

Comparison of two indirect techniques for local delivery of a high dose of an antimicrobial in the distal portion of forelimbs of horses

Jason A. Errico, DVM; Troy N. Trumble, DVM, PhD; Aloisio C. D. Bueno, MV, MS;
Jennifer L. Davis, DVM, PhD; Murray P. Brown, DVM, MSc

Objective—To compare isolated limb retrograde venous injection (ILRVI) and isolated limb infusion (ILI) for delivery of amikacin to the synovial fluid of the distal interphalangeal and metacarpophalangeal joints and to evaluate the efficacy of use of an Esmarch tourniquet in standing horses.

Animals—6 healthy adult horses.

Procedures—Horses were randomly assigned in a crossover design. In ILRVI, the injection consisted of 1 g of amikacin diluted to a total volume of 60 mL administered during a 3-minute period. In ILI, the infusion consisted of 1 g of amikacin diluted to 40 mL administered during a 3-minute period followed by administration of boluses of diluent (82 mL total) to maintain vascular pressure. During ILI, the infusate and blood were circulated from the venous to the arterial circulation in 5-mL aliquots. Synovial fluid and serum samples were obtained to determine maximum amikacin concentrations and tourniquet leakage, respectively.

Results—Both techniques yielded synovial concentrations of amikacin > 10 times the minimum inhibitory concentration (MIC) for 90% of isolates (80 µg/mL) and > 10 times the MIC breakpoint (160 µg/mL) of amikacin-susceptible bacteria reported to cause septic arthritis in horses. These values were attained for both joints for both techniques. Esmarch tourniquets prevented detectable loss of amikacin to the systemic circulation for both techniques.

Conclusions and Clinical Relevance—Both techniques reliably achieved synovial fluid concentrations of amikacin consistent with concentration-dependent killing for bacteria commonly encountered in horses with septic arthritis. Esmarch tourniquets were effective for both delivery techniques in standing horses. (*Am J Vet Res* 2008;69:334–342)

In equine practice, local delivery of antimicrobials for the treatment of musculoskeletal sepsis is used to supplement or replace systemic administration. Local delivery techniques are favored because they are capable of achieving high tissue concentrations of an antimicrobial at the site of infection while reducing undesirable systemic effects.^{1,2} The ability to deliver high tissue concentrations of drug for an appropriate time interval results in maximum bacterial killing and reduced bacterial resistance.³⁻⁵

Received April 13, 2007.

Accepted July 12, 2007.

From the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610 (Errico, Trumble, Bueno, Brown); and the Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 (Davis). Dr. Trumble's present address is the Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108.

Supported in part by the University of Florida Resident Research Grant Competition.

Presented in part at the Veterinary Orthopedic Conference, Sun Valley, Idaho, March 2007.

The authors thank Dr. Steeve Giguère for assistance with statistical analysis and Dr. Alison Morton for technical assistance.

Address correspondence to Dr. Errico.

ABBREVIATIONS

ILP	Isolated limb perfusion
ILI	Isolated limb infusion
ILRVI	Isolated limb retrograde venous injection
ILIOI	Isolated limb intraosseous injection
MIC _{BP}	Minimum inhibitory concentration breakpoint
C _{max} :MIC	Ratio of maximum concentration to minimum inhibitory concentration
DIP	Distal interphalangeal
MCP	Metacarpophalangeal
MIC ₉₀	Minimum inhibitory concentration for 90% of isolates
LLOQ	Lower limit of quantification
AUC _{0-∞}	Area under the concentration-time curve extrapolated to infinity

Local drug delivery can be categorized into 2 general approaches (ie, direct delivery and indirect delivery). Both delivery methods are used in equine practice and have yielded good clinical success.⁶⁻⁹ Direct delivery involves administration of a drug directly onto the tissues or into the structure of interest without relying on blood or lymphatic vessels for primary distribution. In contrast, indirect delivery relies entirely on blood and

lymphatic vessels to deliver a drug to target tissues. Indirect delivery can be classified into 4 distinct regional methods (ie, ILP, ILI, ILRVI, and ILIOI).

To our knowledge, ILRVI and ILIOI are the only indirect delivery techniques that have been experimentally and clinically applied to horses.^{7,10-15} Both ILRVI and ILIOI deliver antimicrobial drugs via a single injection into a peripheral vessel or the medullary cavity of a long bone, respectively.^{13,16,17} The techniques rely on the transient pressure and concentration gradients established by tourniquet isolation to distribute a drug to the tissues.¹⁸ The techniques of ILRVI and ILIOI do not involve active circulation of blood or drugs during the administration interval.

The technique of ILP has been used in humans for > 50 years.^{19,20} The technique creates a metabolically stable and isolated environment for drug delivery by establishing extracorporeal cardiopulmonary bypass for the extremity or organ under treatment.^{19,21} Similar to ILP, ILI involves controlled and active circulation of blood and drugs (ie, an infusate) through the vasculature isolated by a tourniquet. Isolated limb infusion was developed as a simple alternative to ILP by replacing the cardiopulmonary bypass equipment with a 3-way stopcock, extension tubing, and syringe to circulate the blood and infusate from the venous to the isolated arterial system.²²⁻²⁴ The technique of ILI requires repeated administration of boluses or a continuous rate infusion of infusate during the treatment interval to maintain vascular pressure and volume.

Historically, studies in horses have referred to ILRVI and ILIOI as perfusion. The term perfusion is probably a misnomer because these techniques involve a single injection that does not maintain vascular pressure or metabolic stability or actively circulate blood and drugs within the isolated vasculature. We suggest use of the term injection, as used in the bovine literature, instead of the term perfusion for these techniques.^{16,17}

In studies^{12,13,25} in horses, mean maximum synovial concentrations of 20 to 55 times the MIC_{BP} for amikacin and gentamicin have been reported for the techniques of ILRVI and ILIOI. Although these concentrations are much higher than the ideal theoretic minimum for maximum efficacy (which is ≥ 10 times the MIC of the bacteria) of a concentration-dependent antimicrobial, concern has been expressed about the reported variability in consistently generating concentrations ≥ 10 times the MIC_{BP} in synovial fluid isolated by a tourniquet.^{11-14,26,27} This variability is unfortunate because local delivery techniques achieve the highest synovial fluid C_{max}:MIC for aminoglycosides.

