was associated with a mass exponent of 0.91:

$$CL = 0.064 \cdot BW^{0.91},\ R^2 = 0.94$$

If the clearance of gentamicin in Miniature Horses had varied allometrically from that of Quarter Horses by the ¾ mass exponent, then the predicted gentamicin clearance in the Miniature Horses would have been 0.90 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$, more than 25% greater than the measured clearance of 0.71 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$. The statistical power (β) of the present study to detect such a difference was 0.97, with s.d. = 0.14 and $\alpha = 0.05$. The other pharmacokinetic parameters tested were similarly unrelated to body weight (elimination rate and AUC) or were directly proportional to body weight (Vd_{ss}, V_c; data not shown).


Summed gentamicin clearance (mL/kg/min)
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
100
200
300
400
500
600
Body weight (kg)

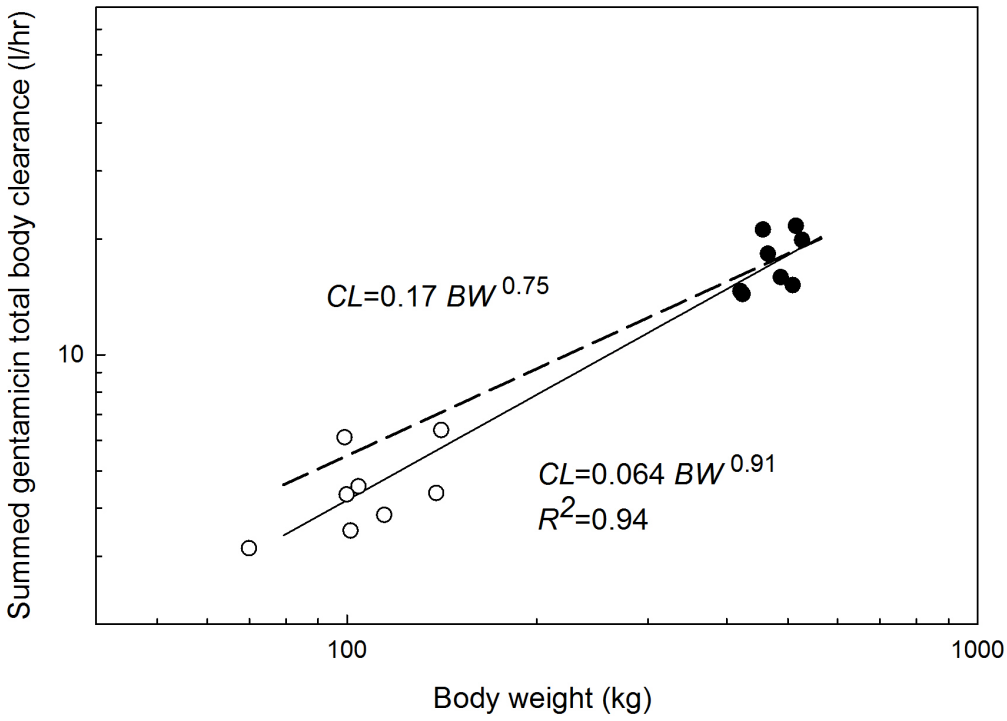

Summed gentamicin total body clearance (l/hr)
10
100
1000
Body weight (kg)
CL=0.17 BW 0.75
CL=0.064 BW 0.91
R2=0.94

Figure 5b: Relationship between summed gentamicin clearance and body weight. a) Mass specific clearance of summed gentamicin versus body weight in Miniature Horses and Quarter Horses, demonstrating that total body clearance was similar between the two groups when normalized to body weight. b) Total body clearance of gentamicin versus body weight in Miniature Horses and Quarter Horses, showing that total body clearance varied proportionally with body weight. The regression line calculated from the data (solid line) is compared to that predicted from standard allometry (dashed line).

