PHARMACOKINETICS OF AMIKACIN AFTER A SINGLE INTRAVENOUS DOSE

AND RESULTANT CONCENTRATIONS IN BODY FLUIDS OF THE HORSE

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

_____

Nelson Pinto

Certificate of Approval:

Jennifer Taintor Assistant Professor Clinical Sciences	John Schumacher, Chair Professor Clinical Sciences
Sue Duran Professor Clinical Sciences	George T. Flowers Dean Graduate School

PHARMACOKINETICS OF AMIKACIN AFTER A SINGLE INTRAVENOUS DOSE AND RESULTANT CONCENTRATIONS IN BODY FLUIDS OF THE HORSE

Nelson Pinto

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
August 10, 2009

PHARMACOKINETICS OF AMIKACIN AFTER A SINGLE INTRAVENOUS DOSE

AND RESULTANT CONCENTRATIONS IN BODY FLUIDS OF THE HORSE

Nelson Pinto

_____

Signature of Author

_____

Date of Graduation

THESIS ABSTRACT

PHARMACOKINETICS OF AMIKACIN AFTER A SINGLE INTRAVENOUS DOSE AND RESULTANT CONCENTRATIONS IN BODY FLUIDS OF THE HORSE

Nelson Pinto

Master of Science, August 10, 2009
(DVM, Universidad Nacional de Colombia, 2000)

Typed Pages

Directed by John Schumacher

Amikacin, an aminoglycoside antibiotic, has been used extensively in humans and other animals to treat infections produced by Gram-negative bacteria. The pharmacokinetics of amikacin has been studied in foals, but because of its high cost it has not been studied in adult horses. The present study was designed to determine if a single intravenous dose of amikacin (10 mg/kg) will reach therapeutic concentrations in plasma of adult horses (n=6) and to evaluate the concentration of amikacin in synovial, peritoneal, and interstitial fluid with the same dosage.

Serum concentrations of amikacin averaged 115.93 ± 8.254 μg/ml at three minutes, and after 60 minutes the mean concentration was 39.311 ± 5.052 μg/ml. The area under the curve (AUC) was 139.34 ± 34.02 μg*h/ml; the elimination half-life ($t_{½\beta}$) was 1.346 ± 0.4 hours; the total body clearance was 1.251 ± 0.281 ml/min/kg, and the

mean residual time (MRT) was 1.814 ± 0.5 hours. The concentration of amikacin in serum for all horses was below the minimum detectable concentration for the assay used at 24 hours.

The mean maximum concentration (C_{max}) of amikacin after a single intravenous injection was 19.78 ± 7.14 μg/ml and 21.44 ± 4.39 μg/ml for synovial and peritoneal fluid, respectively. The time to achieve maximum concentration (T_{max}) was 65 ± 12.24 minutes for synovial fluid and 115 ± 12.24 minutes for peritoneal fluid. The area under the curve to the infinite ($AUC_{0-\infty}$) was 100.02 ± 39.89 μg*h/ml for synovial and 139.99 ± 25.88 μg*h/ml for peritoneal fluid. Amikacin in the interstitial fluid reached a mean C_{max} of 10.82 ± 5.33 μg/ml, and after 24 hours the mean concentration was 3.316 ± 1.69 μg/ml.

Based on the results of the pharmacokinetic analysis, it will appear that a single dose of amikacin (10 mg/kg) in adult horses would be therapeutic in infections caused by susceptible bacteria with a minimum inhibitory concentration (MIC) of ≤8 μg/ml. Due to individual variation, close drug monitoring is recommended.

ACKNOWLEDGMENTS

The author would like to thank Drs. John Schumacher, Jennifer Taintor, and Sue Duran for their mentorship throughout this project. Special thanks to Dr. Dawn Boothe and Dr. Fred DeGraves for their advice and support.

I cannot thank enough Mary, who gave me the courage and the inspiration to accomplish this challenge. To my parents, Olga and Alirio, thanks for the freedom and all the love.

Without the collaboration of the following individuals this project could not be finished: Debie Burelle, Tiffany Finch, Dave Storrs, Michael Owen, Susan Agnew, Sam Thrower, Heather Edwards, Kelly Smith, Julie Dorough, and Michelle Brown.

Style manual of journal used: Equine Veterinary Journal

Computer software used: Microsoft Word for Windows XP

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. INTRODUCTION

Bacterial infections are common causes of disease in adult horses and affect the respiratory, digestive, and musculoskeletal systems more commonly than other systems. Broad spectrum antimicrobial therapy is aimed as treatment of infection caused by Gram-positive, Gram-negative, and anaerobic microorganisms. The most common bactericidal antimicrobial drugs used to treat horses with infection caused by Gram-negative bacteria are the fluorquinolones and aminoglycosides. The aminoglycoside most commonly administered to equine patients is gentamicin sulfate, but amikacin, streptomycin, tobramycin, or neomycin are occasionally administered. Several studies have evaluated the pharmacokinetics of fluorquinolones and gentamicin in both adult horses and neonates [44-50], but little is known about the pharmacokinetics of amikacin in the adult horse.

Several studies described the pharmacokinetics of intravenous amikacin in normal neonates and neonates suffering from conditions such as hypoxia, azotemia, or sepsis [36-41]. It has been shown that amikacin dosed at 21 mg/kg [41] and 25 mg/kg [39] once daily provides serum concentrations ten times the minimum inhibitory concentration (MIC) for most Gram-negative equine pathogens and reduces the potential for aminoglycoside-induced nephrotoxicity in neonates .

Intravenous dosage of amikacin in adult horses has been evaluated in only two studies. The results of those studies showed that at 6.6 mg/kg [42] and 6 mg/kg [43], amikacin maintained concentration above the MIC for most equine pathogens (4 ug/mL) for 8 hours, suggesting that repeated doses every 8 [43] or 12 [42] hours would be therapeutic.

More recently it was recognized that aminoglycosides are concentration-dependent antibiotics with a prolonged postantibiotic effect (PAE) indicating that high doses administered once a day are effective and less nephrotoxic [41].

Currently amikacin is recommended for use in the adult horse at the once daily dose recommended for foals, making treatment of adult horses cost prohibitive for many owners. Based on pharmacokinetic data collected while treating several adult horses with amikacin at the currently recommended dose, it was suspected that an effective dose of amikacin in adult horses may be much lower than that of foals (Pinto, unpublished data).

The objectives of this study were to: 1) describe the plasma concentration-time profiles after intravenous administration of amikacin; 2) describe pharmacokinetic parameters after administration of amikacin, and 3) determine the concentration of amikacin in synovial, peritoneal, and interstitial fluid following intravenous administration.

II. LITERATURE REVIEW

History

Aminoglycoside antibiotics were first derived from *Streptomyces* bacteria in 1944. Since then other antibiotics in this family have been isolated from different species of *Streptomyces* bacteria or produced synthetically. Streptomycin, which came from *Streptomyces griseus,* was the first aminoglycoside discovered and five years later neomycin was isolated from *Streptomyces fradiae*. In 1957 kanamycin was isolated from *Sreptomyces kanamyceticus*. Gentamicin was isolated from *Micromonospora purpurea* in 1963. In 1967 tobramycin was produced from *Streptomyces tenebrarius*. Amikacin was produced in 1972 as a semisynthetic derivate of kanamycin [1].

The aminoglycoside structure consists of two or more amino sugars attached by glycosidic bonds to an amino-cyclitol nucleus that can be streptidine or 2-deoxystreptamine [1, 2]. Chemically, aminoglycosides are basic, polar molecules, highly soluble in water, and not lipid soluble. With these characteristics they cannot cross the blood-brain barrier nor are they absorbed through the intestine. Their antibacterial capacity is enhanced in basic pH rather than in acidic conditions. The aminoglycosides are positively charged, which allow them to bind negative molecules such as

lipopolysaccharide in the bacterial cell wall and other intracellular molecules such as DNA, RNA, and phospholipids, thus facilitating their mechanism of action [2].

Mechanism of Action

The aminoglycosides are bactericidal because they have the ability to impair the protein synthesis and change the integrity of the bacterial cell membrane. The uptake of aminoglycoside by bacteria involves several steps: 1.) The aminoglycoside initially binds to negative charged molecules in the outer membrane of Gram-negative bacteria and then diffuses through the outer cell membrane of growing bacteria. 2.) The phase I transport system allows the aminoglycoside to cross the inner cytoplasm. The affinity between the phase I transport system and the drug is low and can be blocked by ions such as magnesium and calcium, hyperosmolarity, anaerobic conditions, and low pH [1, 2].

In the cytoplasm the drug binds to a site of high affinity, the 30S ribosomal subunit in the cytosol through the phase II transport system [1, 2]. By binding to the ribosomal 30S the proofreading processes for production of protein are impaired which causes inaccurate translation of protein rather than stopping the protein synthesis. As a result, defective proteins are inserted in the cellular membrane of the bacterium leading to changes in permeability which allows more aminoglycoside to enter the cell.

The ribosome is a very complex structure with three RNA molecules and more than fifty proteins; it has been suggested that aminoglycosides can bind to more than one site in that complex structure [15]. As a consequence the exact mechanisms of action of aminoglycosides are still under investigation. The specific interaction of the

aminoglycoside with the 16S rRNA has been studied most. In this interaction the aminoglycoside binds to the A-site of the 16S rRNA in the decoding region where it affects the translation process by altering the recognition of tRNA by rRNA [16] and by the stabilization of the complex mRNA and tRNA [17].

