Plasma Peak and Trough Gentamicin Concentrations in Hospitalized Horses Receiving Intravenously Administered Gentamicin

J.R. Bauquier, R.C. Boston, R.W. Sweeney, P.A. Wilkins, and R.D. Nolen-Walston

Background: Gentamicin is an aminoglycoside antimicrobial commonly used in horses at 6.6 mg/kg IV once daily. Therapeutic drug monitoring (TDM) can confirm desired peak concentration is reached for common bacterial isolates, and detect toxicosis associated with high trough values.

Objectives: Determine the relationship between gentamicin dose and plasma concentration in hospitalized horses, and identify a starting dose range to achieve peaks > 32 μg/mL.

Animals: Sixty-five horses (2002–2010) receiving once-daily gentamicin with TDM performed (N = 99 sets).

Methods: Retrospective study. Data from hospitalized horses including weight, dose, plasma peak, and trough gentamicin concentration, creatinine concentrations and presence of focal or systemic disease were collected from medical records. Peak concentrations measured 25–35 minutes after administration were included (N = 77). Data were divided into low (<7.7 mg/kg), medium (7.7–9.7 mg/kg) and high (>9.7 mg/kg) dose groups, and were grouped by the horse having focal or systemic disease.

Results: Peak concentrations resulting from doses ≥7.7 mg/kg were 5.74 μg/mL (SE 2.1 μg/mL) greater than peaks from doses <7.7 mg/kg (*P* = .007). Peak concentrations was 3.6 times more likely to be >32 μg/mL if dose was ≥7.7 mg/kg (*P* = .04). There were no significant effects of dose on trough or creatinine concentration. At a given dose, horses with focal disease had higher peaks than those with systemic disease (*P* = .039).

Conclusions and Clinical Importance: These data suggest gentamicin dosage should be individually determined in horses using TDM, but support an initial once-daily dose of 7.7–9.7 mg/kg IV to achieve peaks >32 μg/mL and trough concentrations <2 μg/mL. Further studies evaluating the safety of doses >6.6 mg/kg are required.

Key words: Aminoglycoside; Equine; Pharmacokinetics; Therapeutic drug monitoring.

Gentamicin is an aminoglycoside antimicrobial commonly used to treat Gram negative infections in humans and many veterinary species, including horses.[1–3] It is a water-soluble, bactericidal, concentration-dependent antimicrobial, and is most commonly administered intravenously (IV) to adult horses at 6.6 mg/kg every 24 hours, based on pharmacokinetics in healthy horses.[2,4,5] However, dose calculations often need to be adjusted after plasma peak and trough levels are obtained in hospitalized equine and human patients,[6–8] although these equine studies used 6–12 hourly dosing regimens. Once-daily dosing has been examined in surgical colics,[9] but not a wider population

Abbreviations:

TDM	therapeutic drug monitoring
MIC	minimum inhibitory concentration

of hospitalized horses. Gentamicin is reportedly most effective when its concentration : minimum inhibitory concentration (MIC) ratio is 8–10 : 1 (gentamicin : MIC).[10] The MIC of most equine pathogens is ≤2 μg/mL, with 4 μg/mL for organisms with intermediate susceptibility.[11] Due to concerns with gentamicin resistance, the MIC for intermediate resistance is used in our hospital where gentamicin treatment is indicated before bacterial culture and antimicrobial sensitivity results are available. Using an MIC of 4 μg/mL gives a target peak range of 32–40 μg/mL (8–10× MIC), while a trough value of <2 μg/mL is considered indicative of adequate renal clearance,[12] reducing the risk of acute tubular necrosis.[1] Lower target trough values of <0.5–2 μg/mL have been suggested[5,13] and there is no consensus on target trough values for horses. Previous studies investigating gentamicin use in systemically ill horses investigated 6-, 8-, or 12-hourly dosing regimens,[7,8] which are no longer recommended. As once-daily dosing in hospitalized patients has been studied only in surgical colics,[9] investigation of the once-daily dosing regimen in a wide range of hospitalized equine patients is warranted.

Routine performance of TDM for gentamicin in our hospital suggests that an IV, once-daily dose >6.6 mg/kg is generally required to achieve a target peak concentration of >32 μg/mL, when measured 30 minutes after administration of gentamicin. Based on this observation, our specific aims for undertaking this study were first to determine whether a once-daily IV dose of gentamicin >6.6 mg/kg is required to achieve plasma levels

>32 μg/mL in hospitalized horses; second to determine whether TDM results lead to dose adjustments and repeat TDM; and, finally, to determine whether systemic, as opposed to focal disease, affects the dose required to achieve plasma levels >32 μg/mL.