The objective of the study reported here was to compare ILRVI and ILI for delivery of amikacin to the synovial fluid of the DIP and MCP joints of standing horses. We hypothesized that for a 1-g dose of amikacin, ILI would achieve synovial fluid concentrations of amikacin ≥ 10 times the MIC₉₀ (80 $\mu\text{g/mL}$) and ≥ 10 times the MIC_{BP} (160 $\mu\text{g/mL}$) in both joints.²⁸⁻³¹ We hypothesized that ILI would achieve higher and less variable amikacin concentrations in synovial fluid than would be achieved with ILRVI and that an Esmarch tourniquet would prevent loss of antimicrobial into the systemic circulation for both techniques in standing

horses. Our goal was to determine whether ILI could function as an effective and practical replacement technique for ILRVI.

Materials and Methods

Animals—Six healthy Thoroughbred mares ranging from 8 to 20 years of age (mean \pm SD, 14 \pm 4.2 years) and weighing 484 to 627 kg (mean, 574 \pm 54 kg) were selected from the University of Florida College of Veterinary Medicine blood donor herd. All horses were free of obvious musculoskeletal disease and lameness in the distal portions of the limbs as determined by palpation and by trotting the horse in a straight line. All horses were housed in paddocks with unlimited access to hay and water throughout the study. The study was approved by the Animal Care and Use Committee at the University of Florida.

Study design—Each horse was randomly assigned to initially receive ILRVI or ILI in a crossover design; there was a minimum 14-day washout period between subsequent techniques. One forelimb was randomly assigned to receive 1 technique, with the contralateral forelimb receiving the alternate technique. Each horse was evaluated once daily for 72 hours after each technique for evidence of lameness and signs of infection or thrombosis of catheterized vessels. All horses were assessed for lameness immediately prior to the 12-hour arthrocentesis and at 24-hour intervals after the last arthrocentesis. Lameness was scored by use of the American Association of Equine Practitioners grading scale.³²

Experimental protocol for ILRVI—After each horse was sedated with xylazine hydrochloride^a (0.3 to 0.5 mg/kg, IV), the treatment forelimb was prepared for perineural anesthesia. Perineural anesthesia at the level of the median and ulnar nerves was chosen to facilitate catheter placement and eliminate pain and movement associated with the tourniquet, repeated arthrocentesis, and pressurization of the vasculature distal to the tourniquet. Perineural anesthesia was achieved with a 50:50 mixture of 2% lidocaine hydrochloride^b:0.5% bupivacaine hydrochloride^c; total volume of local anesthetic did not exceed 50 mL/forelimb. Once effects of perineural blockade were evident, detomidine hydrochloride^d (0.006 to 0.010 mg/kg, IV) was administered as necessary to minimize movement of horses throughout the procedure.

The forelimb was clipped from the coronary band to the proximal portion of the metacarpus and aseptically prepared. A pretreatment blood sample (time 0) was collected from a jugular vein into a sterile clot tube, and a pretreatment synovial sample was collected from the DIP and MCP joints. A new Esmarch tourniquet^e was centered over the proximal portion of the metacarpus, which isolated the digital flexor tendon sheath immediately distal to the tourniquet. The entire length of the tourniquet was used. Tourniquet application was always performed by 1 author (JAE) to minimize variation. Tourniquet application involved 2 initial circumferential wraps of moderate tension before placement of 2 sets of gauze sponges (each set consisted of three 4 \times 4-inch gauze sponges). Gauze sponges were folded in half and positioned over the medial and lateral neu-

rovascular bundles (middle of the metacarpus). The folded gauze sponges were not placed directly on the skin in an attempt to avoid displacement of the gauze in the direction of wrapping during tourniquet application. The tourniquet was applied as tightly as possible encompassing a full 360° with each stretch of the tourniquet.

A 22-gauge, 1-inch, over-the-needle catheter^f with an injection port^g was inserted into both the medial and lateral proper palmar veins in a proximal-to-distal direction at the level of the proximal sesamoid bones. Catheters were secured in place with cyanoacrylate glue^h and flushed with 3 mL of a 0.6% sodium citrate solution (500 mL of 0.9% NaClⁱ solution with 90 mL of 4% sodium citrate^j solution). A fluid pressure gauge^k was attached to the medial catheter by means of a pressure monitoring line,^l and an 18-gauge, 1-inch needle was secured to the limb with adhesive tape.^m The isolated distal portion of the limb was injected via the lateral catheter with 1 g of sterile amikacin sulfateⁿ diluted to a total volume of 60 mL by the addition of 0.6% sodium citrate solution. The injection was delivered during a 3-minute period by use of a 60-mL syringe; an extension line^o; and an 18-gauge, 1-inch needle. The extension line was flushed with 3 mL of sodium citrate solution before the needle was removed from the injection port. The tourniquet was maintained on the limb for 30 minutes after the initiation of amikacin administration. Pressure measurements were recorded before treatment with the tourniquet in place (time 0), 3 and 5 minutes after initiation of the treatment, and then at 5-minute intervals throughout the duration of the ILRVI technique until the tourniquet was removed. A blood sample was collected from the jugular vein immediately before the tourniquet was removed (at 30 minutes).

Synovial fluid samples were collected by repeated aseptic arthrocentesis accomplished by use of a 20-gauge, 1.5-inch needle at 0.5, 1, 2, 12, and 24 hours after amikacin injection. Samples of synovial fluid at 0.5 hours were obtained immediately after the tourniquet was removed.

All catheters were removed 10 minutes after the tourniquet was removed, and firm manual pressure over each vessel was applied for 10 minutes. After collection of the samples at 2 hours, the distal portion of the limb was bandaged and the horse returned to the paddock. The bandage was removed only for collection of samples; it was then reapplied. After samples were obtained at 24 hours, the bandage was not reapplied. All horses received a single dose of phenylbutazone^p (4.4 mg/kg, PO) immediately after collection of the 24-hour synovial sample.

Experimental protocol for ILI—Amikacin dose, sample collection techniques, and time points for the ILI technique were identical to those for the ILRVI technique. For ILI, the lateral palmar artery and medial palmar vein were catheterized with 22-gauge, 1-inch over-the-needle catheters^f with injection ports^g (Figure 1). The artery was catheterized before application of the tourniquet because of ease of palpation of the artery and insertion of the catheter. The vein was catheterized before or after application of the tourniquet, depending on vein distention. The extracorporeal portion of the

infusion system consisted of two 18-gauge needles used to establish access to the arterial and venous catheter injection ports. Three extension sets^{o,q,r} were connected in series to form the extracorporeal circuit between the catheterized vein and artery. A 3-way stopcock^s was positioned on the venous side of the circuit for attachment of syringes and to direct blood and infusate from the vein to the artery during circulation. Twenty-milliliter syringes were used to inject infusate through the 3-way stopcock into the proper palmar artery. A 6-mL syringe was used to circulate infusate and blood through the extracorporeal circuit from the vein to the artery. A fluid pressure gauge^k with a pressure monitoring line^l was attached to the extracorporeal circuit with a 3-way stopcock to direct flow to the pressure gauge only during measurement periods. All pressure measurements were obtained with the circuit open at both stopcocks.

