Clinical and pharmacokinetic effects of regional or general anaesthesia on intravenous regional limb perfusion with amikacin in horses

by

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DEDICATION

To my wife, Celia:

Met of sonder, solank dit saam met jou is...

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LIST OF ABBREVIATIONS

%	Percentage
µg/ml	Micrograms per millilitre
ANOVA	Analysis of variance
AUC_{0-24}	Area under the concentration-time curve over 24 hours post-administration
$AUMC_{0-24}$	Area under the concentration-first-moment-time curve over 24 hours post-administration
bwt	Body weight
CFU	Colony forming unit
cm	Centimetre
C_{max}	Maximum concentration
CNT	Standing sedation without regional anaesthesia (treatment group)
CV	Coefficient of variation
g	Grams
g	Standard gravity
GA	General anaesthesia (treatment group)
HR	Heart rate
i.v.	Intravenous
IVA	Standing sedation with intravenous regional anaesthesia (treatment group)
IVRLP	Intravenous regional limb perfusion
kg	Kilogram
L	Litre
L/min	Litre/minute
LIFT	Number of times the limb was lifted
mg	Milligrams
MIC	Minimum inhibitory concentration
mL	Millilitre
mmHg	Millimetres of mercury
MPC	Mutant protection concentration
MRT	Mean residence time

No	Number
PAE	Post-antibiotic effect
PNA	Standing sedation with perineural regional anaesthesia (treatment group)
RLP	Regional limb perfusion
rPkp	Regional pharmacokinetic parameters
RR	Respiratory rate
SD	Standard deviation
T0	Time before administration
T0.5	0.5 hours post-administration
T1.5	1.5 hours post-administration
$T_{1/2}$	Terminal elimination half-life
T12	12 hours post-administration
T24	24 hours post-administration
T6	6 hours post-administration
USA	United States of America
VAS	Visual analogue scale of discomfort
λ_z	Lamda-Z

SUMMARY

Clinical and pharmacokinetic effects of regional or general anaesthesia on intravenous regional limb perfusion with amikacin in horses

Mahne, AT *et al.* University of Pretoria, 2012

Introduction: Orthopaedic infection in horses is a common and life-threatening condition, which requires early and aggressive treatment. Apart from systemic antimicrobial therapy, local antimicrobial therapy is often added to the treatment regime with the aim to obtain high levels of antimicrobials in the affected areas. This includes intravenous regional limb perfusion (IVRLP) with antimicrobials.

Antimicrobial IVRLP consists of the infusion of an antimicrobial agent under pressure into the vascular system of a portion of a limb isolated from the systemic circulation by the use of a proximally placed tourniquet. The infusion can take place via a peripheral blood vessel or the medullary cavity of a bone. Synovial antimicrobial concentrations after IVRLP are often > 50 times the minimum inhibitory concentration (MIC) for clinically-relevant bacterial organisms.

Intravenous regional limb perfusion can be performed on the standing, sedated horse or with the horse under general anaesthesia. In an attempt to increase the comfort of the standing horse and decrease movement due to discomfort, a local anaesthetic agent is often administered as perineural regional anaesthesia or intravenous regional anaesthesia. Some authors regard antimicrobial IVRLP under general anaesthesia to be superior to that on the standing sedated horse due to better efficacy of the tourniquet and absence of movement of the animal.

This study aimed to evaluate the effects of regional or general anaesthesia on the animal's comfort and synovial pharmacokinetic parameters of amikacin administered by IVRLP to horses.

Materials and methods: Eight healthy horses received 4 treatments of amikacin IVRLP in a randomised, blinded, cross-over design: under standing sedation without

regional anaesthesia (CNT), under standing sedation with intravenous regional anaesthesia (IVA), under standing sedation with perineural regional anaesthesia (PNA) or under general anaesthesia (GA). For all treatments, a tourniquet was applied proximal to the carpus and the cephalic vein was used for injection of the perfusate. Synovial fluid amikacin concentrations in the middle carpal joint were measured over 24 hours and the regional synovial pharmacokinetic parameters (rPkp) calculated. Heart and respiratory rates, visual analogue scale (VAS) of discomfort, number of times the limb was lifted (LIFT) and number of additional sedations administered were recorded. ANOVA cross-over analysis was applied with significance level at $P < 0.05$.

Results: One horse was removed from the analysis due to recumbency during IVA and CNT treatments. Amikacin concentrations and rPkp did not differ significantly among treatments. Maximum amikacin concentrations (mean ± CV; µg/mL) in middle carpal joint synovial fluid were 239 ± 0.97, 172 ± 0.55, 344 ± 1.25 and 503 ± 1.26 for CNT, IVA, PNA and GA groups, respectively. Scores of VAS (mean ± SD) were significantly lower with PNA (19 ± 16) versus IVA (69 ± 36) or CNT (81 ± 14). Significantly lower LIFT (mean ± SD) occurred with PNA (20 ± 22) versus CNT (54 ± 24). No horses with PNA treatment required any additional sedations during the procedure, while treatments CNT and IVA required a median (range) of 1.5 (0-3) and 1 (0-4) additional sedations, respectively.

Conclusions: This study concluded that PNA was most effective in providing comfort to horses undergoing IVRLP under standing sedation. General or regional anaesthesia with IVRLP did not have any significant effect on synovial amikacin concentrations or rPkp.

Relevance: The comfort of horses undergoing standing IVRPL can be increased by performing perineural anaesthesia prior to the treatment. Use of general anaesthesia to improve antimicrobial synovial concentrations during IVRLP is not justified based on this study.