Materials and Methods

This study was conducted retrospectively using records from horses >3 months of age presented to the University's large animal hospital from 2002 to 2010. Clinical pathology logs were used to identify cases with TDM performed, then medical records were evaluated in detail. Data collected included age, breed, sex, whether the horse had focal (for example musculoskeletal or ophthalmic) or systemic (likely to cause a systemic inflammatory response, for example pleuropneumonia, colitis, surgical colic, metritis) disease, body weight, dose of gentamicin administered, time of sampling for TDM after administration, peak gentamicin plasma concentration, trough gentamicin plasma concentration, whether the dose was adjusted after TDM was performed, duration of gentamicin treatment, creatinine concentration on presentation, and any subsequent creatinine values obtained. Peak gentamicin concentrations were only included if they were taken between 25 and 35 minutes after injection. Cases were excluded if actual dose (mg/kg) could not be determined because of the horse's weight not being recorded.

Data were analyzed by a priori division of peak and trough values into 3 groups by dose: a low dose group receiving <7.7 mg/kg, a medium dose group receiving 7.7–9.7 mg/kg, and a high dose group receiving >9.7 mg/kg. Initial dose administered was determined based on clinician preference. As weight was often estimated at the time dose was determined, a wide range of initial doses were given when calculated using the horse's actual weight.

Plasma gentamicin concentrations were measured using a competitive binding immunoassay (2002–2005; analyzer 1),[a] or a latex-enhanced immunosorbent assay (2008–2010; analyzer 2).[b] Analyzer 1 was validated by the manufacturer; however, these data (other than limit of quantification) were not available because of the age of the machine meaning it is no longer supported by the manufacturer. Limit of quantification for this analyzer was 0.27 μg/mL. Analyzer 2 was validated by the manufacturer for human serum but not equine plasma. Human validation data for this analyzer are: intra-assay coefficient of variation 2.99%, inter-assay coefficient of variation 8.76%, and limit of quantification 0.85 μg/mL. Data on limit of detection were not available. Accuracy and precision was ensured through compliance with the College of American Pathologists' quality assurance program. Gentamicin TDM logs from 2006–2007 were not available and were not included. Creatinine concentration was measured in plasma.[c] Troughs were taken at 22 hours post administration. For trough samples obtained earlier than 22 hours (7–20 hours), the anticipated 22 hour trough was calculated by extrapolation from the peak and trough data points, using the formula:[14]

$$C_{\text{trough 22}} = C_{\text{peak}} \times e^{(-22\,\text{h} - \text{peak time}) \times \beta}$$

where $C_{\text{trough 22}}$ is the plasma trough concentration at 22 hours, C_{peak} is the plasma peak concentration, e is the base of the natural logarithm, peak time is the time in hours that the peak was taken after gentamicin administration, and β is the slope of the ln (concentration) versus time plot (that is, elimination rate constant for the single compartment model). Where the measured trough value was below the quantification limit of the assay, the limit of quantification was used (0.27 μg/mL for analyzer 1, and 0.85 μg/mL for analyzer 2).

Peak and trough values were also divided into two groups based on whether the horse from which the value came had had focal or systemic disease. The focal disease group comprised horses with diagnoses such as infected synovial structures, lacerations, and ophthalmic disease that were otherwise healthy, and the systemic disease group comprised horses with diagnoses such as surgical colic, pleuropneumonia, metritis, and colitis. There were no elective surgical cases included in the focal disease group; however, gentamicin might have been given primarily as perioperative prophylaxis to some cases in both groups, most commonly ophthalmic cases and surgical colics.

Statistical Analysis

All analyses were performed using commercial software.[d] To determine whether any differences in sex, breed, age, and weight existed between groups, analysis was performed using Chi squared tests of association with Somers' D procedure or clustered regression analysis. Normality of data was tested using a Shapiro-Wilk test. Regression analysis was used to determine the relationship between dose group on peak gentamicin concentration. Where needed and the regression model allowed, clustering was used to account for multiple peaks coming from individual horses. Robust regression was used to assess the consequences of outlying data points on the regression results. Where distinct dose groups were very similar with regard to items measured, consideration was given to amalgamating the groups. Logistic regression, again with clustering, was used to quantitate associations between dichotomous outcomes and study predictors. Somers' D procedure[15] was also used to confirm any strong associations that were detected with logistic regression. Trough data were non-normally distributed and not susceptible to normalization, therefore these data were analyzed using the Kruskal-Wallis rank test. Duration of treatment data were not normally distributed and were square root transformed to achieve normality, after which robust regression was used for analysis, and results back transformed. Highest creatinine value measured for any particular horse was normally distributed and regression methods were used to analyze the relationship between dose and highest creatinine value obtained. Data for change in creatinine (Δcreatinine) were not normally distributed, and the relationship between dose and Δcreatinine was analyzed using Spearman's rank correlation coefficient. Somers' D was deferred to in order to protect against type 1 errors because of the underestimation of observation variance (compared with Spearman's correlation tests of association). The level of significance was set at <0.05.