Volume of the extracorporeal circuit (minus the pressure gauge and volume of the extension line) was 12 mL. The infusate consisted of 1 g of sterile amikacin diluted (total volume of 40 mL) by the addition of 0.6% sodium citrate solution and was administered as a bolus during a 3-minute period. The initial bolus of infusate was followed by boluses of 20 mL of 0.6% sodium citrate solution, which were administered during a 30-second period at 5, 10, and 15 minutes. A 10-mL bolus was administered during a 15-second period at 20 minutes. At 25 minutes, 12 mL of 0.6% sodium citrate solution was used to clear amikacin from the external portion of the extracorporeal circuit. The infusate and blood were circulated from the venous to the arterial circulation in 5-mL aliquots starting at 3 minutes and

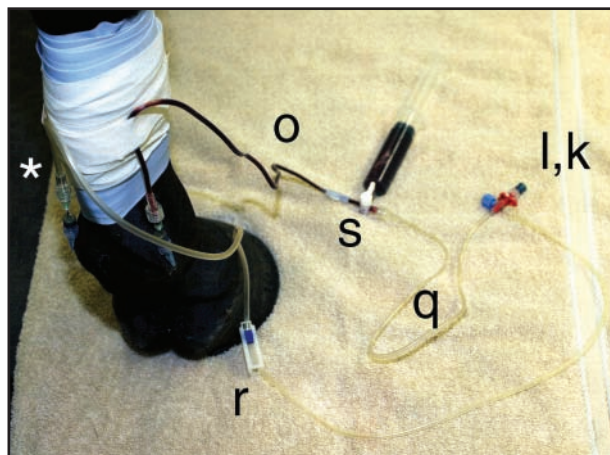


Figure 1—Photograph of the instrumentation (without pressure monitoring equipment attached) used to perform ILI in the forelimb of a standing, sedated horse with perineural anesthesia of the median and ulnar nerves. A new Esmarch tourniquet is positioned under high tension over the proximal portion of the metacarpus with the digital flexor tendon sheath completely isolated distal to the tourniquet. The medial catheter (white asterisk) is used to collect blood and infusate from the limb into the extracorporeal circuit, and the lateral catheter is used for infusion of blood and infusate into the artery. A 3-way stopcock and syringe are used to provide boluses of infusate into the artery and direct flow from the vein into the artery during circulation. o = 21-inch extension set with male luer-lock adapter (to lateral arterial catheter). s = 3-way stopcock with male luer-slip adapter. q = 20-inch interlink system with 3-way stopcock (l and k represent a pressure gauge and monitoring line, respectively, that would be attached to the 3-way stopcock of q). r = 41-inch blood collection set (to venous catheter).

Table 1—Mean \pm SD values for vascular pressure in the proper palmar vein during ILRVI and for extracorporeal circuit pressure during ILI in 6 horses.

Technique	Time (min)							
	0	3	5	10	15	20	25	30
ILRVI (mm Hg)	115 \pm 18	460 \pm 36	220 \pm 73	122 \pm 30	95 \pm 15	89 \pm 14	77 \pm 16	73 \pm 9
ILI (mm Hg)	95 \pm 21	275 \pm 27	315 \pm 27	318 \pm 32	292 \pm 40	300 \pm 32	287 \pm 21	134 \pm 30

Time 0 = Start of infusion.

continuing until 25 minutes. Circulation of the infusate was stopped only during pressure measurements, bolus injections, and the interval between 25 and 30 minutes. Pressure measurements were recorded at the same time points as for the ILRVI technique, except that an additional measurement was obtained just before each of the bolus administrations.

The water pressure gauge was tested against a pressure transducer connected to a physiologic monitor[†] for pressures that ranged from 100 to 300 mm Hg (20 pressure trials). The value for the water pressure gauge was always within \pm 5 mm Hg of the value for the pressure transducer connected to the physiologic monitor.

Sample analysis—Blood samples were allowed to clot and then were centrifuged at 1,000 \times g for 10 minutes. For each sample, serum was harvested, placed in a sterile plastic microcentrifuge tube,^u and stored at -80°C until assayed. Each synovial fluid sample was placed in a sterile plastic microcentrifuge tube^u and centrifuged at 11,300 \times g for 10 minutes; supernatant was then collected and transferred to another sterile plastic microcentrifuge tube.^u Samples were stored at -80°C until assayed.

Amikacin concentrations were determined by use of a fluorescence polarization immunoassay analyzer.^v Before analysis of serum samples was conducted, calibration of the analyzer was performed by use of internal standards provided by the manufacturer. Serum samples were analyzed directly without modification in accordance with a validated method.¹² The LLOQ was 0.8 μg of amikacin/mL of serum.

For synovial fluid samples, internal standards were assayed before evaluation of unknown samples. In addition, a calibration curve was made by fortifying blank synovial fluid with an amikacin reference standard^w dissolved in water. An aliquot (200 μL) of a fortified sample was then mixed with 10 μL of hyaluronidase^x (25 mg/mL) from bovine testes, vortexed, and injected directly onto the machine. The calibration curve included concentrations between 1 and 100 $\mu\text{g}/\text{mL}$. Accuracy of the method at 50, 10, and 5 $\mu\text{g}/\text{mL}$ was within (mean \pm SD) 2.9 \pm 2.5% of the expected value, and precision was within 2.7 \pm 1.4% of the mean. The LLOQ for synovial fluid was 1 $\mu\text{g}/\text{mL}$.

Synovial fluid samples obtained during the study at 0, 12, and 24 hours were analyzed in accordance with the manner described for the calibration curve. Because of the high concentrations of drug in the earliest samples, samples were diluted with blank synovial fluid prior to the addition of hyaluronidase. Samples obtained at 0.5 and 1 hour were diluted 1:20, and samples obtained at 2 hours were diluted 1:4. Calibration

samples were diluted in a similar manner to determine the effects of dilution on drug detection. All diluted samples were within 5.56% and 13.8% of the expected value for the 1:4 and 1:20 dilution, respectively.

Pharmacokinetic analysis—Pharmacokinetic values were determined for the DIP and MCP joints on the basis of amikacin concentrations for each method of drug delivery. Pharmacokinetic values were determined by use of computerized software.^y Noncompartmental analysis was used to generate the values for C_{max} in synovial fluid, time to C_{max} , elimination half-life, and mean residence time. The $\text{AUC}_{0-\infty}$ was calculated by use of the trapezoidal rule and based on the slope of the terminal phase of the concentration-time curve.