Rapidly induced damage to the outer cell membrane of *Pseudomonas spp*. independent to its ribosomal action has been reported for gentamicin [18]. Other disturbances within bacteria caused by aminoglycosides include changes in cellular concentration of ions and abnormal synthesis of DNA and RNA [2].

Dosing

Proper use of an antibiotic demands the understanding of specific characteristics of the drug and the relationship between the drug, the patient, and the pathogen. Characteristics of the patient such as immune status, organ functions, and body weight are very important considerations. Characteristics of the bacteria to consider include its pattern of susceptibility to antibiotics and ability to generate antibiotic resistant subpopulations. Other considerations for selection of an antibiotic include antibacterial activity, clinical efficacy, tissue penetration, protein binding, metabolism, elimination, and potential for drug interactions. Toxic effects of the antibiotic on the patient must be evaluated as well [8].

The length of time of bacterial exposure to an antibiotic and the concentration of that antibiotic in relation to the minimum inhibitory concentration (MIC) for that particular bacteria are very important variables when considering the choice of antibiotic

for therapy. Based on those two factors, antibiotics can be classified into two groups: concentration-dependant and time-dependant [6, 7].

For antibiotics that have concentration-dependent activity, the concentration of antibiotic and the bacterial killing rate are directly related. Therefore, the most important pharmacokinetic/pharmacodynamic index is the antibiotic concentration/MIC ratio; for aminoglycosides it is the maximum concentration (Cmax)/MIC ratio and for fluorquinolones, azithromycin, ketolides, daptomycin, and linezolid it is the area under the curve (AUC)/MIC ratio [6, 7].

Aminoglycosides likely have a two-phase mechanism of action. The first phase is rapid killing of bacteria, which depends directly on the maximum concentration, and the second phase is slower killing of bacteria, which is independent of the concentration. This second phase is also known as the postantibiotic effect (PAE) [4].

Postantibiotic effect is the suppression of bacterial growth by exposure of bacteria to antibiotic concentrations below the MIC. The PAE is affected by factors that include type of bacteria, class and concentration of the antibiotic, length of antimicrobial exposure, and presence of antimicrobial combinations [5, 6, 8]. Aminoglycosides cause ribosomal damage resulting in a prolonged PAE which, for amikacin and gentamicin, allows an effective once-daily dosing of these drugs [4]. Aminoglycosides have PAE for both Gram-positive and Gram-negative bacteria.

There is also a direct relationship between concentration of antibiotic and length of the PAE. The reported duration of the PAE for aminoglycosides varies between 0.5 to 10 hours depending on the Cmax/MIC ratio and the bacterial species targeted [5, 8].

Clinical and pharmacological characteristics of the aminoglycosides such as the concentration-dependent bactericidal activity, extended PAE, decreased risk of adaptive resistance, and diminished accumulation of the aminoglycoside in renal tubules and inner ear make the high once-daily dose a superior alternative to the low multi-daily dose [4, 9, 12, 13]

In summary, the goal of therapy with aminoglycosides is to achieve the highest peak concentration necessary for bacterial death without toxic effects of the aminoglycoside on the patient. Several studies have shown that higher concentrations of aminoglycosides in plasma are correlated with increase in survival and improved clinical response in people with Gram-negative sepsis and pneumonia [5-8]. Resolution of infection and significant decrease in selection and regrowth of resistant subpopulations of bacteria present in the initial inoculum have been associated with Cmax/MIC ratios ranging from 6 to 10 [4-8]. People treated for common infections with gentamicin, amikacin, or tobramycin at doses that reached peak concentrations 10 times above the MIC had reduced the length of treatment by 3 to 4 days for common infections [19, 51].

Advantages of the single daily dose include: 1) increased efficacy in the treatment of many types of infection (intra-abdominal, respiratory, genitourinary, skin, and staphylococcal and streptococcal endocarditis) in people [5]. 2) No measurable serum concentration of aminoglycoside in patients with normal renal function in a period of seven to eight hours, thus decreasing the accumulation of aminoglycoside in the kidney [6, 8, 9, 52]. 3) Minimizes cost, simplifies administration, and improves prognosis [9].

Resistance

The mechanisms by which bacteria resist the effect of aminoglycosides include: 1) reduction of the intracellular concentration of the drug by efflux pumps in the bacterial cell membrane, decreased inner membrane transport, and drug trapping, 2) changes in the molecular target, the 30s ribosomal subunit, mediated by gene mutation or substitution for an exogenous gene, 3) enzymatic inactivation of the aminoglycosides by acetyltransferases, nucleotidyltransferases, and phosphotranferases, 4) methylation of the aminogycoside binding site [2, 3]. The development of resistance to an aminoglycoside requires long periods of exposure or large inoculums of organisms and is less common than bacterial resistance to third-generation cephalosporins [2].

Toxicity

Aminoglycosides are known to be nephrotoxic, ototoxic, and possibly toxic to the retinal epithelium. Nephrotoxicity caused by aminoglycosides develops after several days of constant exposure of the kidney to the drug and is the consequence of tubular and glomerular damage [11]. Aminoglycoside-induced nephritis is seen clinically as non oliguric renal failure, characterized by an increase in serum creatinine along with hypoosmolar urine [10].

After being filtrated by the glomeruli, the aminoglycosides are attached to acidic phospholipids, which are abundant in the brush border of the tubular epithelial cells. The aminoglycoside is then transferred to a transmembrane protein, the megalin, and together

they are internalized in endosomes [10]. The megalin has the capacity to bind to polar molecules and is found in different epithelia such as renal tubular, retinal and inner ear [10, 11, 13]. Megalin appears to be responsible for the uptake of aminoglycosides in those cellular lines [10]. The megalin transport system can be saturated by gentamicin, amikacin and netilmicin, but not tobramicin [10]. Evidence of the saturability feature of the system is seen when a large dose of the drug is administered once daily and a lower percentage of the total dose enters the renal cell, in contrast with lower doses administered multiple times a day which leads to a higher intracellular concentrations in the renal tubules [12].

Within the epithelial cell the aminoglycoside accumulates in the endosomal and lysosomal vacuole and within the Golgi complex. Within lysosomes, aminoglycosides induce the accumulation of myeloid bodies (polar lipids) by inhibition of phospholipases and aggregation of lipid bilayers. Neither of those events explain the epithelial cell death but they correlate with the nephrotoxic potential of aminoglycosides. Clinicopathologic signs of renal tubular dysfunction include formation of urinary casts, an increased concentration in urine of enzymes released from the brush border, and lysozymes of tubular cells. There is also an increase in protein, glucose, phospholipids, and electrolytes in urine caused by decreased reabsorption of these substances after filtration. These urinary changes precede and accompany the presence of myeloid bodies, which contain phospholipids and proteins [10]. Accumulation of the drug (5%-10) occurs in the epithelial lining of the proximal tubule after filtration by the glomerule [10-13].

Different hypothesis have been presented to explain the necrosis of renal tubular cells caused by administration of an aminoglicoside: 1) Toxicity occurs in direct relation

to concentration of the aminoglycoside in the renal tubular cell, and lysosomal changes are responsible for the toxicity, 2) Aminoglycosides become toxic when lysosomes release their contents after undergoing structural changes or when threshold concentrations of aminoglycosides within lysosomes have been reached, 3) Aminoglycosides stored in lysosomes are non-toxic, but the aminoglycoside that reaches non-lysosomal targets can induce toxicity [10].

The glomerular toxicity of the aminoglycosides is a consequence of mesangial cell contraction, leading to changes in glomerular filtration, and stimulation of mesangial cell proliferation and apoptosis. These changes are believed to be mediated by an increase within the mesangial cell of calcium, phospholipase A2, platelet-activator factor and thromboxane A2 [11].

The nephrotoxicity of aminoglycosides varies: neomycin > framycetin = paramomycon > gentamicin > sisomycin = amikacin = kanamycin > tobramycin > netilmicin [1, 12]. Risk factors for nephrotoxicity include type of aminoglycoside administered, duration of therapy, administration route and dosing schedule, total aminoglycoside dose, sepsis, hypotension, dehydration, hypovolemia, and concurrent administration of other nephrotoxic drugs [1, 12].

Aminoglycosides can induce permanent bilateral hearing loss (high-frequency sensorineural) and temporary vestibular hypofunction. They cause ototoxicity by damaging the hair cells in the cochlear and in the apex of the cristae and striolar regions and eventually damaging hair cells in the periphery of the vestibular receptor [1, 14]. Ototoxic aminoglycosides include amikacin, streptomycin, gentamicin, tobramycin, and

kanamycin. The nephrotoxicity and ototoxicity effects of aminoglycosides are independent.

Bacterial Susceptibility to Aminoglycosides

Bacteria sensitive to the effects of aminoglycoside antibiotics include Gram-negative aerobic bacilli, Gram-positive cocci (Staphylococci), and some species of mycobacteria. Anaerobic bacteria are resistant to the effect of aminoglycosides due to failure in the transport of the antibiotic [54].