Discussion:

Therapeutic drug monitoring of aminoglycosides is performed rarely in veterinary teaching hospitals in the United States and Canada. The few hospitals that do have the capability to perform TDM in house analyze very few samples annually using an antibody-based assay, with the vast majority of samples being from horses. The emphasis on TDM of aminoglycosides in horses is consistent with the recommendation in the literature that therapeutic drug monitoring be performed routinely even in healthy foals to ensure that the desired peak and trough concentrations are being achieved, especially due to the inherent individual variation in the pharmacokinetic parameters of gentamicin (Burton et al., 2012). However, the infrequent performance of TDM in veterinary medicine is not consistent with the importance of this technique as demonstrated in human studies (van Lent-Evers et al., 1999; Rea et al., 2008). A retrospective study in humans demonstrated that the majority of medical intensive care unit patients would not be predicted to achieve a pharmacodynamic target based on minimum inhibitory concentrations (Rea et al., 2008), emphasizing the potential effect of TDM in this population. A multicenter prospective study in humans compared an ‘active’ TDM strategy using pharmacokinetic dosage optimization, subsequent adaptive control, and ongoing patient follow-up with a ‘standard’ TDM strategy using attending physician dosing and TDM on request only, and showed shorter hospitalization times, reduced nephrotoxicity, reduced mortality in patients admitted with an infection, and lower total costs with the ‘active’ TDM strategy (van Lent-Evers et al., 1999). In the equine species, a study that measured serum gentamicin concentrations in

equine patients considered at high-risk for developing toxicosis found that nine of the twelve horses studied required dosage adjustment to optimize therapeutic concentrations (Sojka and Brown, 1986). The reason for the current infrequent performance of TDM in veterinary medicine is unclear, but it is likely that it is correlated with the low availability of the test and the expense associated currently with the fluorescent polarization immunoassay.

The present study employed an improved high performance liquid chromatography method that allowed the sensitive and specific determination of the concentrations of the three main gentamicin components in equine plasma. The sensitivity of the method was slightly improved as compared to the previously reported HPLC method, with the limits of quantification of the components of 0.2 $\mu g \cdot mL^{-1}$ (C1a and C2) the same the previous report, and 0.1 $\mu g \cdot mL^{-1}$ (C1) lower than the previous report of 0.4 $\mu g \cdot mL^{-1}$ (C1) (Steinman et al., 2002). The stated accuracy, precision, and recovery of the novel assay were all within acceptable limits. The use of a two compartment model describing gentamicin pharmacokinetics in horses predominates in the literature (Jones et al., 1998; Magdesian et al., 1998; Martin-Jimenez et al., 1998), but the increased sensitivity of the assay in the present study with a limit of detection of 0.05 $\mu g \cdot mL^{-1}$ compared to the limit of detection of 0.16 $\mu g \cdot mL^{-1}$ noted in previous work (Jones et al., 1998) allowed the definition of a third compartment. The articles of the US Pharmacopoeia stipulate the following limits for the component composition of gentamicin: C1a, 10-35%, C2, 25-55%, and C1, 25-50% (Vydrin et al., 2003). The proportions obtained in the present study using the HPLC method were 24% C1a, 26% C2, and 50% C1. In contrast, the proportions obtained in the present study using the NMR analysis appeared to be similar at 22% C1a, 30% C2, 32% C1, and 16% C2a. The authors hypothesize that the HPLC peak identified as C1 was actually a combination of components C1 and C2a, which would then be the sum of these components on NMR analysis,48%, which was very similar to the 50% composition determined for C1 by HPLC.

Therefore, all the percentages obtained via HPLC were within 14% of the percentages obtained via NMR, and were within the limits specified by the US Pharmacopoeia.

The values calculated for clearance for the horses in the present study were lower than those reported previously for gentamicin components in light-breed horses (Steinman et al., 2002). Specifically, the mean mass specific clearance of gentamicin components C1a, C2, and C1 for all horses in the present study of 0.64, 0.65, and 0.67 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$, respectively, were lower than the clearance for gentamicin components C1a, C2, and C1 of 1.63, 1.10, and 1.03 $mL \cdot min^{-1} \cdot kg\ bwt^{-1}$ respectively reported previously in light-breed horses given the same dose of gentamicin (Steinman et al., 2002). Additionally, the reported AUC values for Quarter Horses in present study were higher, and the V_c values for Quarter Horses in the present study were lower than those reported previously (Steinman et al., 2002). The design of the present study allowed better definition of the initial distributive phase via sampling at 3 minutes and 6 minutes after gentamicin administration, instead of taking the first sample at 10 minutes as did the previous study (Steinman et al., 2002). Since the lower limit of quantification of the present study also allowed quantification of all three gentamicin components for a longer duration after administration, it is hypothesized that these differences in measured clearance, AUC, and V_c are due to the improved definition of all three compartments of gentamicin modeling in the present study..The clearance of gentamcin for the horses in the present study was also lower than that of approximately 1.04 $mL \cdot min^{-1} \cdot kg$ determined commonly in studies using fluorescence polarization immunoasay bwt^{-1} (Jones et al., 1998). Comparisons with studies that utilize this method of analysis are difficult since the gentamicin components are not analyzed separately with this method, and it is likely that the individual component analysis and low limit of quanitification of the present study allowed a more precise definition of clearance.