Results

Ninety-nine peak values, 92 raw trough values, and 84 trough values either taken at 22 hours (n = 9) or extrapolated to 22 hours (n = 75) were obtained from 65 horses, with 27 horses having TDM performed more than once. Not all data points were available for all horses. Troughs could not be extrapolated to 22 hours if time of sampling was not available. Twenty-two peaks were excluded because of sample collection being outside the defined 25–35 minute timeframe, giving 77 peaks from 53 horses for the peak analyses. Of these 77 peaks, 6 were obtained at 25 minutes, 66 at 30 minutes, and 5 at 35 minutes. There were 23 horses with peaks measured at 25–35 minutes more than once. Ages ranged from 3 months to 20 years, and there were 26 females, 29 geldings, 8 intact males, and 2 horses for which sex was not recorded. Weight was not significantly different between dose

groups. Females were over-represented in the medium dose group, and geldings in the low dose group ($P = .002$). Horses in the low dose group were significantly older than in the other 2 groups ($P = .04$). As peak data were extremely similar between the medium and high dose groups, they were combined posthoc into a single group, and, the significant differences in age no longer existed ($P = .45$). Breeds classed as "other" were not matched across dose groups as other breed categories were, with significantly higher numbers in the low dose group ($P = .028$). Horses with TDM performed more than once were often split across dose groups (caused by dosage adjustments in response to TDM), and this was accounted for statistically by using the Somers' D procedure and clustering by horse.

Data for duration of treatment were available for 27 horses, 7 of which had peak values taken across multiple dose groups. The highest dose group that the horse had a peak value recorded for was used for analysis. On average, horses in the low dose group were treated for 1.2 days, whereas horses in both the medium and high dose groups were treated for 6.1 days ($P = .00$). For the two different analyzers used, 72, 60 and 67% of analyses were performed with analyzer 1 in the low, medium and high dose groups, respectively. Median (range) doses for each dose group were 6.7 (4.3–7.6) mg/kg for the low dose group, 8.8 (7.7–9.6) mg/kg for the medium dose group and 11.0 (9.8–15.0) mg/kg for the high dose group.

Twenty-three of the 53 horses that had peaks measured between 25–35 minutes after gentamicin administration had TDM performed on 2 (22 cases) or 3 (1 case) occasions. Of the 77 total peaks, 51 (66%) were below the minimum target peak of 32 μg/mL. Of these 51 peaks of <32 μg/mL, a dose adjustment was made 69% of the time (35 out of 51 peaks). In the low dose group, 83% of peaks were <32 μg/mL, in the medium dose group 57% of peaks were <32 μg/mL, and in the high dose group 63% of peaks were <32 μg/mL. Peak and trough values in the high dose group came entirely from horses that had had an upwards dose adjustment after TDM was previously performed, resulting in the proportion of peaks above 32 μg/mL increasing from 0/11 (0%) to 4/11 (36%). An additional 3 horses reached peaks just below 32 μg/mL, achieving peak concentrations of 31.0, 31.25 and 31.4 μg/mL (Fig 1). Thirty-six peaks and 38 trough values came from horses with focal disease, and 41 peaks and 43 troughs came from horses with systemic disease.

Mean (±SD) peak for the low dose group was 26.7 (±7.7) μg/mL (n = 24), for the medium dose group was 33.7 (±11.4) μg/mL (n = 42), and for the high dose group was 32.1 (±5.5) μg/mL (n = 11). The medium and high dose groups were combined into a single group (dose ≥ 7.7 mg/kg) as these groups in isolation were found to be very similar in the logistic regression model. Therefore, this resulted in two dose groups, one for doses <7.7 mg/kg and another for doses ≥ 7.7 mg/kg. Peaks in the ≥7.7 mg/kg dose group were on average 5.7 μg/mL (standard error 2.1 μg/mL) greater than peaks in the <7.7 mg/kg group ($P = .007$). Peaks were 3.6 times more likely to be >32 μg/mL in the
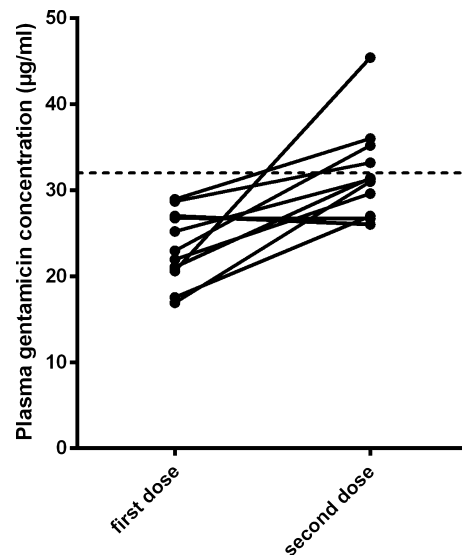
Fig 1. Peak gentamicin concentrations obtained from horses with peaks in the high dose group, which consisted entirely of peaks achieved after therapeutic drug monitoring (TDM) had previously been performed and a dose adjustment made. Dashed line represents the desired minimum peak of 32 μg/mL. Before dose adjustment, 0/11 (0%) of peaks were above 32 μg/mL, and after dose adjustment 4/11 (36%) of peaks were above 32 μg/mL. An additional 3 peaks were measured at 31–32 μg/ml, making a total of 7/11 peaks that were 31 μg/ml or above.
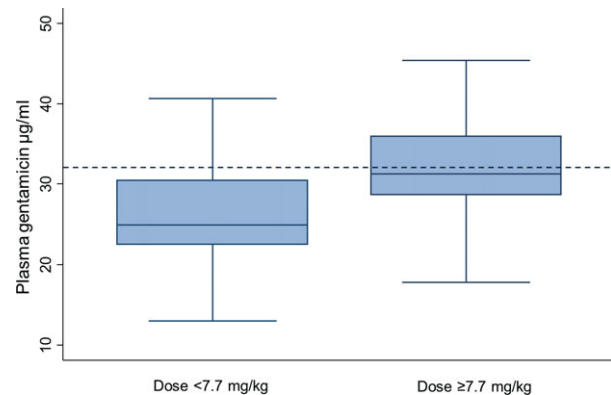
Fig 2. Effect of dose group on peak plasma gentamicin concentrations. Boxes represent the interquartile ranges from the 25th to the 75th percentiles. Horizontal lines within the boxes indicate median values; whiskers represent the 10th (lower whiskers) and 90th (upper whiskers) percentiles. Peaks from the ≥7.7 mg/kg group were on average 5.7 μg/mL higher than those in the <7.7 mg/kg group ($P = .007$), and were 3.6 times more likely to be >32 μg/mL ($P = .047$). Dashed line represents the desired minimum peak concentration of 32 μg/mL.