For treatment of people, aminoglycosides are chosen for their potent antimicrobial capacity in a concentration dependant manner, low cost, chemical stability, lack of allergic reaction, synergistic action with other antibiotics (especially true for some Gram-positive bacteria), and activity towards a large number of Gram-negative aerobes. Amikacin, which is structurally different from the other aminoglycosides, is often effective in treatment of bacterial infections even when the bacteria are resistant to other aminoglycosides [2, 5].

Bacterial Infection in Adult Horses and Neonates

Infectious diseases caused by bacteria are probably the most important cause of morbidity and mortality in horses, and these diseases have been reported to involve all body cavities and systems. Many studies have looked at the most common bacterial

isolates in these diseases and their antimicrobial susceptibility to provide valuable information for initial treatment of horses with bacterial infections.

In the last 25 years some studies looked at bacterial isolates cultured from horses with bacterial infections without discrimination in age of the horse or location of the infection. In these studies the most common gram-negative bacterial isolates were *E. coli*, *Enterobacter*, *Klebsiella*, *Salmonella*, *Pseudomonas*, *Actinobacillus*, *Bordetella* and *Proteus* which were all susceptible to amikacin [21, 22, 27], with the exception of *Pseudomonas,* for which 4.4% of the isolates were resistant to amikacin [20]. Within the last ten years there has been a significant increase in resistance of these species of bacteria to trimethroprim-sulfamethoxazole, penicillin, tetracycline, and gentamicin, but not to amikacin [22]. In one study, isolates of *E. coli*, *Staphylococcus spp*., and *Pseudomonas spp* were more sensitive to amikacin than to gentamicin or enrofloxacin [21]. In the case of *Staphylococus spp* all isolates were reported susceptible to amikacin [21, 27] and resistance was only found in one study for only 4% of the isolates [22].

Numerous studies of bacterial isolates from blood cultures in equine neonates indicated that Gram-negative sepsis was more frequently diagnosed [23-25], but in a recent study Gram-positive bacteria were more commonly isolated from blood cultures in equine neonates [26]. The most common Gram-negative isolates were *E. coli*, *Enterobacter*, *Actinobacillus*, *Acinetobacter*, *Pseudomonas*, *Salmonella*, *Klebsiella*, and *Pantoea* and all were susceptible to amikacin [23-26].

Bacterial infection of the lower portion of the respiratory tract of adult horses was most commonly associated with aerobic/facultative anaerobic cocci (*Streptococcus equi subsp zooepidermicus* and others) followed by Gram-negative bacilli (*Pasteurella, E. coli*

and others), anaerobic bacilli, anaerobic Gram-negative bacilli, anaerobic Gram-positive cocci and aerobic Gram-positive bacilli. Most of these Gram-negative bacteria were susceptible to amikacin [28, 29].

Actinobacillus, a Gram-negative pleomorphic bacillus, has been reported to be associated with sepsis in foals [53] and also with peritonitis [31], pericarditis [30], and post-operative infections in adult horses [32]. In these reports the resistance of *Actinobacillus* against amikacin varied from moderate [30] to none [31, 32].

A survey of horses with orthopedic infections reported that the most common isolate was *E. coli*, followed by *Streptococcus,* and *Staphylococcus* and *Pseudomonas*. *Pseudomonas* and *Staphylococcus spp*. were found to be very susceptible to amikacin [33]. In the same report the antibiotic susceptibility was reevaluated for these organisms ten years later and it was found that *Staphylococcus* and *E. coli* had developed resistance to other antibiotics different to amikacin.

In two reports of horses with septic arthritis/tenosinovitis or osteomyelitis, *Enterobacteriaceae* was the most common group isolated, followed by *Streptococcus* and *Staphylococcus*. Amikacin was effective against Gram-negative bacteria (*Enterobacter*, *Salmonella*, *Actinibacillus* and *Pseudomonas*) and *Staphylococcus* [34, 35].

Most studies of equine bacterial pathogens indicate that Gram-negative bacteria and isolates of *Staphylococcus* are very common causes of equine bacterial disease. In these studies bacterial pathogens showed consistent susceptibility to amikacin.

Objectives of Current Study

We hypothesized that 10 mg/kg of amikacin administered intravenously daily in adult horses will reach therapeutic concentrations in plasma and synovial, peritoneal, and interstitial fluids. Objectives of the study included: (a) describing the plasma concentration-time profiles after intravenous administration of amikacin; (b) describing pharmacokinetic variables after administration of amikacin; (c) determining synovial, peritoneal, and interstitial fluid concentration of amikacin following intravenous administration.

III. MATERIALS AND METHODS

Experimental Animals

Six adult Quarter horse and Thoroughbred horses (5 geldings and one mare) from the Auburn University teaching herd were used for the study. The horses had no history of recent disease. They weighed from 423 to 590 kg [517.16 ± 60.18 (SEM) kg] and their age ranged from 8 to 22 years [18.61 ± 5.63 (SEM) years]. The horses were confined in individual stalls in the research barn facility at the College of Veterinary Medicine. They were offered coastal Bermuda hay and water *ad libitum*, and 1.5 kg of grain, which was 12% protein, twice daily, until the experiment ended.

Intravenous indwelling catheters were placed aseptically in each jugular vein. The right jugular catheter was used to administer the single dose of amikacin and the left jugular catheter was used to collect blood for sampling. All experiments were approved by the Auburn University Institutional Animal Care and Use Committee.

Experiment 1a: Pharmacokinetics of Amikacin

A single dose of 10 mg/kg of amikacin (amikacin sulphate, Amiglyde®, Fort Dodge Animal Health, Fort Dodge, IA) was administered as a bolus through a 14-gauge

catheter placed in the right jugular vein, followed by 5 ml of heparin solution (10 USP Units/ml, heparin from porcine intestinal mucosa, Heparin Lock Flush solution, USP, Hospira, Inc, Lake Forest, Il). Blood samples were collected from a 14-gauge x 5-inch catheter that had been placed aseptically in the left jugular vein. Before drawing the sample of blood for analysis, 5 ml of blood was aspirated and discarded along with the syringe. Blood was collected at 0 (before administration),3,6,10,15,25,40,60,and 90 minutes, 2, 2.5, 3, 4, 5, 6, 8, 12, and 24 hours. The plasma was stored at -80°C until assayed to determine concentration of amikacin.

Experiment 1b: Synovial Fluid Concentration of Amikacin

Synovial fluid from a randomly assigned radiocarpal joint of each horse was collected at 0 (before intravenous administration of amikacin), 30, 60, and 90 minutes, and 2, 6, 12, and 24 hours later. After surgical preparation of the site of arthrocentesis, synovial fluid was collected using a 22-gauge, 1-inch disposable hypodermic needle. Samples of synovial fluid were stored at -80°C until assayed to determine the concentration of amikacin.

Experiment 1c: Peritoneal Fluid Cconcentration of Amikacin

Peritoneal fluid was collected from each horse at 0 (before intravenous administration of amikacin), 30, 60, and 90 minutes, and 2,6,12, and 24 hours later. After surgical preparation of the site of abdominocentesis, peritoneal fluid was collected using

an 18-gauge, 1 1/2 inch disposable hypodermic needle. Samples of peritoneal fluid were stored at -80°C until assayed to determine the concentration of amikacin.

Experiment 1d: Interstitial Fluid Concentration of Amikacin

Twelve hours prior the administration of a single dose of amikacin all horses were placed in stocks and sedated with a single dose of xylazine hydrochloride (Anased®, Loyd Laboratories, Shenandoah, IA) administered intravenously at 0.5 mg/kg. After subcutaneous infiltration of local anesthetic solution in the left side of the neck cranial to the scapula, an ultrafiltration probe (RUF-3-12 Reinforced Ultrafiltration Probes, Bioanalytical Systems Inc, West Lafayette, IN) was placed subcutaneously through a 0.5 cm skin incision. The probe was connected to a tube with vacuum (Vacutainer, Beckton Dickenson) which was collected and replaced at -2, 0 (before administration), 1.5, 4, 8, 12, and 24 hours after intravenous administration of amikacin. Samples of interstitial fluid collected in vacuum tubes at each of these time-points were stored at -80°C until assayed to determine the concentration of amikacin.

Amikacin Assay Procedure

The concentration of amikacin was measured in all samples (plasma, synovial fluid, peritoneal fluid, and interstitial fluid) using a fluorescence polarization immunoassay (DTx®, Abbott Laboratories, Abbott Park, IL). The sensitivity of the assay or the lowest measurable amikacin concentration was 0.8 μg/mL.

Pharmacokinetic Analysis

The concentrations of amikacin vs. time were plotted on a semilogarithmic graph, for all different fluids. Pharmacokinetic software (WinNonlin 4.0.1.Pharsight Coroporation, Mountain View, CA) was used to fit the time vs. concentration data points to a one-, two-, and three-compartment pharmacokinetic model. Using the Aikakie's Information Criterion a 2-compartment model was most appropriate for the interpretation of the amikacin plasma concentration curves vs. time. The plasma concentration (C) of amikacin after intravenous administration was described by using an equation as follows:

$$C(t) = A^{-\alpha t} + Be^{-\beta t}$$

where *e* is the base of the natural logarithm; *t* is the time after administration; A and B are the *y*-axis intercepts for the distribution and elimination phases of the curve respectively; and α and β are the slopes for the distribution and elimination phases of the curve respectively.