Statistical analysis—Statistical analyses were performed by use of a computer program.^z Normality of the data and equality of variances were assessed by use of the Kolmogorov-Smirnov and Levene tests, respectively. A 2-way ANOVA for repeated measurements was used to determine the effects of technique (ILRVI vs ILI), site of sample collection (DIP vs MCP joints), and the interactions between technique and site of sample collection on each measured and calculated pharmacokinetic variable. Variables that did not meet the assumptions of the ANOVA were rank-transformed prior to analysis. When appropriate, multiple pairwise comparisons were conducted by use of the Holm-Sidak test. A value of $P < 0.05$ was considered significant.

Results

Animals—None of the horses developed complications related to the regional limb techniques. All horses stood quietly for clipping, aseptic preparation of the skin, vessel catheterization, tourniquet application, and antimicrobial administration during median and ulnar perineural anesthesia. After application of both techniques, all horses had evidence of lameness (grade 3/5) for the treatment limb once perineural anesthesia became ineffective at the 12-hour arthrocentesis. None of the horses had visible evidence of lameness in the forelimbs when walking or trotting during evaluations conducted 48 and 72 hours after each technique. Placement of vascular catheters was not associated with complications, except in 1 horse in which the lateral palmar artery could not be catheterized and thus the medial palmar artery was used instead. On the basis of digital palpation at 24, 48, and 72 hours after catheters were removed, all palmar vessels appeared to remain patent.

Pressure measurements—Mean \pm SD maximum pressure for ILRVI was 460 \pm 36 mm Hg at 3 minutes,

which was the end of the injection period (Table 1). After the initial injection was complete, there was a rapid loss of venous pressure during the next 7 minutes, which resulted in a pressure of 122 ± 30 mm Hg at 10 minutes. Mean maximum pressure for ILI was 318 ± 32 mm Hg at 10 minutes. Mean pressure measurements obtained at 3, 5, 15, 20, and 25 minutes were similar to the mean pressure obtained at 10 minutes.

Serum concentrations of amikacin—Use of the Esmarch tourniquets prevented detectable movement of amikacin into the systemic circulation in all horses for both techniques. All amikacin concentrations obtained before (time 0) and at 30 minutes (before tourniquet removal) were less than the LLOQ for the assay (ie, < 0.8 $\mu\text{g/mL}$).

Synovial fluid concentrations of amikacin—The highest synovial fluid concentrations of amikacin were measured in samples obtained at 30 minutes (immediately after removal of the tourniquet), except for those joints in which a synovial fluid sample could not be col-

lected at that time point ($n = 1$) or in which there was obvious blood contamination of the sample to cause dilution (3). For all joints, the highest recorded concentration of amikacin in synovial fluid was used for data analysis (Figure 2; Table 2). For the ILI technique, all values for DIP joints of 1 horse were excluded from calculations because of blood contamination in synovial fluid samples obtained from 0.5 through 12 hours. Mean \pm SD C_{max} amikacin values for ILRVI were 542 ± 173 $\mu\text{g/mL}$ for the DIP joint and 445 ± 171 $\mu\text{g/mL}$ for the MCP joint. Mean C_{max} values for ILI were $1,090 \pm 170$ $\mu\text{g/mL}$ for the DIP joint and 514 ± 170 $\mu\text{g/mL}$ for the MCP joint. Use of ILI resulted in significantly ($P = 0.002$) higher amikacin concentrations in the DIP joint, compared with concentrations in the MCP joint. Concentrations of amikacin in the DIP joint for ILI were significantly ($P = 0.002$) higher than concentrations in the DIP and MCP joints for ILRVI. The coefficient of variation for ILRVI was 32% and 38% for the DIP and MCP joints, respectively, whereas the coefficient of variation for ILI was 16% and 33% for the DIP and MCP joints, respectively. Both techniques maintained detectable concentrations of amikacin in the synovial fluid of both joints for the entire 24-hour sampling period.

Discussion

The ILRVI and ILI are simple and safe procedures to perform on standing, sedated horses that have been administered appropriate nerve blocks. Use of both techniques did not result in complications. All catheterized vessels remained patent throughout the study. Administration of the infusate through the arterial system, as described in the study reported here, did not result in complications, although it has been questioned⁶ whether the arterial route can be used safely as a method for administration. An anticoagulant was required for ILI because blood was circulated through a simple extracorporeal circuit. Sodium citrate solution appeared to be a logical choice because it was a component of the amikacin formulation used in the study. Sodium citrate solution functioned adequately as an anticoagulant for ILI.

The pharmacodynamic variables that determine antimicrobial efficacy depend on the mechanism of bacterial killing. There are 2 primary patterns of anti-

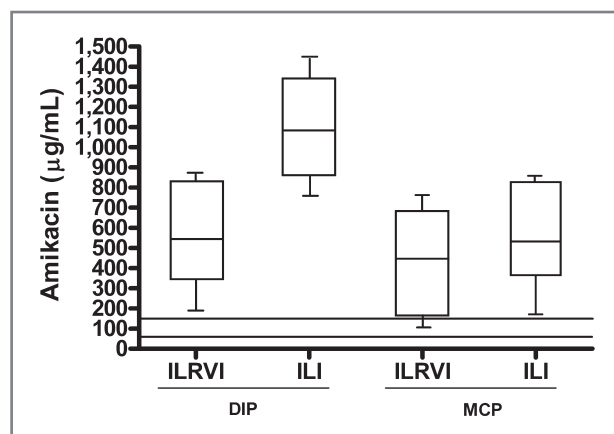


Figure 2—Mean, range, and 2 SD values for C_{max} amikacin concentrations in synovial fluid obtained from the DIP and MCP joints of 6 horses during ILRVI and ILI. Values for the DIP joint for ILI represent results for only 5 horses. The horizontal line within each box represents the mean. The upper and lower limits of each box represent the range, and the bars represent 2 SD. Horizontal lines at 160 and 80 $\mu\text{g/mL}$ represent 10 times the MIC_{BP} and 10 times the MIC_{90} , respectively.

Table 2—Mean \pm SD pharmacokinetic values for amikacin in synovial fluid of 6 horses after ILRVI and ILI.