≥7.7 mg/kg dose group than the <7.7 mg/kg dose group ($P = .047$, 95% CI 1.0–12.4) (Fig 2).

Median (range) of extrapolated or actual 22 hour trough values were 0.01 (0.00–0.85) μg/mL (n = 25) for the low dose group, 0.01 (0.00–0.85) μg/mL (n = 45) for the medium dose group and 0.00 (0.00–1.10) μg/mL (n = 14) for the high dose group. The upper limit of the range of 0.85 reflects the limit of quantification of

analyzer 2, therefore the actual upper limit of the range may have been lower. Values below the limits of quantification of the analyzers are derived from the extrapolation calculations. Raw trough data were taken between 7 hours and 23 hours 45 minutes after gentamicin administration. Time of trough sample collection after gentamicin administration was 7–10 hours (median 1.2 μg/mL, range 0.2–6.5 μg/mL; n = 58), 10–13 hours (median 0.8 μg/mL, range 0.2–1.7 μg/mL; n = 21) and 18–24 hours (median 0.5 μg/mL, range 0–1 μg/mL; n = 13); none were taken between 13–18 hours. Seven of the 92 raw trough values were greater than the maximum desired trough value of 2 μg/mL (median 2.9 μg/mL, range 2.2–6.5 μg/mL), which were all taken between 7 hours 20 minutes and 8 hours 40 minutes after the gentamicin administration. All extrapolated or actual 22 hour troughs in all dose groups were <2 μg/mL, with a maximum trough value of 1.1 μg/mL. Median trough values were not significantly different between dose groups (P = .600).

Values for peak creatinine concentration and Δcreatinine were available for 21 horses: 8 and 13 in the low and medium dose groups, respectively. Creatinine values were recorded for the initial dose group to which the horse was assigned. Therefore, as all horses in the high dose group initially received lower doses, their creatinine values were recorded against the initial dose administered and no creatinine parameters were recorded in this group. Median (range) Δcreatinine was 0.0 (0.0–1.1) mg/dL for the low dose group and 0.0 (0.0–0.2) mg/dL for the medium dose group. There was no significant relationship between peak creatinine (P = .957) or Δcreatinine (P = .956) and dose group, and no correlation between Δcreatinine and dose (Spearman's ρ = 0.013, P = .954). In 18/21 cases for which more than one creatinine value was recorded, peak creatinine was the value taken at admission. Only two horses had an increase in creatinine that exceeded the upper limit of the reference range of 1.6 mg/dL; one in each of the low and medium dose groups. In the low, medium and high dose groups, respectively, 42% (10/24), 60% (25/42) and 55% (6/11) had systemic disease. Horses with systemic disease had significantly lower peaks (Fig 3) (P = .039, Somers' D coefficient −0.25, 95% CI −0.49 to 0.01). There was no association between focal or systemic disease and trough (P = .60, Somers' D coefficient 0.04, 95% CI −0.203 to 0.118).