The synovial and peritoneal fluids were interpreted using a non-compartmental analysis. The area under the curve to infinite ($AUC_{0-\infty}$) was calculated with the following formula:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/k_e$$

where C_t was the last measured concentration and k_e was calculated by applying a log-linear regression to at least the last 3 quantifiable concentrations of amikacin.

Statistical Analysis

The pharmacokinetic parameters were presented as mean ± standard deviation (SD), and coefficient of variation (CV) for experiments 1a, 1b and 1c.

IV. RESULTS

Experiment 1a: Pharmacokinetics of Amikacin

The intravenous dose of amikacin administered at 10 mg/kg to the horses was compatible with a two-compartment model (Figure 1).

A summary of the pharmacokinetic parameters of amikacin after a single intravenous dose is presented in Table 1. The predicted maximum concentration (C_{max}) was approximately 143.61 μg/ml. The half life for the distribution phase ($t_{½\alpha}$) was described as approximately 0.097 hours followed by an elimination phase half-life ($t_{½\beta}$) of 1.346 hours. The total body clearance was calculated as 1.251 ± 0.281 ml/min/kg. The MRT averaged 1.814 hours with a range of 1.171 to 2.77 hours. The volume of distribution at steady state (V_{dss}) was 128.69 ± 12.43 ml/kg.

The concentration of amikacin in serum was below the minimum detectable concentration for the fluorescence polarization immunoassay used for all horses at 18 hours with exception of Horse 1 (1.21 μg/ml), which was below the minimum detectable concentration after 24 hours of administration.

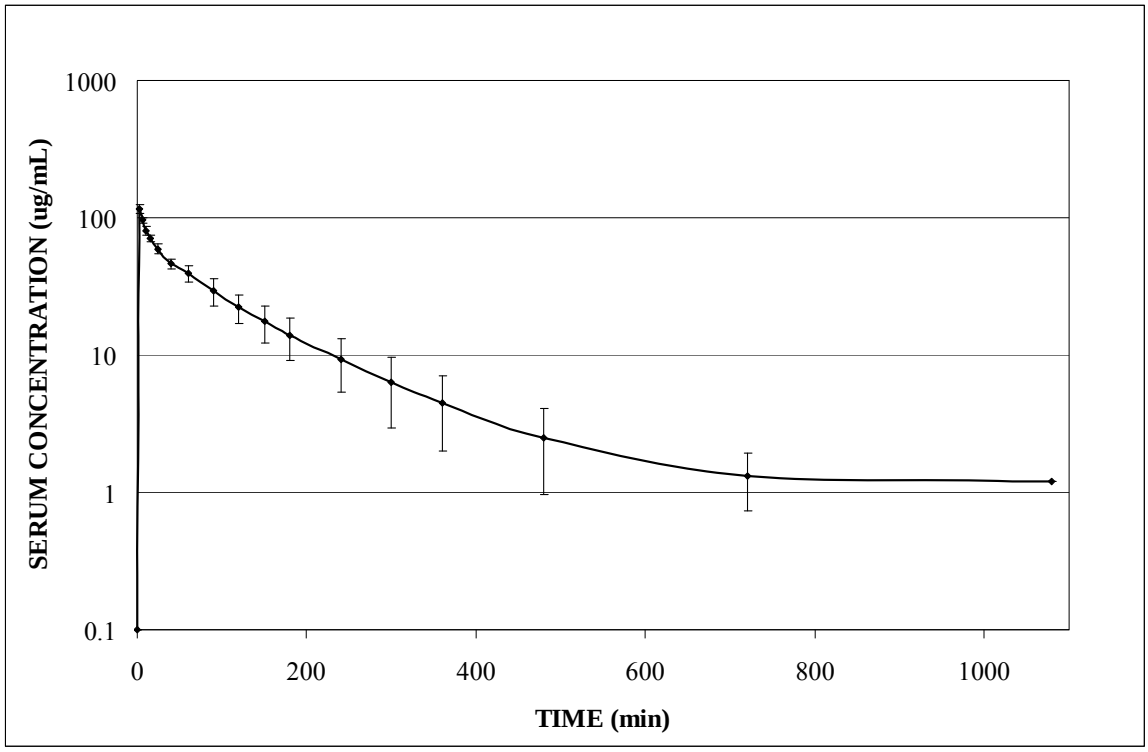

Figure 1: Depicts the mean concentration (n = 6) at individual time points following treatment with Amikacin (initiated at Time 0) at doses of 10 mg/kg. Data represent means ± standard deviation (SD).

PARAMETER	UNITS	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	MEAN	SD	CV
AUC	µg *h /mL	200.421	98.671	120.715	144.593	134.168	137.525	139.349	34.027	0.244
K_{10} HL	min	64.461	23.34	32.391	39.156	48.415	43.093	41.81	14.108	0.337
Alpha	1/min	0.092	0.223	0.149	0.167	0.067	0.127	0.138	0.055	0.403
Beta	1/min	0.005	0.013	0.011	0.008	0.008	0.008	0.009	0.002	0.295
$t_{½\alpha}$	h	0.124	0.051	0.077	0.068	0.171	0.09	0.097	0.043	0.449
$t_{½\beta}$	h	2.031	0.876	0.985	1.337	1.426	1.42	1.346	0.408	0.303
A	µg/mL	64.917	103.909	76.013	82.883	56.878	70.081	75.78	16.428	0.216
B	µg/mL	64.388	71.906	78.979	70.69	58.370	62.641	67.829	7.448	0.109
C_{max}	µg/mL	129.305	175.816	154.993	153.574	115.249	132.722	143.61	21.879	0.152
K_{10}	1/min	0.01	0.029	0.021	0.017	0.014	0.016	0.018	0.006	0.359
MRT	h	2.77	1.171	1.329	1.824	1.868	1.921	1.814	0.561	0.309
CL	ml/min/kg	0.831	1.689	1.38	1.152	1.242	1.211	1.251	0.281	0.224
V_{ss}	ml/kg	138.22	118.677	110.146	126.2	139.229	139.686	128.693	12.434	0.096
K_{12}	1/min	0.038	0.107	0.058	0.076	0.023	0.054	0.059	0.029	0.496
K_{21}	1/min	0.048	0.099	0.082	0.081	0.038	0.064	0.069	0.022	0.331
V1	ml/kg	77.336	56.877	64.518	65.115	86.768	75.345	70.993	10.805	0.152
V2	ml/kg	60.884	61.8	45.627	61.084	52.460	64.34	57.699	7.151	0.123

Table 1: Pharmacokinetic parameters in six adult horses after a singe intravenous dose of amikacin (10 mg/kg).

Experiment 1b: Synovial fluid concentration of amikacin

The pharmacokinetics of amikacin in synovial fluid after a single intravenous injection was assessed under a non compartmental analysis. The mean concentrations of amikacin in synovial fluid are shown in Figure 2.

Calculated pharmacokinetic parameters for amikacin in synovial fluid after intravenous injection are presented in Table 2. The mean peak synovial concentration of amikacin (C_{max}) was 19.78 μg/ml with a range from 10.8 to 28.2 μg/ml. The time to achieve maximum concentration of amikacin (T_{max}) was 65 minutes. The mean residual time to the infinite observed ($MRT_{0-\infty}$) was 5.85 ± 3.364 hours. The area under the curve to the infinite ($AUC_{0-\infty}$) was 100.28 ± 39.892 μg*h/mL.

Horse 1 and horse 5 were the only individuals with detectable concentrations of amikacin in synovial fluid at 24 hours (1.22 and 0.83 μg/ml respectively).

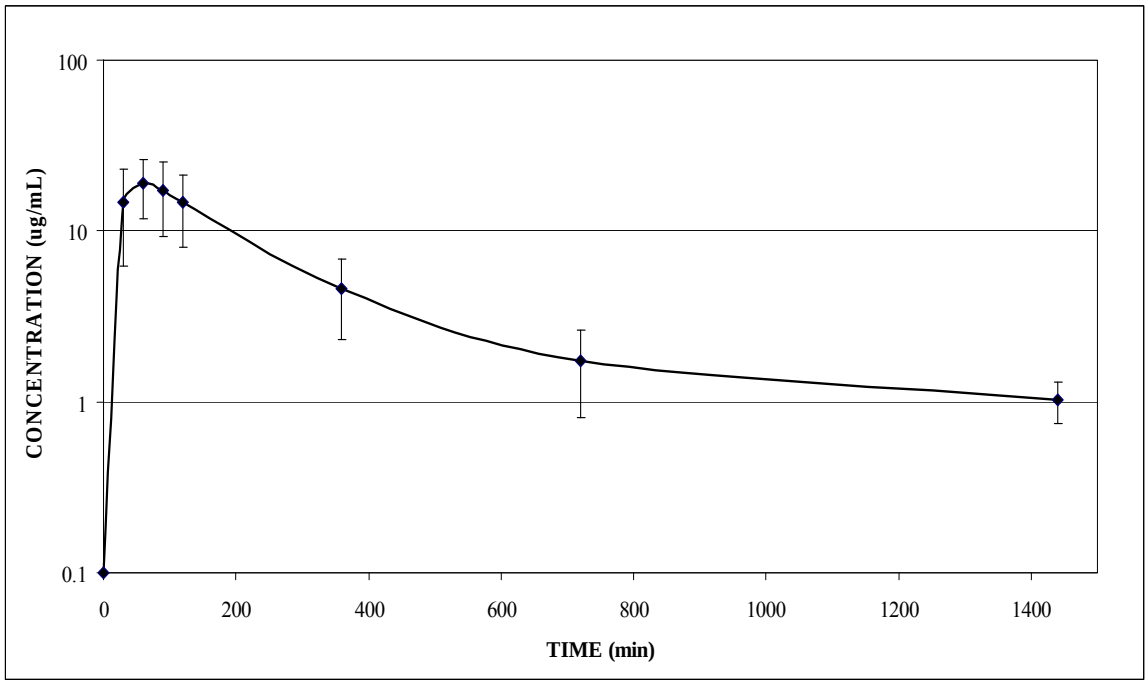

Figure 2: Mean synovial fluid concentration of amikacin in adult horses (n=6) after a single intravenous dose (10 mg/kg). Data represent mean (n=6) ± SD.