Variable	DIP joint		MCP joint	
	ILRVI	ILI*	ILRVI	ILI
C_{max} ($\mu\text{g/mL}$)	542 ± 173	$1,090 \pm 170^\dagger$	445 ± 171	514 ± 170
T_{max} (h)	0.58 ± 0.18	$0.50 \pm 0.0^\ddagger$	0.75 ± 0.56	0.58 ± 0.19
$t_{1/2}$ (h)	6.11 ± 2.80	3.94 ± 0.83	3.5 ± 0.38	3.74 ± 0.58
$\text{AUC}_{0-\infty}$ ($\text{h} \times \mu\text{g/mL}$)	$1,601 \pm 1,622$	$2,360 \pm 1,471$	$1,519 \pm 531$	$1,810 \pm 687$
λ_z (1/h)	0.14 ± 0.07	0.18 ± 0.04	0.19 ± 0.22	0.19 ± 0.03
MRT (h)	2.52 ± 0.88	2.07 ± 0.71	2.97 ± 0.37	3.32 ± 0.44
Range ($\mu\text{g/mL}$)	322–837	865–1,338	171–697	352–836
CV (%)	32	16	38	33

*Values for ILI in the DIP joint represent results for only 5 horses. † Within a row, value differs significantly ($P = 0.002$) from other values. ‡ All T_{max} values were recorded at 0.5 hours.

T_{max} = Time until C_{max} ; $t_{1/2}$ = Half-life of elimination phase; λ_z = Slope of the terminal phase of the concentration-time curve; MRT = Mean residence time; CV = Coefficient of variation.

microbial killing (ie, concentration-dependent killing and time-dependent killing).³³ For concentration-dependent drugs, such as the aminoglycosides, the higher the drug concentration, the faster and more complete the eradication of pathogens. Drug concentrations of 10 times the MIC appear to be generally accepted as the minimum C_{\max} :MIC necessary to result in pure concentration-dependent killing for these agents.⁴ The goal of treatment with aminoglycosides should be optimization of peak concentrations by use of the highest possible nontoxic dose.³⁴

Techniques for local administration improve drug delivery to the site of an infection, which helps achieve a microbiological cure and reduces resistance selection.² For aminoglycosides, limiting resistance selection and achieving a microbiological cure are most accurately predicted by tissue concentrations of drug at the site of an infection.³ Direct and indirect methods of antimicrobial delivery achieve drug concentrations in tissues at the site of an infection that are orders of magnitude greater than those achieved by systemic administration. Reported gentamicin C_{\max} values in synovial fluid after systemic (IV) administration of 1 g, indirect administration via ILRVI, and direct intra-articular injection are 2.86, 589, and 64,535 $\mu\text{g/mL}$, respectively.^{10,25,35} These concentrations are 0.72, 147, and 16,134 times as high as the gentamicin MIC_{BP} of 4 $\mu\text{g/mL}$.³⁰ Analysis of these data reveals that local administration techniques are capable of delivering extremely high drug concentrations to the tissues, compared with concentrations achieved by use of systemic administration. Protocols for indirect delivery in horses have varied with regard to dose of antimicrobial, total volume administered, rate of administration, type of tourniquet, amount of time tourniquet is applied, sampling procedures, and positioning of the horse.⁶ Multiple protocols have yielded inconsistent antimicrobial concentrations in synovial fluid within and among studies. Such reported variability limits confidence in use of these techniques.

In the study reported here, use of ILRVI and ILI resulted in mean C_{\max} values in synovial fluid of both joints well in excess of 10 times the MIC_{BP} of amikacin (160 $\mu\text{g/mL}$). These values are consistent with pure concentration-dependent killing. Although reported mean C_{\max} values are important, it is essential to have consistency in generating C_{\max} :MIC ratios of ≥ 10 to reliably cause maximum bacterial killing and decrease resistance selection. The C_{\max} :MIC ratios were evaluated 3 ways for each technique. First, the lowest C_{\max} value for each joint in both techniques was compared to 10 times the MIC_{BP} for amikacin (160 $\mu\text{g/mL}$) and 10 times the MIC_{90} for amikacin (80 $\mu\text{g/mL}$). The lowest recorded C_{\max} for each joint in the ILRVI and ILI techniques was $> 160 \mu\text{g/mL}$. Second, the SD calculated for each joint in both techniques was multiplied by 2 to yield a theoretic range that would account for 95% of predicted amikacin concentrations in synovial fluid on the basis of the experimental data. Calculations for ILI resulted in predicted concentrations $> 160 \mu\text{g/mL}$ for both joints, whereas calculations for ILRVI resulted in predicted concentrations $> 160 \mu\text{g/mL}$ for the DIP joint but not for the MCP joint. All values for both techniques and both joints were $> 80 \mu\text{g/mL}$. Third,

the coefficient of variation for the joints examined in the study reported here ranged between 16% and 38%. These values are less variable and compare favorably with results of other studies^{12,13,25,27} that yielded coefficient of variation values between 36% and 116% for the same joints.

Despite the recorded low variability for amikacin concentrations within both joints for both techniques, it is interesting that there was low variability between joints for the ILRVI and high variability between joints for the ILI. Historically, authors in several ILRVI studies^{12,13,27} have reported higher concentrations of antimicrobial in the DIP joint, compared with concentrations in the MCP joint. In contrast, use of ILIOI appears to result in a higher concentration of antimicrobial in the MCP joint than in the DIP joint.^{13,14} In the study reported here, use of ILRVI did not result in significant differences in amikacin concentrations between the DIP and MCP joints; however, a higher mean C_{\max} was determined for the DIP joint, compared with the C_{\max} for the MCP joint. In contrast, ILI resulted in a significantly higher concentration of amikacin in the DIP joint than in the MCP joint. Other authors^{26,27,36} have attributed the variability between joints to the Fick principle, differences in synovial fluid volume, differences in synovial membrane surface area, or differences in the location of the site of antimicrobial administration. Our study was not designed to determine the cause for this phenomenon; however, our results support the contention that concentrations of antimicrobial in a joint may be a function of the administration location.^{26,27,36} In studies in which ILIOI yielded higher antimicrobial concentrations in the synovial fluid of MCP joints, investigators used the distal aspect of the third metacarpal bone for administration. In studies in which ILRVI yielded higher antimicrobial concentrations in the synovial fluid of DIP joints, investigators used the proper palmar vein for administration. It would seem plausible that the discrepancy in antimicrobial concentrations in joints is related to proximity of the administration site and direction of flow. It is plausible that ILI accentuated this difference by maintaining vascular pressure and directing infusate flow to the DIP joint preferentially over the MCP joint for a longer period. Despite the variability between joints, both techniques reliably attained amikacin concentrations in synovial fluid consistent with concentration-dependent killing for the DIP and MCP joints.