Discussion

These results show that in hospitalized horses, a once-daily IV gentamicin dose of ≥7.7 mg/kg is more likely to result in a peak of >32 μg/mL, and that the 6.6 mg/kg dose was generally inadequate to reach that target peak concentration. The clinical relevance of the difference in mean peak values (32.6 versus 27.2 μg/mL) between these doses is unknown. This is in contrast to previous work, where a once-daily IV dose of 6.6 mg/kg in horses that had undergone abdominal surgery was documented to result in plasma gentamicin concentrations >32 μg/mL.[9] It has also been reported
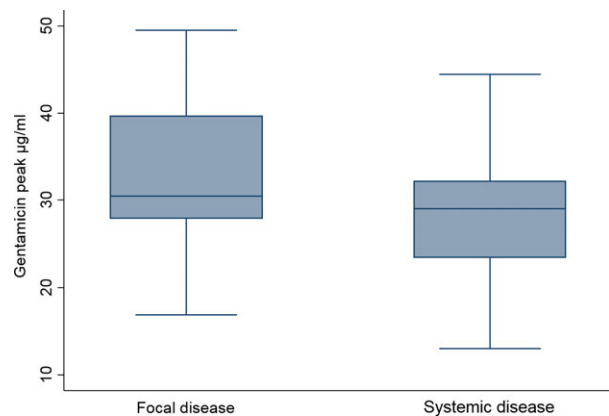
Fig 3. Effect of focal versus systemic disease on plasma gentamicin peak values. Boxes represent the interquartile ranges from the 25th to the 75th percentiles. Horizontal lines within the boxes indicate median values; whiskers represent the 10th (lower whiskers) and 90th (upper whiskers) percentiles. Horses with focal disease that were considered systemically healthy had significantly higher peaks than those with systemic disease (P = .039).

that a calculated IV dose of 6.8 mg/kg could be predicted to achieve optimum plasma concentrations if an MIC of ≤4 μg/mL was used, and mean plasma concentrations of 40.71 μg/mL were achieved 20 minutes after intravenous administration of a 6.6 mg/kg IV dose.[9] Gentamicin peak concentrations are classically obtained 30–60 minutes after dosing,[16] when the distribution phase ends and the elimination phase begins. As peak concentrations can change rapidly during the distribution phase, and because of the difference in sampling time points, no direct comparisons can be made between peak concentrations in this and other studies.

The 25–35 minute post-gentamicin administration range for sampling of peaks may have meant some peaks were taken within the distribution phase. As only peak and trough samples were taken, the exact point on the distribution/elimination curve that each individual horse was at the time of peak sampling cannot be determined. It should also be noted that the ideal time for peak sampling for TDM of gentamicin in horses is unknown, therefore the 25–35 minute sampling timeframe might not be ideal. However, only including peaks taken within a short timeframe allows for some consistency, enabling comparison of peak data.

The peak range of 32–40 μg/mL is based on a target peak concentration of 8–10× the MIC of 4 μg/mL, rather than the lower target peak range of 16–20 μg/mL based on an MIC of ≤2 μg/mL. While the MIC for veterinary pathogens has been reported as being ≤4 μg/mL, recent guidelines from the Clinical Laboratory Standards Institute (CLSI) document MIC of susceptible equine pathogens as ≤2 μg/mL, with the MIC for intermediately susceptible pathogens being 4 μg/mL.[11,17,18] Therefore, it should be noted that the target peak range used in our study is higher than that recommended based on the CLSI guidelines, and that lower doses are likely adequate to reach this lower target peak range. After Streptococcus species, gram negative pathogens are the most common isolates from hospitalized

horses.[19] While gentamicin remains a common first line antimicrobial, there are relatively high proportions (26–50%) of common gram negative equine pathogens reported as not susceptible to gentamicin.[20–22] Not all of these studies used broth microdilution methods in which an MIC was determined, making comparison difficult. However, in one study using broth microdilution,[22] the breakpoint used was 4 μg/mL. Therefore, resistance might even be problematic at higher concentrations. In another study, 30% of *Actinobacillus suis* isolates, the most commonly isolated gram negative pathogen in the population sampled, were not susceptible to gentamicin.[23] This lack of susceptibility ranged up to 44% for other gram negative isolates reported in that study.[23] Again, the disk diffusion method was used, making comparison to MIC difficult. In some populations there is evidence of increasing resistance to gentamicin in commonly isolated equine pathogens.[24] Therefore, where pathogens might be expected to have an MIC that falls between the susceptible and resistant breakpoints, the intermediate susceptibility MIC breakpoint may be used to determine desired target plasma peak concentration.[25] However, knowledge of resistance patterns of expected nosocomial organisms or culture and susceptibility testing should be used to guide perioperative antimicrobial choice and individual treatment decisions. Because of the risk of treatment failure with aminoglycoside monotherapy, specific studies evaluating the ability of high doses of aminoglycosides to prevent resistance in clinical patients are lacking.[26]

All TDM data in the high dose group came from horses that had TDM previously performed which indicated need for dose increase. The high dose group therefore most likely comprised horses that had higher volumes of distribution or greater clearance. The medium and high dose groups in this study were combined posthoc into one group of TDM values resulting from doses of ≥7.7 mg/kg. Despite the high dose group comprising entirely of second or subsequent TDM values, combining these groups was considered justified because of the data in the 2 groups being extremely similar, indicating these groups were better represented by combining them for analysis.