PARAMETER	UNIT	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	MEAN	SD	CV
Lambda z	1/min	0.002	0.003	0.004	0.004	0.001	0.003	0.003	0.001	0.394
HL Lambda z	min	346.341	181.044	150.1	156.432	486.064	180.334	250.053	136.638	0.546
T_{max}	min	60	90	60	60	60	60	65	12.247	0.188
C_{max}	µg/mL	22.05	11.83	19.86	25.81	10.86	28.2	19.768	7.144	0.361
T_{last}	min	1440	720	720	720	1440	720	960	371.806	0.387
C_{last}	µg/mL	1.22	1.17	0.95	1.49	0.83	2.4	1.343	0.565	0.421
$AUC_{0-\infty}$	µg*h/mL	153.224	64.163	71.928	119.349	59.41	132.094	100.028	39.8926	0.398
$MRT_{0-\infty}$	h	7.838	4.23	3.238	3.728	11.851	4.214	5.85	3.364	0.575

Table 2: Pharmacokinetic variables for amikacin in the synovial fluid after a single intravenous dose (10 mg/kg).

Experiment 1c: Peritoneal fluid concentration of amikacin

Mean concentration values of amikacin in peritoneal fluid after a single intravenous injection (10 mg/kg) are displayed in Figure 3. The pharmacokinetics of amikacin in peritoneal fluid after a single intravenous injection was assessed under a non compartmental analysis.

Calculated pharmacokinetic parameters for amikacin in peritoneal fluid after intravenous injection are presented in Table 3. The mean peak synovial concentration of amikacin (C_{max}) was 21.44 μg/ml with a range from 15.48 to 26.02 μg/ml. The time to achieve maximum concentration of amikacin (T_{max}) was 115 minutes. The mean residual time to the infinite observed ($MRT_{0-\infty}$) was 4.873 ± 0.776 hours. The area under the curve to the infinite was 139.99 ± 25.88 μg*h/mL. The concentration of amikacin in peritoneal fluid was below the minimum detectable concentration for the fluorescence polarization immunoassay used at 24 hours for all six horses. The mean concentration of amikacin in the peritoneal fluid at 12 hours was 2.24 μg/mL.

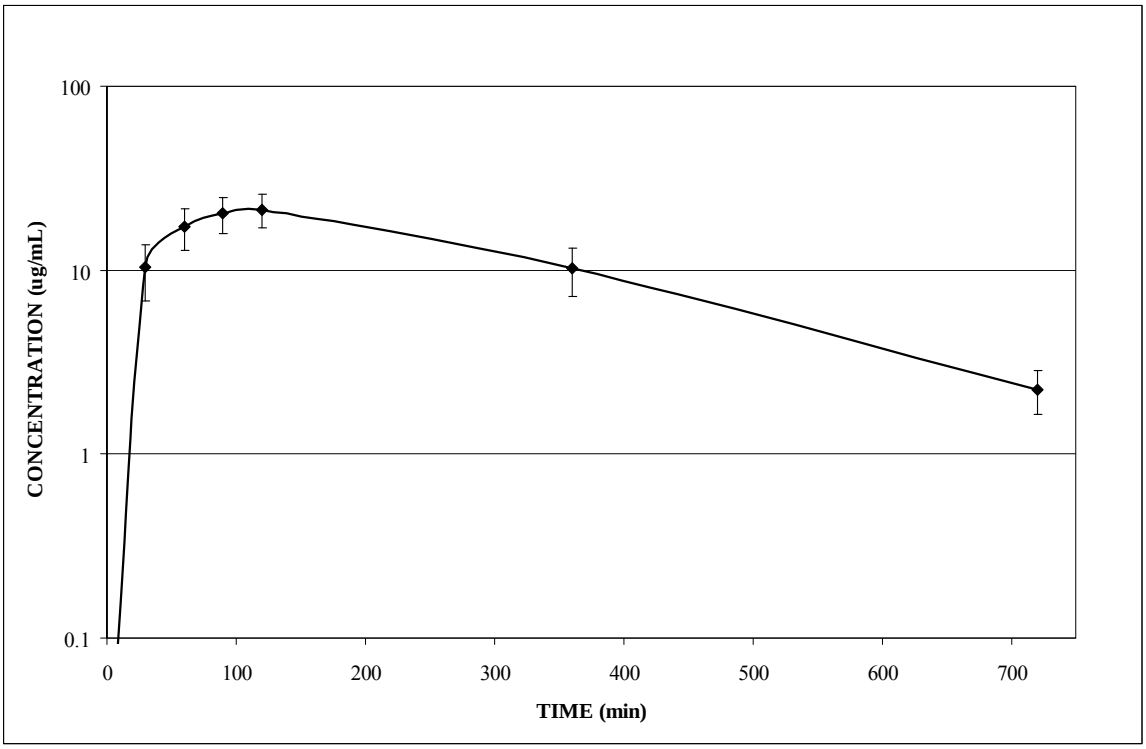

Figure 3: Mean peritoneal fluid concentration of amikacin in adult horses after a single intravenous dose (10 mg/kg). Data represent mean (n=6) ± SD.

PARAMETER	UNIT	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	MEAN	SD	CV
Lambda z	1/min	0.003	0.003	0.004	0.004	0.003	0.004	0.003	0.0006	0.159
HL Lambda z	min	212.804	224.163	164.244	159.471	202.136	155.747	186.428	30.088	0.161
T_{max}	min	120	120	90	120	120	120	115	12.247	0.106
C_{max}	µg/mL	22.31	15.48	21.68	26	17.15	26.02	21.44	4.393	0.204
T_{last}	min	720	720	720	720	720	720	720	0	0
C_{last}	µg/mL	3.32	2.46	1.62	1.98	2.23	1.83	2.24	0.605	0.27
$AUC_{0-\infty}$	µg*h/mL	174.27	114.694	118.822	159.937	117.961	154.303	139.998	25.888	0.184
$MRT_{0-\infty}$	h	5.673	5.746	4.115	4.362	5.262	4.083	4.873	0.776	0.159

Table 3. Pharmacokinetic variables for amikacin in the peritoneal fluid after a single intravenous dose (10 mg/kg).

Experiment 1d: Interstitial fluid concentration of amikacin

The concentrations of amikacin in interstitial fluid are displayed for each horse in Figure 4 and the mean concentration (n=5) is displayed in Figure 5.

Major failure of the vacuum system attached to the microfiltration probe resulted in inadequate collection of the samples in horse 1, data from this individual was not included in this experiment. The vacuum system failed for the first two samples in horse 2. A summary of the amikacin concentration in interstitial fluid is displayed in Table 4.

The pharmacokinetic analysis in this experiment was declined due to the low number of horses and the high variability in the concentrations among horses.

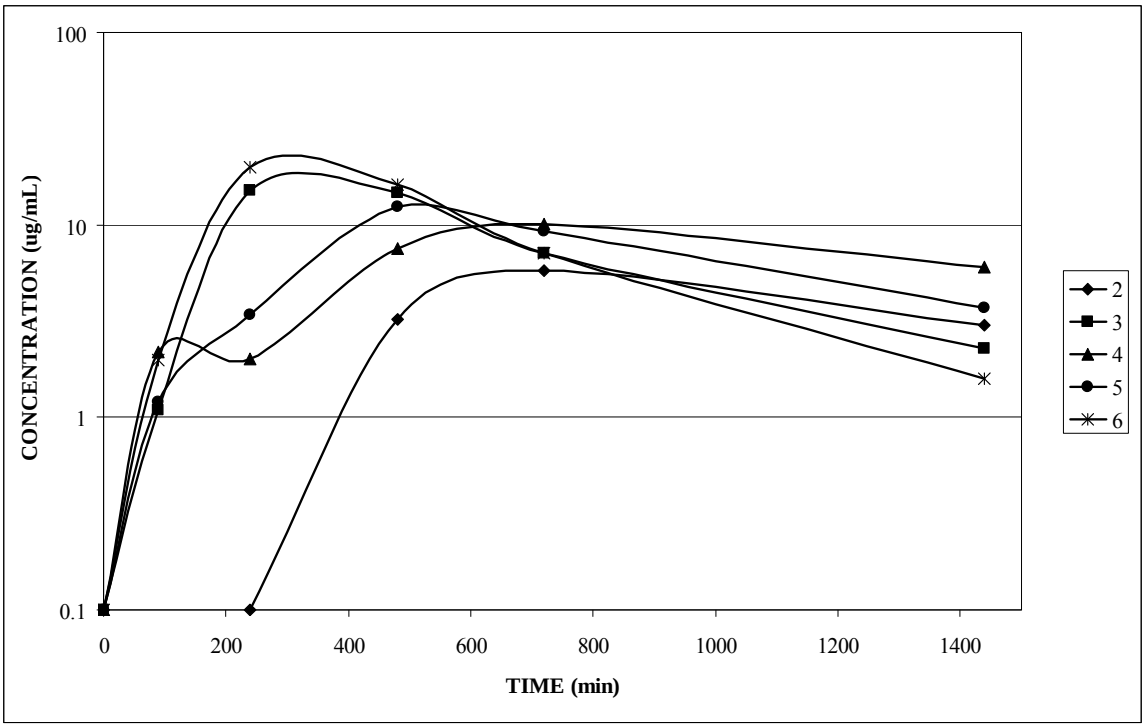

Figure 4: Concentration of amikacin in the interstitial fluid after a single intravenous injection of amikacin (10 mg/kg).