Although ILRVI is widely practiced clinically, to our knowledge, the study reported here is the first to validate the ability of an Esmarch tourniquet to prevent movement of antimicrobial into the systemic circulation in standing horses. Esmarch tourniquets were used because pneumatic tourniquets are expensive and are not commonly owned by equine practitioners. An appropriately sized tourniquet for accurate pressure gauge measurements and efficient pressure transfer to the underlying tissues should be approximately 20% wider than the diameter of the limb around which it is placed.³⁷ The stretched Esmarch tourniquet we used adequately approximated the appropriate size for the proximal portion of the metacarpus of a typically sized adult horse. This calculation is supported by a study²⁷

in which investigators used a similar tourniquet in anesthetized horses.

In the study reported here, vascular pressures distal to the tourniquet were recorded throughout the treatment period for each regional technique. These vascular pressures were recorded with the intent of relating the pressures to the specific horses that had systemic concentrations of amikacin during the treatment period. Detection of systemic concentrations of amikacin during the treatment period would have indicated loss of amikacin from the treatment region and failure of the Esmarch tourniquet. If there had been detectable leakage, it would have been possible to determine the maximum vascular pressure under the tourniquet before leakage was evident in standing horses. This may have provided a means of preventing systemic leakage by maintaining vascular pressure below a specific value. In this study, we did not detect systemic leakage, although this was limited to the detection capacity of the serum amikacin assay used; thus, an actual maximum vascular pressure was not identified for the Esmarch tourniquet in standing horses. Despite the fact that our study did not identify a maximum vascular pressure, it did establish 2 protocols that reliably prevented detectable systemic leakage of amikacin from the isolated distal portion of the forelimbs and a vascular pressure of 500 mm Hg distal (measured distal to the tourniquet) as a safe maximum to prevent loss of drug to the systemic circulation.

Studies³⁸⁻⁴⁰ in humans have revealed that pressure from application of an Esmarch tourniquet is directly related to the number of times the tourniquet is wrapped around the extremity, and studies in dogs in which Esmarch tourniquets were tested have yielded tissue pressures > 1,000 mm Hg. Studies^{7,9,10,14,15,27,36,41-43} in horses in which Esmarch tourniquets were used have not described the length of tourniquet or number of wraps around a limb. In the study reported here, the entire length of the Esmarch tourniquet was used to maximize the number of wraps around the limb and, as a result, the tissue pressure. In the study, the size and type of tourniquet, method of tourniquet application, site of vascular access, injection volume, and injection rate were standardized to limit variability. In addition, perineural anesthesia proximal to the tourniquet allowed all standing horses to bear weight without pain. This prevented actions such as pawing, walking, and striking. Preliminary studies conducted by our laboratory group revealed that sudden weight shifts on the forelimbs more than doubled intravascular pressures distal to the tourniquet (data not shown). Sudden large increases in vascular pressures distal to the tourniquet (ie, higher than the attained tourniquet tissue pressure) can lead to systemic leakage.³⁷ Our method of Esmarch application and perineural anesthesia for regional limb isolation yielded reliable isolation of the distal portion of the forelimbs for ILRVI and ILI in standing horses.

The establishment of appropriate dosing intervals was beyond the scope of our study, but their determination may improve efficacy by allowing time for adaptive resistant bacteria to revert to being susceptible before subsequent administration of antimicrobials. This is important because bacteria in an adaptive resistant

state can have transient reversible resistance to a second dose of aminoglycoside up to concentrations 128 times the MIC.⁴⁴ However, these same bacteria revert to an original susceptibility when exposed to drug-free media. The duration of adaptive resistance depends on the strain of bacteria, type of aminoglycoside, initial peak antimicrobial concentrations, and duration of exposure.⁴⁵⁻⁴⁷ Both techniques maintained detectable amikacin concentrations in synovial fluid for the 24-hour sampling period in all horses for both joints. This suggests that administration intervals should be > 24 hours to help prevent administering a dose to an adaptively resistant population.

Our study had some limitations. Collection of synovial fluid samples by repeated arthrocentesis may cause a variable degree of synovial hemorrhage. This may have affected our results in 2 ways. First, collection of a control sample may have initiated synovial hemorrhage that was exacerbated by an anticoagulant and high vascular pressure during the treatment period. This could have resulted in falsely increased amikacin concentrations in the synovial fluid caused by accumulation of blood that contained amikacin in the joints during treatment. Second, continued synovial hemorrhage as a result of repeated arthrocentesis may have led to dilution artifacts in the synovial fluid samples collected after treatment. These are valid limitations to the potential interpretation of our study. On the basis of gross observation of the synovial fluid at the time of collection and after centrifugation and evaluation of the resulting amikacin concentrations, it was determined that < 13% of the samples collected at 0.5 hours were overtly contaminated with blood. In addition, samples collected at 0.5 hours were obtained after removal of the tourniquet and aseptic preparation of the skin, which would allow time for amikacin washout from the vasculature in the distal portion of the limbs. Synovial fluid samples collected at 0.5 hours that were grossly contaminated with blood had dilution artifacts and were removed from all calculations, which resulted in use of synovial samples collected at 1 hour for determination of C_{max} . Global contamination of synovial samples was based on an evaluation similar to that used for the samples obtained at 0.5 hours and revealed an overall gross contamination of < 6% of all synovial samples collected. Blood contamination of collected synovial samples did not appear to be a limiting factor to the overall interpretation of our study.

When evaluating the efficacy of the Esmarch tourniquet, the likelihood of detecting minor loss of amikacin into the systemic circulation is low based on an LLOQ of 0.8 $\mu\text{g/mL}$ for the assay. Detection of slow partial leakage of the low regional dose of amikacin during the treatment period, compared with detection for a full systemic dose for which this assay was developed, may be beyond the limits of the assay. Despite this potential for low sensitivity, the aminoglycoside assay appears to be the criterion-referenced standard for detecting antimicrobial leakage attributable to inadequate tourniquet application in horses.¹²⁻¹⁵

In the study reported here, all horses had a grade 3/5 lameness in the treatment limb at the 12-hour evaluation, regardless of the indirect delivery method

used. Lameness was transient and not evident at the 48- and 72-hour posttreatment evaluations. Our study design did not provide for determining location of the lameness, so it was not possible to definitively determine whether the lameness was attributable to repeated arthrocentesis, the regional limb technique, or the Esmarch tourniquet. Evaluation of the tourniquet site for 72 hours after application did not reveal heat, swelling, or evidence of pain. We believe the lameness was attributable to repeated arthrocentesis during a short period. This idea is supported by a study¹⁰ that attributes lameness to trauma caused by multiple synovial samplings. In addition, other reports^{6,10,36} indicate a likelihood for lameness when repeated arthrocentesis is performed. In contrast, use of a temporary needle in an anesthetized animal or a synovial catheter is not associated with lameness.^{13,48} This would suggest that it is the trauma of repeated arthrocentesis and not the tourniquet or the injection-infusion technique that causes lameness. If these techniques were applied to affected animals in clinical situations, repeated arthrocentesis during a 24-hour period and clinical evaluation of patients during trotting would not be part of the treatment strategy. Therefore, we believe that the transient lameness was not clinically relevant and was a result of the sampling technique, rather than attributable to the antimicrobial delivery.