The hypothesis of the present study that gentamicin would follow an allometric relationship in the horse was based on the large variation in body size between Miniature Horses and Quarter

Horses. The efficacy of gentamicin is well correlated to its plasma concentration, and it is primarily eliminated in an unchanged form via glomerular filtration, characteristics which make it one of a number of drugs that have been reported to scale allometrically between species (Riviere et al., 1997). Indeed, the elimination half-life of gentamicin correlates with body weight with a coefficient of determination of 0.86 in comparisons between a range of animal species and was therefore expected to scale allometrically within a species. However, allometric scaling of the pharmacokinetic parameters of gentamicin was not demonstrated within horses in the present study. Comparisons between clinically relevant pharmacokinetic parameters that have been previously shown to scale allometrically with body weight (Cox et al., 2004; Dinev, 2008; Gebru et al., 2011) did not show a significant difference between the horse breeds employed in the current study. To determine if there was sufficient range in body weight and statistical power in the present study to reveal a standard allometric relationship in which drug clearance varies with body weight raised to the ¾ power, the predicted gentamicin clearance for Miniature Horses using standard allometry was compared with the actual measured total body clearance for the summed gentamicin components (Figure 5). As the power of the present study to show this relationship was high at 0.97, it can be confidently stated that gentamicin does not follow standard allometry in Miniature Horses as compared to Quarter Horses. This result is similar to the only other study comparing the pharmacokinetics of Miniature Horses to Quarter Horses, in which flunixin was demonstrated to be without any allometric effect in these breeds (Lee & Maxwell, in press). The clearance of gentamicin was also plotted against body weight on a double $\log_{10}$ plot to examine the possibility of a nonproportional relationship following a scaling factor other than standard allometry, such as the 2/3 power commonly used in body surface area calculations (Gouma et al., 2012). The mass exponent of this regression was 0.91 and did not significantly differ from unity ($P>0.05$). Although clearance was approximately proportional to body weight, the mass exponent value less than unity does suggest that a weak allometric relationship between gentamicin clearance and body weight may be present within horses.

However, if such an allometric relationship is truly present it is weak and unlikely to be of clinical significance in dose determinations for gentamicin in Miniature Horses.

The efficacy and toxicity of gentamicin have not been compared between breeds in the horse. However, it has been demonstrated that pharmacokinetic/pharmacodynamic indices for antibiotics such as gentamicin in humans vary greatly with different patient ages and with reduction in clearance (Nielsen et al., 2011). Since the efficacy and toxicity of gentamicin in patient populations can primarily be explained by disease factors that impact pharmacokinetic parameters, dosage recommendation for gentamicin administration to Miniature Horses can primarily rely upon their pharmacokinetic similarity to Quarter Horses.

The results of the present study do not support a need to adjust the dose rate of gentamicin for Miniature Horses. As neither gentamicin nor flunixin disposition depended upon body weight when comparing Miniature Horses to a common light-breed of horses, it is likely that allometric effects need not be taken into account when designing dosing regimens for Miniature Horses. Veterinarians may therefore confidently administer gentamicin and other drugs to Miniature Horses using typical equine dosing regimens. However, TDM is recommended as part of the treatment protocol for the administration of aminoglycosides in both human and veterinary medicine (Burton et al., 2012; Sojka and Brown, 1986), and the administration of gentamicin to Miniature Horses is no exception.