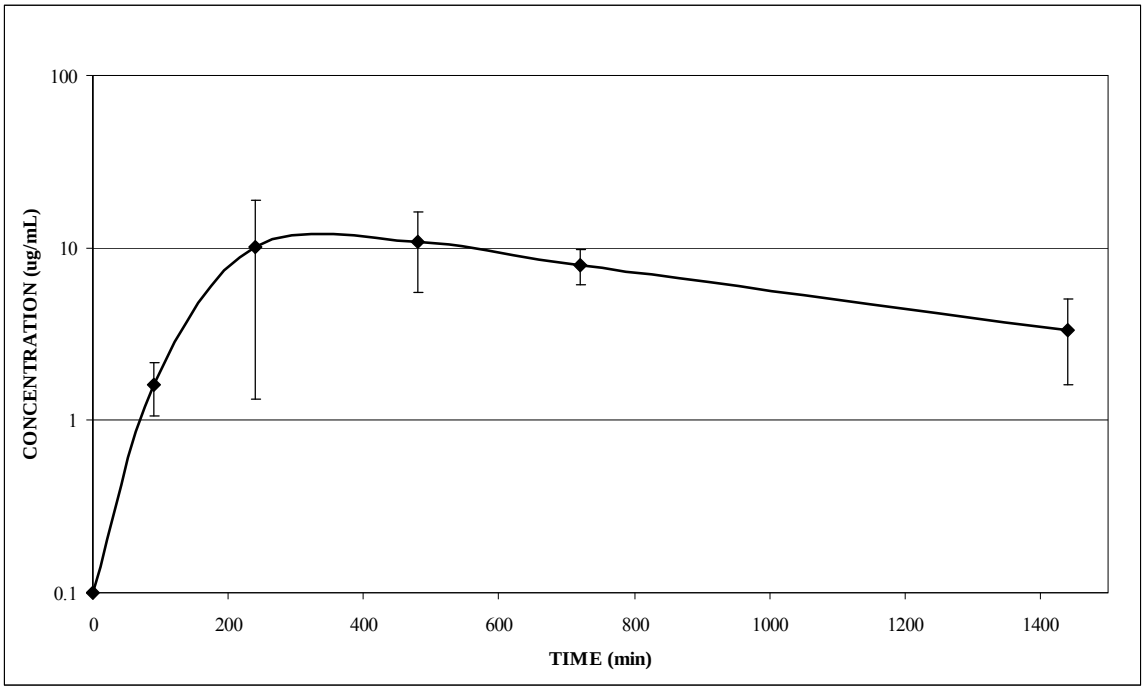

Figure 5: Mean interstitial fluid concentration of amikacin in adult horses after a single intravenous dose (10 mg/kg). Data represent mean (n=5) ± SD.

TIME OF COLLECTION	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	MEAN	SD
0	0	0	0	0	0	0	0
1-90		1.09	2.18	1.2	1.99	1.615	0.55
91-240		15.17	2.02	3.42	20.04	10.162	8.839
241-480	3.25	14.74	7.55	12.48	16.09	10.822	5.336
481-720	5.79	7.13	10.14	9.33	7.15	7.908	1.779
721-1440	3.01	2.28	6	3.71	1.58	3.316	1.698

Table 4. Concentration of amikacin in the interstitial fluid (μg/ml) after a single intravenous injection of amikacin (10 mg/kg).

V. DISCUSSION

Within the last two decades it has been demonstrated in human and veterinary medicine that aminoglycosides antibiotics administered once a day to reach a Cmax/MIC ratio between 6 to 10 are more effective and less nephrotoxic than if they are administered several times a day[4, 5, 8, 9, 12, 13, 19, 51]. In the horse, the pharmacokinetic data for amikacin administered once a day has been available only for neonatal foals [39 and 41]. Studies concerning the pharmacokinetics of amikacin in adult horses used a multiple daily dosing schedule [42, 54, 55, and 56] and concluded that amikacin should be administered to adult horses at doses between 4.4 to 6.6 mg/kg, IV three times a day [42 and 54] or 7 mg/kg, IM twice a day IM [55].

A comparison of pharmacokinetic parameters after a single intravenous dose of amikacin (10 mg/kg) in adult horses reported in this study with those found in other studies in adult horses [42, 54, 55] is shown in Table 5.

In our study a single intravenous injection of amikacin (10 mg/kg) in adult horses resulted in dose independent pharmacokinetic parameters similar to previous studies performed in adult horses using 11 and 6.6 mg/kg IV [42], 6 mg/kg IV [43] and 7 mg/kg administered intramuscularly [55]. The clearance value (1.25 ml/min/kg) reported in our study was similar to those found in two other studies (1.27, 1.41 and 1.33 ml/min/kg) [42 and 55]. Other parameters such as $t_{½\beta}$ and volume of distribution of the central

compartment (V_c) in our study were also similar to those reported by Orsini [42] but not by Brown [55] or Horspool [43]. The MRT was significantly lower that the one of 4 hours previously reported [43]. The differences in AUC, $t_{½\beta}$ and MRT can result form different route of administration of the drug and also from different protocols of sampling (9 to 12 samples collected en less than 12 h) used in other studies [42, 43, 55].

Dose dependant parameters such as concentration at time zero (C_0), the *y* axis intercept of the elimination phase (B), and AUC calculated in this study after intravenous administration of 10 mg/kg of amikacin were between values found by Orsini *et al*, who administered amikacin at 6.6 and 11 mg/kg IV. When the values for C_0 obtained in our study for amikacin administered intravenously at 10 mg/kg are compared to values for C_0 for the drug administered at 11 mg/kg [42] a very large difference is noted (126.9 vs 254.4 μg/mL); such a difference can be explained by individual variation and/or low statistical power (n=3) of the other study [42].

A comparison of pharmacokinetic parameters found in this study with those parameters found in foals after administration of high doses (21 and 25 mg/kg IV) once a day [39 and 41] is displayed in Table 6. Parameters such as $t_{½\alpha}$, $t_{½\beta}$, V_{dss}, MRT, and AUC were significantly lower in our study than those reported for foals; however the clearance (CL) values were similar for both and the B value was higher in adult horses. It was also found that the serum concentrations of amikacin were very similar from 0 to 4 hours, then the concentration of amikacin in serum of adult horses declined rapidly when compared with concentration of amikacin in foals.

It has been shown in foals, that the clearance and renal elimination of aminoglicosides (amikacin or gentamicin), increase with age; resulting in lower values

for MRT, $t_{½\alpha}$, $t_{½\beta}$, and AUC in older foals [39, 40, 41, and 57]. Another parameter that decrease in foals as they get older is the volume of distribution [39].

In this study MRT, $t_{½\alpha}$, $t_{½\beta}$, AUC, and volume of distribution were lower than those reported for foals [39 and 41]. The difference in volume of distribution between foals and adult horses reported here may be a result of the difference in extracellular volume among those age groups. The adult horse's low $t_{½\beta}$ value is more likely the result of the low volume of distribution, which is directly proportional to $t_{½\beta}$. On the other hand, the lower values for MRT and a serum concentration of amikacin below 0.8 μg/mL after 24 hours are consistent with an adequate elimination of the drug with minimal accumulation.