Both indirect delivery protocols used in the study reported here appeared to be safe and reliable for the delivery of amikacin to the DIP and MCP joints of standing horses. Because of the variability in aminoglycoside concentrations of synovial fluid reported in other studies, we investigated ILI as a possible replacement technique for ILRVI. On the basis of our results, ILRVI or ILI may be used for amikacin delivery to the DIP and MCP joints in standing adult horses. Our study differs from other studies because we used a novel and successful indirect technique for local delivery of antimicrobial in horses, improved the reliability for the delivery method in attaining synovial fluid concentrations of amikacin consistent with pure concentration-dependent killing in normal joints, and obtained these results in standing horses by use of a tourniquet technique that prevented detectable systemic leakage during administration. These findings have clinical relevance because, in our hospital, horses treated by indirect delivery of antimicrobials routinely receive more than a single treatment. Typically, only the first treatment is performed while a horse is anesthetized, and subsequent treatments are almost always performed in standing horses. The results of the study reported here support the use of ILRVI or ILI for attaining ideal amikacin concentrations in synovial fluid of the DIP and MCP joints of standing horses by use of an Esmarch tourniquet to isolate the distal portion of the forelimb. From a procedural perspective, the technically less demanding technique of ILRVI appears to be the most practical technique for indirect delivery when vascular access can be secured.

- a. AnaSed injectable, Lloyd Laboratories, Shenandoah, Iowa.
- b. Lidocaine 2%, Phoenix Pharmaceuticals, St Joseph, Mo.
- c. 0.5% Bupivacaine, Abbott Laboratories, Abbott Park, Ill.
- d. Dormosedan, Pfizer Animal Health, Exton, Pa.
- e. Esmark bandage, 6-inch × 12-feet, Ownes & Minor, Richmond, Va.

- f. BD Vialon, Becton-Dickinson, Sandy, Utah.
- g. Injection adapter, Medex, Dublin, Ohio.
- h. Super Glue, Loctite Corp, Avon, Ohio.
- i. 0.9% NaCl solution, Baxter Healthcare, Deerfield, Ill.
- j. Na citrate 4% solution, Jorvet Jorgensen Laboratories Inc, Loveland, Colo.
- k. Low-pressure gauge series 83KD, Marshalltown Instruments, Hastings, Neb.
- l. 36-inch double MLL monitoring line, Medex, Dublin, Ohio.
- m. White tape, 1 inch, Johnson & Johnson, New Brunswick, NJ.
- n. Amikacin sulfate, 500 mg/2 mL, Sicor Pharmaceuticals Inc, Irvine, Calif.
- o. 21-inch extension set, male luer-lock adapter, Baxter Healthcare, Deerfield, Ill.
- p. Phenylbutazone tablets, 1 gram, Animal Health, St Joseph, Mo.
- q. 20-inch Interlink System Novex 3-way stopcock, Baxter Healthcare, Deerfield Ill.
- r. 41-inch blood collection set, Baxter Healthcare, Deerfield, Ill.
- s. Three-way stopcock with male luer-slip adapter, Baxter Healthcare, Deerfield, Ill.
- t. Datascope Passport, Datascope Corp, Paramus, NJ.
- u. SealRite microcentrifuge tubes, 1.5 mL, USA Scientific, Ocala, Fla.
- v. TDx amikacin assay, Abbott Laboratories, Abbott Park, Ill.
- w. Amikacin, Sigma-Aldrich Chemicals, St Louis, Mo.
- x. Hyaluronidase, Sigma-Aldrich Chemicals, St Louis, Mo.
- y. WinNonlin, version 4.0, Pharsight, Cary, NC.
- z. Sigma Stat, Systat Software Inc, Richmond, Calif.

References

1. Streppa HK, Singer MJ, Budsberg SC. Applications of local antimicrobial delivery systems in veterinary medicine. *J Am Vet Med Assoc* 2001;219:40–48.
2. Hanssen AD, Osmon DR, Patel R. Local antibiotic delivery systems: where are we and where are we going? *Clin Orthop Relat Res* 2005;437:111–114.
3. Craig WA. Choosing an antibiotic on the basis of pharmacodynamics. *Ear Nose Throat J* 1998;77:7–11.
4. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis* 1998;26:1–10.
5. Stratton CW. Dead bugs don't mutate: susceptibility issues in the emergence of bacterial resistance. *Emerg Infect Dis* 2003;9:10–16.
6. Rubio-Martínez LM, Cruz AM. Antimicrobial regional limb perfusion in horses. *J Am Vet Med Assoc* 2006;228:706–712.
7. Whitehair KJ, Bowersock TL, Blevins WE, et al. Regional limb perfusion for antibiotic treatment of experimentally induced septic arthritis. *Vet Surg* 1992;21:367–373.
8. Santschi EM, Adams SB, Murphy ED. How to perform equine intravenous digital perfusion, in *Proceedings*. 44th Annu Meet Am Assoc Equine Pract 1998;198–201.
9. Palmer SE, Hogan PM. How to perform regional limb perfusion in the standing horse, in *Proceedings*. 45th Annu Meet Am Assoc Equine Pract 1999;124–127.
10. Whitehair KJ, Blevins WE, Fessler JF, et al. Regional perfusion of the equine carpus for antibiotic delivery. *Vet Surg* 1992;21:279–285.
11. Whitehair KJ, Adams SB, Parker JE, et al. Regional limb perfusion with antibiotics in three horses. *Vet Surg* 1992;21:286–292.
12. Murphey ED, Santschi EM, Papich MG. Regional intravenous perfusion of the distal limb of horses with amikacin sulfate. *J Vet Pharmacol Ther* 1999;22:68–71.
13. Butt TD, Bailey JV, Dowling PM, et al. Comparison of 2 techniques for regional antibiotic delivery to the equine forelimb: intraosseous perfusion vs. intravenous perfusion. *Can Vet J* 2001;42:617–622.
14. Mattson S, Boure L, Pearce S, et al. Intraosseous gentamicin perfusion of the distal metacarpus in standing horse. *Vet Surg* 2004;33:180–186.
15. Parra-Sanchez A, Lugo J, Boothe DM, et al. Pharmacokinetics and pharmacodynamics of enrofloxacin and a low dose of amikacin administered via regional intravenous limb perfusion in standing horses. *Am J Vet Res* 2006;67:1687–1695.
16. Navarre CB, Zhang L, Sunkara G, et al. Ceftiofur distribution