CHAPTER IV

CONCLUSION

The large variation in body size within the equine species led to the hypotheses that intraspecific allometric scaling plays a role in the disposition of several important therapeutics of the horse, and that flunixin and gentamicin would scale allometrically with body weight in the horse. However, the results of the present study do not support pharmacokinetic scaling of either drug in the horse. Statistical comparisons between Miniature Horses and Quarter Horses were performed on those pharmacokinetic parameters used commonly in allometric calculations and deemed clinically relevant to dose calculations (Cox et al., 2004; Dinev, 2008; Gebru et al., 2011). These key pharmacokinetic parameters showed no significant differences between Miniature Horses and Quarter Horses for either drug. When the measured clearance for each drug in Miniature Horses was plotted against body weight on a double $\log_{10}$ plot, the mass exponent of each comparison was similar to unity, further demonstrating the absence of a clinically relevant allometric effect.

There could be several reasons for the inability of the present study to demonstrate a clinically relevant allometric effect in the horse. Although the inclusion of draft horses in the study would have allowed comparison across a full order of magnitude in body weight, instead of the ½ order of magnitude present between Miniature Horses and Quarter Horses, it is unlikely that Miniature Horses follow standard allometry, as the study was adequately powered to detect such a difference between the breeds utilized. It is possible, however, that a weak allometric effect may

have been demonstrated by inclusion of horses over a wider range in body weights and with a larger number of horses.. There are several possible explanations for the lack of pharmacokinetic scaling reported in the present studies on flunixin and gentamicin disposition in Miniature Horses. First, the equine species demonstrates a smaller range in body weights than does the dog, in which intraspecific allometry in drug disposition has been demonstrated (Satyanarayana Achanta, personal communication). There is also a much smaller span in orders of magnitude within body weight in the equine species than is commonly investigated in interspecific comparisons, many of which span the eight orders of magnitude present in mammals (Savage et al., 2008). This smaller span in orders of magnitude within body weight may hinder demonstration of an allometric effect because it is difficult to detect a statistical difference between unity and the standard allometric exponent across a narrower range. The only previous paper to demonstrate an allometric effect in equids compared clearance of phenylbutazone in miniature donkeys to previously reported clearance in standard donkeys (Matthews et al., 2001). While this study may be overly reliant on previously reported data, it is also possible that the metabolism of phenylbutazone in donkeys is different from that in horses, or that phenylbutazone is more prone to exhibit allometric scaling than is flunixin. Finally, it is possible that there are breed effects within the equine species that supersede the influence of allometric relationships in both physiology and pharmacokinetics. This could have an effect on the ability of the Quarter Horses in the present study to be representative of all light-breed horses, and could have an effect on the uniformity of pharmacokinetics within the Miniature Horse breed since it is the result of the selective breeding of various light horse breeds. Further studies investigating allometry in horses may include the comparison of the clearance of gentamicin in Miniature Horses and draft horses, the comparison of the clearance of phenylbutazone in Miniature Horses and Quarter Horses, and the comparison of the glomerular filtration rates of Miniature Horses, Quarter Horses, and other light and draft breeds of horses.

Novel high performance liquid chromatography methods were employed in the present study for the determination of the concentration of flunixin meglumin and gentamicin in equine plasma. The sensitivities of the methods used in the present study were improved as compared to previously reported methods (Soma et al., 1988; Steinman et al., 2002). The improved sensitivity of the method for flunixin analysis allowed the reliable quantification of flunixin for up to 24 hours after administration for the first time, which demonstrated the presence of a third compartment as compared to previous studies that described a two compartment model (Chay et al., 1982; Semrad et al., 1985; Lees et al., 1987; Soma et al., 1988). The sensitivity of the method for gentamicin analysis with the individual analysis of the three main components of gentamicin also allowed the demonstration of a third compartment as compared to the two compartment model described previously in the literature (Magdesian et al., 1998; Martin-Jimenez et al., 1998). Both methods described in the present study were deemed robust and feasible for use in future pharmacokinetic studies.

Although active therapeutic drug monitoring strategies for aminoglycosides have been demonstrated to allow shorter hospitalization times, reduced nephrotoxicity, reduced mortality in patients admitted with an infection, and lower total costs in human medicine (van Lent-Evers et al., 1999), therapeutic drug monitoring is presently performed infrequently in veterinary teaching hospitals in the United States and Canada. The few veterinary teaching hospitals that do have the capability to perform TDM within their institution analyze very few samples annually using an antibody-based assay, with the vast majority of samples being from equine patients. The reason for the infrequent performance of TDM in veterinary medicine is unclear, but it is likely that it is correlated with the low availability of the test in currently used clinical analyzers and the expense associated with currently available assays. Dosage recommendations for gentamicin should include a recommendation for TDM in all horses, including Miniature Horses.