Variable peaks within dose groups (especially the low and medium dose groups) highlight the need to perform TDM on each patient when confirmation of peak concentrations is indicated. This is supported by results from the high dose group (consisting entirely of horses with an upwards dose adjustment after a peak of <32 μg/mL obtained by previous TDM). The much smaller standard deviation for peak concentration of this group indicates that TDM allows more accurate dose determination, providing more predictable peak concentrations for specific patients. In human patients receiving aminoglycoside antimicrobials in intensive care units, TDM is considered essential because of decreased probability of these patients to achieve therapeutic concentrations at initial doses given.[3,6,27,28] The low proportion of peak concentrations >32 μg/mL in our study suggests this applies to our population of equine patients. Similar results were previously found in systemically ill horses

treated with gentamicin, however, these studies evaluated the older 6–12 hourly dosing regimens.[7,8]

Despite the low percentage of peaks that were >32 μg/mL, TDM did not always elicit a dose adjustment. While the majority of TDM results where the peak was <32 μg/mL were followed by a dosage adjustment, 31% of cases had no action taken. Therefore, sometimes clinicians do not make dose adjustments despite peak values being below the target range. This may be caused by a perceived clinical improvement at the current dose, and that TDM was performed primarily to ensure adequate renal clearance. Horses in the low dose group had significantly shorter treatment duration compared to those in the medium and high dose groups. For horses with TDM performed more than once, the highest dose group they qualified for was used for this analysis. Therefore, this difference is not simply caused by the horses in the low dose group moving up to a higher dose group after TDM was performed. It is possible that shorter treatment duration in the low dose group was because of a perceived lack of response to treatment after which antimicrobial class was changed, or because this group comprised a smaller percentage of horses with systemic disease, and may therefore have been changed to an oral antimicrobial or discontinued antimicrobial treatment sooner.

No effect of gentamicin dose was seen on either highest creatinine value or Δcreatinine, although available creatinine data were limited. A major limitation is the small number of horses that had more than one creatinine value collected from the medical record for evaluation of Δcreatinine during gentamicin treatment. As most creatinine values were taken at admission, often before gentamicin treatment being started, peak creatinine was not considered to be affected by gentamicin treatment. Time between initial creatinine and subsequent creatinine values was not collected and this further limits interpretation of Δcreatinine results. Because of low numbers, this study is likely underpowered to detect a difference. More sensitive markers of nephrotoxicity such as urine GGT : creatinine ratio[1] were not evaluated and therefore subtle indications of nephrotoxicosis may have been missed. However, an increased urine GGT:creatinine ratio does not necessarily indicate gentamicin treatment requires alteration, therefore its clinical usefulness in this situation is questionable as it may be too sensitive.[29] Further limiting the study was that creatinine values for 3 horses were split across dose groups. These horses had TDM performed more than once, after which dose adjustments were made and TDM repeated. The creatinine values for these horses were assigned to the dose group for the first dose of gentamicin administered, as most commonly the highest creatinine value obtained was on admission. It must be emphasized that no inference on safety of gentamicin doses >6.6 mg/kg can be made from this study because of the small number of horses for which Δcreatinine was recorded, and the relatively short duration of treatment for many of these horses.

Increases in creatinine concentrations occurred in 2 horses. The first was a 4 month-old foal in the low dose

group whose creatinine increased from 1.1 to 2.2 mg/dL. This foal, considered systemically healthy at the time of TDM, developed antimicrobial-associated colitis which coincided with the creatinine increase. Antimicrobial treatment was then discontinued. The increase in creatinine may have been pre-renal because of effects of colitis, caused by gentamicin treatment, or both. The second horse was in the medium dose group and had a creatinine increase from 1.6 to 1.7 mg/dL. This horse was receiving other nephrotoxic drugs (phenylbutazone and regional limb perfusion with amikacin) and was also systemically healthy. The increase was considered clinically insignificant as the creatinine decreased to 1.3 mg/dL before discontinuation of gentamicin.

All 22 hour troughs were below the maximum target trough value of 2 µg/mL, and were not significantly different between dose groups. Troughs measured before 22 hours were extrapolated to a 22 hour value to allow comparison of values. It is possible that calculated troughs underestimate the actual value, because extrapolation from two data points implies a single compartment pharmacokinetic model and ignores the potential effect of multi-compartment kinetics. Specifically, our data points did not take third compartment accumulation into consideration. Therefore, effects of higher doses on renal function cannot be determined from this study. Low 22 hour values are expected with once-daily administration, because typically >10 half-lives of gentamicin will elapse during the intertreatment interval.[30,31] However, there is no consensus of how low troughs should be. In the human and veterinary literature, recommended trough concentrations range from <0.5–2 µg/mL.[1,13,32] Therefore, whether all troughs of <2 µg/mL are adequately low should be interpreted with caution, as actually some troughs, although <2 µg/mL, may be increased and indicative of inadequate renal clearance. Troughs from horses with normal renal function tend to be close to zero at 22–24 hours after gentamicin administration.[5] Further studies are required to determine an appropriate maximum trough concentration in horses.