- in plasma and joint fluid following regional limb injection in cattle. *J Vet Pharmacol Ther* 1999;22:13–19.
17. Gagnon H, Ferguson JG, Papich MG, et al. Single-dose pharmacokinetics of cefazolin in bovine synovial fluid after intravenous regional injection. *J Vet Pharmacol Ther* 1994;17:31–37.
 18. Langer K, Seidler C, Partsch H. Ultrastructural study of the dermal microvasculature in patients undergoing retrograde intravenous pressure infusions. *Dermatology* 1996;192:103–109.
 19. Schaadt J, Crowley R, Miller D, et al. Isolated limb perfusion: a literature review. *J Extra Corpor Technol* 2002;34:130–143.
 20. Ariyan S. Regional isolated perfusion of extremities for melanoma: now a 26-year experience with drugs other than L-phenylalanine mustard. *Plast Reconstr Surg* 2003;111:1257–1261.
 21. Fraser M, Marentay P, Bertha R. A collaborative approach to isolated limb perfusion. *Aorn J* 1999;70:642–653.
 22. Thompson JF, Kam PC, Waugh RC, et al. Isolated limb infusion with cytotoxic agents: a simple alternative to isolated limb perfusion. *Semin Surg Oncol* 1998;14:238–247.
 23. Mian R, Henderson MA, Speakman D, et al. Isolated limb infusion for melanoma: a simple alternative to isolated limb perfusion. *Can J Surg* 2001;44:189–192.
 24. Bonenkamp JJ, Thompson JF, de Wilt JH, et al. Isolated limb infusion with fotemustine after dacarbazine chemosensitisation for inoperable loco-regional melanoma recurrence. *Eur J Surg Oncol* 2004;30:1107–1112.
 25. Werner LA, Hardy J, Bertone AL. Bone gentamicin concentration after intra-articular injection or regional intravenous perfusion in the horse. *Vet Surg* 2003;32:559–565.
 26. Scheuch BC, Van Hoogmoed LM, Wilson WD, et al. Comparison of intraosseous or intravenous infusion for delivery of amikacin sulfate to the tibiotarsal joint of horses. *Am J Vet Res* 2002;63:374–380.
 27. Rubio-Martínez LM, López-Sanroman J, Cruz AM, et al. Evaluation of safety and pharmacokinetics of vancomycin after intravenous regional limb perfusion in horses. *Am J Vet Res* 2005;66:2107–2113.
 28. Adamson PJ, Wilson WD, Hirsh DC, et al. Susceptibility of equine bacterial isolates to antimicrobial agents. *Am J Vet Res* 1985;46:447–450.
 29. Hirsh DC, Jang SS. Antimicrobial susceptibility of bacterial pathogens from horses. *Vet Clin North Am Equine Pract* 1987;3:181–190.
 30. NCCL standards. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. 4th ed. NCCLS document M7-A4. Wayne, Pa: National Committee for Clinical Laboratory Standards, 1997.
 31. Schneider RK. Common bacteria encountered in septic arthritis, in *Proceedings*. 44th Annu Meet Am Assoc Equine Pract 1998;152–158.
 32. Swanson T. *Guide for veterinary service and judging of equestrian events*. 3rd ed. Golden, Colo: American Association of Equine Practitioners, 1984.
 33. Nicolau DP, Quintiliani R, Nightingale CH. Antibiotic kinetics and dynamics for the clinician. *Med Clin North Am* 1995;79:477–495.
 34. 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.
 35. Anderson BH, Firth EC, Whittem T. The disposition of gentamicin in equine plasma, synovial fluid and lymph. *J Vet Pharmacol Ther* 1995;18:124–131.
 36. Rubio-Martínez LM, López-Sanroman J, Cruz AM, et al. Evaluation of safety and pharmacokinetics of vancomycin after intraosseous regional limb perfusion and comparison of results with those obtained after intravenous regional limb perfusion in horses. *Am J Vet Res* 2006;67:1701–1707.
 37. Grice SC, Morell RC, Balestrieri FJ, et al. Intravenous regional anesthesia: evaluation and prevention of leakage under the tourniquet. *Anesthesiology* 1986;65:316–320.
 38. McLaren AC, Rorabeck CH. The pressure distribution under tourniquets. *J Bone Joint Surg Am* 1985;67A:433–438.
 39. Grebing BR, Coughlin MJ. Evaluation of the Esmark bandage as a tourniquet for forefoot surgery. *Foot Ankle Int* 2004;25:397–405.
 40. Abraham E, Amirouche FML. Pressure controlled Esmarch bandage used as a tourniquet. *Foot Ankle Int* 2000;21:686–689.
 41. Mattson SE, Pearce SG, Boure LP, et al. Comparison of intraosseous and intravenous infusion of technetium Tc 99m pertechnetate in the distal portion of forelimbs in standing horses by use of scintigraphic imaging. *Am J Vet Res* 2005;66:1267–1272.
 42. Kettner NU, Parker JE, Watrous BJ. Intraosseous regional perfusion for treatment of septic physitis in a two-week-old foal. *J Am Vet Med Assoc* 2003;222:346–350.
 43. Rubio-Martínez L, López-Sanroman J, Cruz AM, et al. Medullary plasma pharmacokinetics of vancomycin after intravenous and intraosseous perfusion of the proximal phalanx in horses. *Vet Surg* 2005;34:618–624.
 44. Gilleland LB, Gilleland HE, Gibson JA, et al. Adaptive resistance to aminoglycoside antibiotics in *Pseudomonas aeruginosa*. *J Med Microbiol* 1989;29:41–50.
 45. Begg EJ, Peddie BA, Chambers ST, et al. Comparison of gentamicin dosing regimens using an in-vitro model. *J Antimicrob Chemother* 1992;29:427–433.
 46. Bryan LE, Van Den Elzen HM. Effects of membrane-energy mutations and cations on streptomycin and gentamicin accumulation by bacteria: a model for entry of streptomycin and gentamicin in susceptible and resistant bacteria. *Antimicrob Agents Chemother* 1977;12:163–177.
 47. McGrath BJ, Lamp KC, Rybak MJ. Pharmacodynamic effects of extended dosing intervals of imipenem alone and in combination with amikacin against *Pseudomonas aeruginosa* in an in vitro model. *Antimicrob Agents Chemother* 1993;37:1931–1937.
 48. Pille F, De Baere S, Ceelen L, et al. Synovial fluid and plasma concentrations of ceftiofur after regional intravenous perfusion in the horse. *Vet Surg* 2005;34:610–617.