Prior to this investigation, the absence of objective studies exploring the pharmacokinetics of any veterinary drug in the Miniature Horse has required the veterinary practitioner to rely on subjective information to support any therapeutic decisions in this breed. Although it remains imperative to adjust the drug dose to body weight when administering any therapeutic to a Miniature Horse, the results of the present study allow veterinarians to confidently administer both flunixin meglumine and gentamicin to Miniature Horses using typical equine dosing regimens. Since elimination half-life is significantly correlated with body weight in drugs such as gentamicin that are cleared via glomerular filtration (Riviere et al., 1997), the absence of a significant allometric effect of body weight on drug disposition makes it unlikely that pharmacokinetic scaling is clinically important within the equine species. Therefore, there is now objective evidence to support the use of typical equine dosing regimens for therapeutics in the Miniature Horse.

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APPENDICES

APPENDIX A – Therapeutic Drug Monitoring Survey

1) To your knowledge, does your hospital perform therapeutic drug monitoring of gentamicin, amikacin, or other aminoglycosides? If your answer to this question is "No", the remaining questions are not applicable. Please email the survey results to Dr. Lee, as described below.

2) Is the assay performed in house or off site? If the assay is performed off site, please specify where it is performed.

3) What assay and instrument is used?

4) Approximately how many samples are analyzed per year?

5) Approximately what proportion of samples is from small animal patients as compared to large animal patients?

6) If you have any further information to share about patient demographics (age or species most commonly subjected to TDM of aminoglycosides), please elaborate

APPENDIX B – Therapeutic Drug Monitoring Survey Results

	Response?	Perform TDM?	In house/off site	Assay	#samples/year	type of patient
Auburn	X	Yes	in house	Seimens Xpand	2012: 15 amikacin, 0 gentamicin; 2011: 1 amikacin, 1 gentamicin; 2010: 15 amikacin, 3 gentamicin	75% small animal for amikacin, majority of gentamicin is large animal
Colorado State	X	Yes	in house	unknown	few	majority equine
Cornell	X	No				
Iowa						
Kansas State	X	No				
Louisiana State	X	No				
Michigan State						
Mississippi State	X	No				
North Carolina State	X	No				
Oklahoma State	X	No				
Oregon State	X	No				
Purdue	X	Yes	off site		3.4	99% horses
Texas A&M	X	Yes	off site		0-1	horses
Ohio State	X	Yes	off site	unknown	unknown	unknown
Tufts	X	No				
Tuskegee						
California, Davis						
Florida	X	Yes	off site	unknown	10 to 15	horses
Georgia						
Illinois	X	Yes	in house	Immunolite 2000	0	predict would be foals
Minnesota						
Missouri	X	Yes	off site		2	foals
Penn						
Tennessee	X	No				
Wisconsin	X	No				
Virginia	X	No				
Washington State	X	No				
Western University	X	No				
Calgary	X	No				
Guelph	X	No				
Montreal	X	Yes	in house	unknown	unknown	unknown
Atlantic	X	Yes	off site		1 to 2	95% horses, usually foals, 5% small animal
Saskatchewan	X	Yes	off site		1	horses

VITA

Crystal Lee

Candidate for the Degree of

Master of Science

Thesis: THE EFFECT OF BODY WEIGHT ON THE DISPOSITION OF FLUNIXIN MEGLUMINE AND GENTAMICIN IN MINIATURE HORSES AND QUARTER HORSES

Major Field: Veterinary Biomedical Science

Biographical:

Education:

Completed the requirements for the Master of Science in Veterinary Biomedical Science at Oklahoma State University, Stillwater, Oklahoma in May 2013.

Completed the requirements for the Doctor of Veterinary Medicine at University of Saskatchewan, Saskatoon, SK, Canada in 2009.

Experience:

Residency in Equine Surgery at Oklahoma State University 2010-2013.

Rotating Internship in Equine Medicine and Surgery at University of Missouri, 2009-2010.

Professional Memberships:

American Association of Equine Practitioners 2005-present