The MIC for the most common gram-negative microorganisms that affect horses is reported to be ≤ 4 μg/mL and 8 μg/mL for coagulase-negative *Staphylococcus spp* [42, 58]. For foals the MIC_{90} for all gram-negative isolates from blood cultures is reported to be 4 μg/mL [39]. Based on those reports the C_{max} obtained after a single dose of amikacin should reach values above 40 μg/mL to enhance the efficacy against gram-negative bacteria and reduce the toxicity of the antibiotic in foals and adult horses. In our study the mean concentration of amikacin at time zero was 126 μg/mL. The mean concentration was maintained above 40 μg/mL for 59.45 ± 13.57 min and at 5.4 ± 1.53 hours was above 4 μg/mL. The bactericidal effect of amikacin during the period of time in which the concentration is below the MIC is maintained by the postantibiotic effect (PAE) of amikacin. The length of the PAE depends on the C_{max}/MIC ratio and also the bacterial species [5, 8]. Postantibiotic effect of 13 hours have been reported [59]. The Clinical Laboratory Standards Institute (CLSI) [60] determined that bacteria with a MIC ≤16

μg/mL are considered susceptible to amikacin; with such a MIC, a C_{max} of 160 μg/mL would be required to provide adequate antimicrobial action. That concentration has been achieved with doses of 11 mg/kg [42] and 21 mg/kg [Pinto, unpublished data] in adult horses. The highest Cmax found in the studies performed in foals when amikacin was administered at high doses (21 and 25 mg/kg) was 121 μg/mL which is very similar to the Cmax reported in this study (126 μg/mL). Amikacin is used successfully for treatment of septicemic neonatal foals at recommended doses (21-25 mg/kg) suggesting that the MIC for most Gram-negative bacteria that affect horses may be lower that 16 μg/mL.

Therapeutic drug monitoring (TDM) through peak and trough concentrations is a very useful way to insure that the concentrations of aminoglycoside antibiotic are adequate to enhance its activity, but also to evaluate the CL, half-life, and MRT as indicators of renal elimination. Optimal peaks for amikacin have not been established at this time either in humans or other animals. The trough concentrations are important to determined accumulation of the drug and risk of nephrotoxicity. The plasma concentration of amikacin on adult horses after 24 hours of a 10 mg/kg IV dose was below 0.8 μg/mL, which was lower than the value reported in foals [41].

Pharmacokinetic parameters such as Cmax, $MRT_{0-\infty}$, and $AUC_{0-\infty}$ in synovial and peritoneal fluid were similar after a single dose (10 mg/kg, IV) of amikacin, showing homogeneus distribution of the drug from the plasma to both fluids. The concentration of amikacin in synovial and peritoneal fluid after amikacin administration at different doses (6.6, and 7 mg/kg) and routes has been measured in the past showing similar concentrations [42, 55], but when compared with the values found in this study those values are significantly lower (Table 8).

In conclusion, the pharmacokinetic values found in this study are very similar to those previously reported in foals, suggesting that amikacin at 10 mg/kg IV once a day, should be effective against the most common gram-negative pathogens that affect horses. More severe infections may require higher once a day dosing. Therapeutic drug monitoring is very important to minimize individual variation, drug accumulation, and nephroroxicity; it also allows the clinician to adjust the dosage based on the MIC of the pathogen involved. Further studies in diseased states are needed to prove the efficacy of the dosage suggested in this study.

	Results	Orsini et al.		Brown et al*	Horspool et al
	10 mg/kg IV (n=6)	6.6 mg/kg IV (n=6)	11 mg/kg IV (n=3)	7 mg/kg IM (n=6)	6 mg/kg IV (n=3)
Number of samples	19	9	9	8	12
Length of study (h)	24	9	9	12	12
C_0 (µg/mL)	126.9 ± 21.8	108.9 ± 22.7	254.4 ± 26	Not reported	Not reported
$t_{½}\beta$ (h)	1.346	1.57	1.14	2.3	2.8
Vss (ml/kg)	128.6	Not reported	Not reported	260	206
Vc (ml/kg)	69.9	51	63	Not reported	Not reported
CL (ml/min/kg)	1.25	1.27	1.41	1.33	0.75
MRT (h)	1.814	Not reported	Not reported	Not reported	4
A (µg/mL)	75.78	83.7	174.1	Not reported	Not reported
B (µg/mL)	67.82	25.2	80.3	Not reported	Not reported
AUC (µg *h /mL)	139	86.2	170	Not reported	Not reported

Table 5. Pharmacokinetic parameters for amikacin in normal adult horses.

* In this study amikacin was administered every 12 hours.

PARAMETER	Results 10 mg/kg IV (n=6)	Madgesian et al. 21 mg/kg IV (n=7)			Bucki et al 25 mg/kg IV (n=5)	
	Adult horse	1 day	5 day	10 day	2-3 day	10-11 day
A (μg/mL)	75.7	69.1	74.8	93.3	62.6	77.5
α (1/h)	8.28	3.3	2.7	3.6	1.22	1.62
B (μg/mL)	67.8	34	28.1	27.4	24.9	17.9
β (1/h)	0.54	0.13	0.17	0.18	0.13	0.13
$t_{½}\alpha$ (h)	0.097	0.25	0.26	0.21	0.5	0.49
$t_{½}\beta$ (h)	1.34	5.49	4.3	3.97	5.07	5.2
AUC (μg *h /mL)	139.3	293	202	181	228	195
Vdss (ml/kg)	128.6	537	560	574	N.R.	N.R.
Cl (ml/min/kg)	1.25	1.3	1.8	2.0	1.82	2.13
MRT (h)	1.81	7.3	5.4	4.9	6.17	4.99
C_0 (μg/mL)	126.9	103	103	121	N.R.	N.R.
$C_{0.5}$ (μg/mL)	53.55	N.R.	N.R.	N.R.	53	58.4
C_1 (μg/mL)	39.3	37.5	32.9	30.6	N.R.	N.R.
C_4 (μg/mL)	9.3	17.9	12.3	10.0	N.R.	N.R.
C_{12} (μg/mL)	1.32	6.6	3.5	2.7	N.R.	N.R.
C_{24} (μg/mL)	N.M.	2.3	1.4	1.2	1.2	0.85

Table 6. Pharmacokinetic parameters for amikacin in normal adult horses and foals.

	30	60	90	120	360	720	1440
Plasma	53.4 ± 3.6	39.3 ± 5	29.3 ± 6.6	22.1 ± 5.2	4.5 ± 2.5	1.3 ± 0.5	
Sinovial Fluid	14.7 ± 8.4	18.9 ± 7.1	17.4 ± 8	14 ± 6.5	4.5 ± 2.2	1.7 ± 0.9	1 ± 0.2
Peritoneal Fluid	10.3 ± 3.4	17.1 ± 4.4	20.3 ± 4.3	21.4 ± 4.3	10.16 ± 1.9	2.24 ± 0.6	

Table 7. Concentrations of amikacin in plasma, synovial fluid, and peritoneal fluid after a single intravenous injection (10 mg/kg).

Study	Fluid	Dosage	60	120	240	360
Orsini et al.	Sinovial	6.6 mg/kg IV	16.8 ± 8.8	9.3 ± 4.3	5.0 ± 1.0	3.1 ± 0.7
Brown et al	Sinovial	7 mg/kg IM	8.7 ± 0.5	10.8 ± 1.4	9.7 ± 0.94	8.7 ± 0.9
Pinto et al	Sinovial	10 mg/kg IV	18.9 ± 7.1	14 ± 6.5	---	4.5 ± 2.2
Orsini et al.	Peritoneal	6.6 mg/kg IV	13.7 ± 3.2	12.3 ± 3.6	12.2 ± 3.4	7.9 ± 1.9
Brown et al	Peritoneal	7 mg/kg IM	11.2 ± 1.0	15.3 ± 1.6	14.2 ± 1.4	9.0 ± 1.2
Pinto et al	Peritoneal	10 mg/kg IV	17.1 ± 4.4	21.4 ± 4.3	---	10.16 ± 1.9

Table 8. Comparison of the concentrations of amikacin in plasma, synovial fluid, and peritoneal fluid in adult horses.

REFERENCES

1. Begg, E.J. and Barclay, M.L., Aminoglycosides- 50 years on. Br J clin Pharmac, 1995(39): p. 597-603.

2. Jana, S. and Deb, J.K., Molecular understanding of aminoglycoside action and resistance. Appl Microbiol Biotechnol, 2006(70): p. 140-150.

3. Shakil, S., et al., Aminoglycosides versus bacteria - a description of the action, resistance mechnism, and nosocomial battleground. Journal of Biomedical Science, 2008(15): p. 5-14.

4. Bartal,C., et al., Pharmacokinetic dosing of aminoglycosides; a controlled trial. Am J Med, 2003(114): p. 194-198.

5. Lacy, M.K., et al., The pharmacodynamics of aminoglycosides. CID, 1998(27): p. 23-27.

6. Frimodt-Moller, N., How predictive is PK/PD for antibacterial agents? International journal of antimicrobial agents, 2002(19): p. 333-339.

7. Nicolan, D.P., Optimizing outcomes with antimicrobial therapy through pharmacodynamic profiling. J Infect Chemother, 2003(9): p. 292-296.

8. McKinnon, P.S. and Davis, S.L., Pharmacokinetic and pharmacodynamic issues in the treatment of bacterial infectious diseases. Eur J Clin Microbiol Infect Dis, 2004(23): p. 271-288.

9. Contopoulos-Ioannidis, D.G., et al., Extended-interval aminoglycoside administration for children: a meta-analysis. Pediatrics, 2004(114): p. 111-118.

10. Mingeot-Leclercq, M. and Tulkens, P.M., Aminoglycosides: nephrotoxicity. Antimicrob Agents chemother, 1999(43): p. 1003-1012.

11. Martinez-Salgado, C., Lopez-Hernandez, F.J., and Lopez-Novoa, J.M., Glomerular nephrotoxicity of aminoglycosides. Toxicology and applied pharmacology, 2007(223): p. 86-98.