A significant effect of focal or systemic disease on peak gentamicin concentration was evident. Previous studies in horses investigating gentamicin pharmacokinetics given at 6–12 hour intervals in systemically ill horses, found horses defined as septic frequently required dosage adjustments.[7,8] The need for TDM and dose adjustments in systemic illness is also emphasized in human medicine. As previously mentioned, TDM of aminoglycosides in human intensive care units is considered essential.[3,6,27,28,33] Further, human studies have shown that volume of distribution in septic patients changes as disease state changes.[33] In our study, volume of distribution was not determined, but no difference in this or other kinetic parameters was seen between septic and healthy equine patients in another study.[8] However, volume of distribution significantly decreased in healthy horses administered endotoxin.[34] Ideally, a control group of healthy horses administered gentamicin would have been included; however, the retrospective nature of the study precluded this. Our results emphasize the need for TDM in hospitalized equine patients, especially those with systemic disease.

The absence of equine validation data for the two analyzers is an obvious further limitation of the study. Validation data for analyzer 1 were not available because of the age of the machine, and has not been retained by our laboratory; however, was validated by the manufacturer at the time it was being used. Analyzer 2 is validated for human serum. While equine plasma validation data are absent, results obtained from this analyzer are considered to be clinically relevant by multiple clinicians requesting gentamicin concentrations in equine patients. Further, the analyzer provides consistent acceptable results for accuracy and precision for samples run through compliance with the College of American Pathologists' quality assurance program.

Concurrent administration of gentamicin and nonsteroidal anti-inflammatory drugs (NSAIDs) was not examined. Phenylbutazone can cause changes in the rate and extent of distribution and elimination of gentamicin in horses[35]; however, the dose used in that study (2.2 mg/kg) was low compared to doses in the current study, and also to the dose now commonly used clinically. Most horses included in our study had a condition that would typically require NSAID administration, and likely received NSAIDs. Concurrent administration of gentamicin and NSAIDs is typical in hospitalized horses; therefore, the results of our study are still applicable to general hospitalized populations.

There was a significant difference in age and sex between dose groups; however, once the medium and high dose groups were combined the difference in age was no longer significant. The difference in sex between the dose groups was unexpected. It is difficult to determine the clinical importance of sex on gentamicin TDM.

In conclusion, using IV gentamicin doses of ≥7.7 mg/kg in hospitalized horses is more likely to achieve plasma peak concentrations >32 µg/mL. Individuals show great variation in peaks achieved from the dose given, making gentamicin TDM advisable for individual horses where possible. Further safety studies are required to determine whether IV gentamicin doses >6.6 mg/kg have substantial renal or other toxic effects. The effect of focal versus systemic disease on the pharmacokinetics of once-daily IV dosing of gentamicin requires further investigation; however, systemically ill horses might benefit most from TDM when receiving gentamicin.

Footnotes

References

1. Dowling PM. Aminoglycosides. In: Giguère S, Prescott JF, Dowling PM, eds. Antimicrobial Therapy in Veterinary Medicine, 5th ed. Ames, IA; Hoboken: Wiley; 2013:233–256.

2. Magdesian KG, Hogan PM, Cohen ND, et al. Pharmacokinetics of a high dose of gentamicin administered intravenously or intramuscularly to horses. J Am Vet Med Assoc 1998;213:1007–1011.

3. Watling SM, Dasta JF. Aminoglycoside dosing considerations in intensive care unit patients. Ann Pharmacother 1993;27:351–357.

4. Plumb DC. Plumb's Veterinary Drug Handbook, 7th ed. Ames, IA; USA: Wiley-Blackwell; 2011.

5. Godber LM, Walker RD, Stein GE, et al. Pharmacokinetics, nephrotoxicosis, and in vitro antibacterial activity associated with single versus multiple (three times) daily gentamicin treatments in horses. Am J Vet Res 1995;56:613–618.

6. Rea RS, Capitano B, Bies R, et al. Suboptimal aminoglycoside dosing in critically ill patients. Ther Drug Monit 2008;30:674–681.

7. Sojka JE, Brown SA. Pharmacokinetic adjustment of gentamicin dosing in horses with sepsis. J Am Vet Med Assoc 1986;189:784–789.

8. Sweeney RW, Divers TJ, Rossier Y. Disposition of gentamicin administered intravenously to horses with sepsis. J Am Vet Med Assoc 1992;200:503–506.

9. Tudor RA, Papich MG, Redding WR. Drug disposition and dosage determination of once daily administration of gentamicin sulfate in horses after abdominal surgery. J Am Vet Med Assoc 1999;215:503–506.

10. Moore RD, Lietman PS, Smith CR. Clinical response to aminoglycoside therapy: Importance of the ratio of peak concentration to minimal inhibitory concentration. J Infect Dis 1987;155:93–99.

11. Watts JL. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Approved Standard, 3rd ed. Wayne, PA: CLSI; 2008.

12. Dowling PM. Pharmacologic principles. In: Reed SM, Bayly WM, Sellon DC, eds. Equine Internal Medicine, 3rd ed. Mayland Heights: Elsevier/Saunders; 2010:148–204.

13. Plajer SM, Chin PK, Vella-Brincat JW, et al. Gentamicin and renal function – Lessons from 15 years experience of a pharmacokinetic service for extended interval dosing of gentamicin. Ther Drug Monit 2015;37:98–103.

14. Welling PG. Graphic methods in pharmacokinetics: The basics. J Clin Pharmacol 1986;26:510.

15. Allison P. Logistic Regression Using SAS: Theory and Application, 2nd ed. Cary, NC: SAS Institute, Inc.; 2012.

16. Papich MG. Therapeutic drug monitoring. In: Riviere JE, Papich MG, eds. Veterinary Pharmacology and Therapeutics, 9th ed. Ames, IA, USA: Wiley-Blackwell; 2009:1323–1330.

17. Amsterdam D. Susceptibility testing of antimicrobials in liquid media. In: Lorian V, ed. Antibiotics in Laboratory Medicine, 4th ed. Baltimore: The Williams & Wilkins Co.; 1996:61–143.

18. Dowling PM. Aminoglycocides. In: Giguère S, Prescott JF, Dowling PM, eds. Antimicrobial Therapy in Veterinary Medicine, 4th ed. Ames, IA: Blackwell; 2006:207–229.

19. Lavoie JP, Couture L, Higgins R, et al. Aerobic bacterial isolates in horses in a university hospital, 1986-1988. Can Vet J 1991;32:292–294.

20. Russell CM, Axon JE, Blishen A, et al. Blood culture isolates and antimicrobial sensitivities from 427 critically ill neonatal foals. Aust Vet J 2008;86:266–271.

21. Orsini JA, Benson CE, Spencer PA, et al. Resistance to gentamicin and amikacin of gram-negative organisms isolated from horses. Am J Vet Res 1989;50:923–925.

22. Wilson WD. Rational selection of antimicrobials for use in horses. In: Proceedings of the 47th AAEP Annual Convention, San Diego: American Association of Equine Practitioners; 2001:75–93.

23. Clark C, Greenwood S, Boison JO, et al. Bacterial isolates from equine infections in western Canada (1998-2003). Can Vet J 2008;49:153–160.

24. Theelen MJ, Wilson WD, Edman JM, et al. Temporal trends in in vitro antimicrobial susceptibility patterns of bacteria isolated from foals with sepsis: 1979-2010. Equine Vet J 2013;1:12130.

25. Papich MG. Antimicrobials, susceptibility testing, and minimum inhibitory concentrations (MIC) in veterinary infection treatment. Vet Clin North Am Small Anim Pract 2013;43:1079–1089.

26. DeRyke CA, Su Young L, Kuti JL, et al. Optimising dosing strategies of antibacterials utilising pharmacodynamic principles: Impact on the development of resistance. Drugs 2006;66:1–14.

27. Bartal C, Danon A, Schlaeffer F, et al. Pharmacokinetic dosing of aminoglycosides: A controlled trial. Am J Med 2003;114:194–198.

28. Buijk SE, Mouton JW, Gyssens IC, et al. Experience with a once-daily dosing program of aminoglycosides in critically ill patients. Intensive Care Med 2002;28:936–942.

29. Rossier Y, Divers TJ, Sweeney RW. Variations in urinary gamma glutamyl transferase/urinary creatinine ratio in horses with or without pleuropneumonia treated with gentamicin. Equine Vet J 1995;27:217–220.

30. Mingeot-Leclercq M-P, Tulkens PM. Aminoglycosides: Nephrotoxicity. Antimicrob Agents Chemother 1999;43:1003–1012.

31. Verpooten GA, Giuliano RA, Verbist L, et al. Once-daily dosing decreases renal accumulation of gentamicin and netilmicin. Clin Pharmacol Ther 1989;45:22–27.

32. Ismail R, Haq AH, Azman M, et al. Therapeutic drug monitoring of gentamicin: A 6-year follow-up audit. J Clin Pharm Ther 1997;22:21–25.

33. Triginer C, Izquierdo I, Fernandez R, et al. Gentamicin volume of distribution in critically ill septic patients. Intensive Care Med 1990;16:303–306.

34. Wilson RC, Moore JN, Eakle N. Gentamicin pharmacokinetics in horses given small doses of *Eschericia coli* endotoxin. Am J Vet Res 1983;44:1746–1749.

35. Whittem T, Firth EC, Hodge H, et al. Pharmacokinetic interactions between repeated dose phenylbuatzone and gentamicin in the horse. J Vet Pharmacol Ther 1996;19:454–459.