12. Rougier, F., et al., Aminoglycoside nephrotoxicity. Current drug targets - infectious disorders, 2004(4): p. 153-162

13. Nagai, J., and Takano, M., Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug metab pharmacokin, 2004(19): p. 159-170.

14. Guthrie, O.W., Aminoglycoside induced ototoxicity. Toxicology, 2008(249): p. 91-96.

15. Michael, K., Wang, H., and Tor, Y., Enhanced RNA binding of dimerized aminoglycosides. Bioorg Med Chem, 1999(7): p. 1361-1371.

16. Noller, H.F., Ribosomal RNA and translation. Annu Rev Biochem, 1991(60): p. 191-227.

17. Cate, J.H., et al., X-ray crystal structure of 70S ribosome functional complexes. Science, 1999(285): p. 2095-2104.

18. Kadurugamuwa, J.L., Clarke, A.J., and Beveridge, T.J., Surface action of gentamicin on *Pseudomonas aeruginosa*. J Bacteriol, 1993(175): p. 5798-5805.

19. Moore, R.D., Smith, C.R., and Litman, P.S., The association of aminoglycoside plasma levels with mortality in patients with Gram-negative bacteremia. J Infect Dis, 1984(149): p. 443-448.

20. Orsini, J.A., et al., Resistance to gentamicin and amikacin of gram-negative organisms isolated from horses. Am J Vet Res, 1989(50): p. 923-925.

21. Clark, C., et al., Bacterial isolates from equine infections in western Canada. (1998-2003). Can Vet J, 2008(49): p. 153-160.

22. Peyrou, M., Higgins, R., and Lavoie, J.P., Evolution de la resistance bacterienne envers certains agents antibacteriens chez les chevaux dans un centre hospitalier veterinaire. Can Vet J, 2003(44): p. 978-991.

23. Corley, K.T.T., et al., Bacteraemia in neonatal foals: clinicopathological differences between Gram-positive and Gram-negative infections, and single organism and mixed infections. Equine Vet J, 2007(39): p.84-89.

24. Russell, C.M., et al., Blood culture isolates and antimicrobial sensitivities from 427 critically ill neonatal foals. Aust Vet J, 2008(86): p. 266-271.

25. Marsh, P., Palmer, J.E., Bacterial isolates from blood and their susceptibility patterns in critically ill foals: 543 cases (1991-1998). J Am Vet Med Assoc, 2001(218): p. 1608-1610.

26. Holis, A.R., et al., Bacteremia in equine neonatal diarrhea: a retrospective study (1990-2007). J Vet Intern Med, 2008(22): p. 1203-1209.

27. Jacks, S.S., Giguere, S., and Nguyen, A., In vitro susceptibilities of Rhodococcus equi and other common equine pathogens to Azithromycin, Clarithromycin, and other 20 antimicrobials. Antimicrob Agents Chemother, 2003(47): p. 1742-1745.

28. Racklyeft, D.J. and Love, D.N., Bcterial infection of the lower respiratory tract in 34 horses. Aust Vet J, 2000(78): p. 549-559.

29. Sweeney, C.R., et al., Aerobic and anaerobic bacterial isolates from horses with pneumonia or pleuropneumonia and antimicrobial susceptibility patterns of the aerobes. J Am Vet Med Assoc, 1991(198): p. 839-842.

30. Bolin, D.C., et al., Microbiologic and pathologic findings in an epidemic of equine pericarditis. J Vet Diagn Invest, 2005(17): p. 38-44.

31. Matthews, S. et al., Peritonitis associated with Actinobacillus equuli in horses: 51 cases. Aust Vet J, 2001(79): p. 536-539.

32. Smith, A.S. and Ross, M.W., Postoperative infection with Actinobacillus spp in horses: 10 cases (1995-2000). J Am Vet Med Assoc, 2002(221): p. 1306-1310.

33. Snyder, J.R., et al., Antimicrobial susceptibility of microorganisms isolated from equine orthopedic patients. Vet Surg, 1987(16): p. 197-201.

34. Moore, R.M., et al., Antimicrobial susceptibility of bacterial isolates from 233 horses with musculoskeletal infection during 1979-1989. Equine Vet J, 1992(24): p. 450-456.

35. Schneider, R.K., Common bacteria encountered in septic arthritis. Proc Am Assoc Equine Practitioners, 1998(44): p. 152-158.

36. Adland-Davenport, P., et al., Pharmacokinetics of Amikacin in critically ill neonatal foals treated for presumed or confirmed sepsis. Equine Vet J, 1990(22): p. 18-22.

37. Green, S.L., et al., Effects of hypoxia and azotemia on the pharmacokinetics of Amikacin in neonatal foals. Equine Vet. J, 1992(24): p. 475-479.

38. Wichtel, M.G., Brehaus, B.A., and Aucoin, D., Relation between pharmacokinetics of Amikacin sulfate and sepsis score in clinically normal and hospitalized neonatal foals. J Am Vet Med Assoc, 1992(200): p. 1339-1343.

39. Bucki, E.P., et al., Pharmacokinetics of once-daily Amikacin in healthy foals and therapeutic drug monitoring in hospitalized equine neonates. J Vet Intern Med, 2004(18): p. 728-733.

40. Golenz, M.R., et al., Effect of route of administration and age on the pharmacokinetics of Amikacin administered by the intravenous and intraosseous routes to 3 and 5-day-old foals. Equine Vet J, 1994(26): p. 367-373.

41. Magdesian, K.G., Wilson, W.D., and Mihalyi, J., Pharmacokinetics of a high dose of amikacin administered at extended intervals to neonatal foals. Am J Vet Res, 2004(65): p. 473-479.

42. Orsini, J.A., et al., Pharmacokinetics of amikacin in the horse following intravenous and intramuscular administration. J Vet Pharmacol Ther, 1985(8): p. 194-201.

43. Horspool, J.L., Taylor, D.J., and Mckellar, O.A., Plasma disposition of Amikacin and interactions with gastrointestinal microflora in Equidae following intravenous and oral administration. J Vet Pharmacol Ther, 1994(17): p. 291-298.

44. Papich, M.G., et al., Pharmacokinetics and endometrial tissue concentrations of enrofloxacin and the metabolite ciprofloxacin after i.v. administration of enrofloxacin to mares. J Vet Pharmacol Ther, 2002(25): p. 343-350

45. Bermingham, E.C., Papich, M.G., and Vivrette, S.L., Pharmacokinetics of enrofloxacin administered intravenously and orally to foals. Am J Vet Res, 2000(61): p. 706-709.

46. Kaartinen, L., Panu, S., and Pyorala, S., Pharmacokinetics of enrofloxacin in horses after single intravenous and intramuscular administration. Equine Vet J, 1997(29): p. 378-381.

47. Steinman, A., et al., Pharmacokinetics of gentamicin C1, C1a and C2 in horses after single intravenous dose. Equine Vet J, 2002(34): p. 615-618.

48. Santschi, E.M., Papich, M.G., Pharmacokinetics of gentamicin in mares in late pregnancy and early lactation. J Vet Pharmacol Ther, 2000(23): p. 359-363.

49. Tudor, R.A., Papich, M.G., and Redding, W.R., Drug disposition and dosage determination of once daily administration of gentamicic sulfate in horses after abdominal surgery. J Am Vet Med Assoc, 1999(215): p. 503-506.

50. Madgesian, K.G., et al., Pharmacokinetics of a high dose of gentamicin administered intravenously or intramusculary to horses. J Am Vet Med Assoc, 1998(213): p. 1007-1011.

51. Moore, R.D., Smith, C.R., and Litman, P.S., Clinical response to aminoglycoside therapy: importanc of the ratio of peak concentration to minimal inhibitory concentration. J Infect Dis, 1987(155): p. 93-99.

52. Vogelman, B., et al., In vivo postantibiotic effect in a thigh infection in neutropenic mice. J Infect Dis, 1988(157): p. 198-287.

53. Stewart, A., et al., *Actinobacillus sp*. Bacteremia in foals: Clinical signs and prognosis. J Vet Intern Med, 2002(16): p. 464-471.

54. Bryan, L.E., Kowand, S.K., and Van Den Elzen, H.M., Mechanism of aminoglycoside resistance in anaerobic bacteria: *Clostridium perfringens* and *Bacteroides fragilis*. Antimicrob Agents Chemother, 1979(15): p 7-13.

55. Brown, M.P., et al., Amikacin sulphate in mares: Pharmacokinetics and body fluid and endometrial concentrations after repeated intramuscular administration. Am J Vet Res, 1984(45): p 1610-1613.

56. Orsini, J.A., Park, M.I., and Spencer, P.A., Tissue and serum concentrations of amikacin after intramuscular and intrauterine administration to mares in estrus. Can Vet J, 1996(37): p 157-160

57. Cummings, L.E., et al., Pharmacokinetics of gentamicin in newborn to 30-day-old foals. Am J Vet Res, 1990(51): p 1988-1992.

58. Adamson, P.J., et al., Susceptibility of equine bacterial isolates to antimicrobial agents. Am J Vet Res, 1985(46): p 447-450.

59. Fisman, D.N. and Kaye, K.M., Once-daily dosing of aminoglycoside antibiotics. Infect Dis Clin North Am, 2000(14): p 475-487.

60. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. CLSI document M100-S19. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.