CHAPTER 1: INTRODUCTION

1.1 Background

Orthopaedic infection in the horse is a common life-threatening condition which necessitates early and aggressive treatment. Elimination of the bacterial organisms from the infected structure by means of flushing or drainage, surgical debridement and antimicrobial therapy are the mainstay of treatment.[1,2] Antimicrobial treatment usually includes both systemic and local administration of the agent.[3] As early as the 1970s, antimicrobial regional limb perfusion (RLP) in combination with systemic antimicrobial therapy, was shown to be superior to systemic antimicrobial administration alone for treatment of orthopaedic infections in humans.[4,5] Since the introduction of equine antimicrobial RLP in 1992,[6,7] this technique has become an important part of management of equine orthopaedic infections.[8-10]

Regional limb perfusion can be performed with the horse under general anaesthesia[11-15] or on standing sedated horses.[16-18] Regional anaesthesia is often administered to standing sedated horses undergoing RLP to increase their comfort and to decrease their movement.[16,17] However the effects of regional anaesthesia on the comfort of the horse and the regional antimicrobial pharmacokinetic parameters have not been objectively evaluated. Likewise, no objective comparison of the regional antimicrobial pharmacokinetic parameters after IVRLP on standing sedated horses versus horses under general anaesthesia is available.

1.2 Objectives

The objectives of this study were to investigate:

- The effects of regional anaesthesia on the comfort of the standing, sedated horse undergoing amikacin IVRLP.
- The effects of regional or general anaesthesia on the synovial pharmacokinetic parameters of amikacin administered by IVRLP to horses.

1.3 Hypothesis

The following hypotheses were made:

- The addition of regional anaesthesia (intravenous or perineural) during amikacin IVRLP would cause a significant increase in the horses' comfort level as well as in the synovial fluid amikacin concentrations compared to IVRLP without regional anaesthesia in standing, sedated horses.
- Amikacin concentrations in synovial fluid after IVRLP on standing, sedated horses with the concurrent use of intravenous or perineural regional anaesthesia would be similar to those obtained after IVRLP under general anaesthesia.

1.4 Benefits

The results of this study would be directly applicable to the clinical setting since antimicrobial IVRLP is commonly used by equine practitioners. If the concurrent use of regional or general anaesthesia affects the pharmacokinetics of the concentration-dependent antimicrobial agent administered by IVRLP, the dosing and treatment regimen in the clinical setting may need to be adapted accordingly. In addition, the results of this study may give base for further research on the concurrent use of a local anaesthetic agent with time-dependent antimicrobial agents, which efficacy is directly related to the maintenance of antimicrobial concentrations above the antimicrobial minimum inhibitory concentration (MIC) for the infecting agent.

Comparison of pharmacokinetic parameters in standing versus anaesthetised horses will answer the question raised by the common belief that antimicrobial RLP under general anaesthesia provides higher antimicrobial concentrations in the regional joints being perfused compared to IVRLP on standing, sedated horses. This difference is thought to be due to better tourniquet efficacy on the anaesthetised horse.

The effect of regional anaesthesia in standing sedated horses during IVRLP on the comfort of the horse may also give rise to recommendations to increase the comfort of equine patients undergoing standing IVRLP.

CHAPTER 2: LITERATURE REVIEW

2.1 Orthopaedic infections in horses

Orthopaedic infection in horses is a life-threatening disease that affects horses of all ages and includes osteomyelitis and synovial infections.[19] Orthopaedic infection can cause chronic lameness and loss of use despite intensive and expensive treatment, with some cases suffering from uncontrollable pain and contralateral limb laminitis, necessitating euthanasia.[9,20,21] Therefore, cases with orthopaedic infections should be treated early and aggressively. Treatment includes drainage, surgical debridement and antimicrobial therapy, both locally and systemically, with targeted local antimicrobial therapy being an integral part.[1,2,9,20,22,23]

2.1.1 Aetiology

Reported causes of orthopaedic infection in horses include:

- Penetrating wounds to a synovial structure. These accounted for 75% of 214 septic synovial structures, from 206 adult horses, presented to two equine referral hospitals.[24]
- Iatrogenic infection, occurring after intrasynovial injection or surgery. Intra-synovial injections have been implicated as cause in 4% of cases treated for septic arthritis[24] and 0.9% of 682 horses undergoing elective arthroscopy developed septic arthritis in a retrospective review study.[25]
- Horses of all ages undergoing orthopaedic operations involving internal fracture fixation are at risk of developing infection, with a reported post-operative surgical site infection of 28 % in a recent study.[9]
- Extension of infection from a limb with cellulitis into a synovial structure, which accounted for 6.4% of 93 infected synovial structures.[23]
- Haematogenous spread of infection. This most commonly occurs in foals as a result of bacteraemia originated from a septic focus.[26] In a recent report, 5.7% of 423 bacteraemic foals suffered from osteomyelitis and 13.5% from septic

arthritis.[26] Haematogenous spread of bacteria to synovial structures in the adult horse is rare,[2,23] and is speculated as cause in those cases where a definitive cause for a synovial infection cannot be determined. Haematogenous spread has been reported in 9.5% of 126 adult horses with septic synovial structures[2] and in 13% of 93 infected synovial structures in a more recent study.[23]

2.1.2 Treatment and prognosis

Treatment for cases of orthopaedic infections includes drainage, debridement, lavage and systemic and local antimicrobial therapy. For septic synovial structures, arthroscopic or tenoscopic lavage and debridement or arthrotomy or tenosynotomy can also be performed.[1,2,9,20,22,23] Local antimicrobial therapies used in clinical cases include intravenous or intraosseous RLP, intrasynovial antimicrobial injection and intrasynovial antimicrobial constant rate infusion.[1,2,20,22,23] Since the introduction of equine antimicrobial RLP in 1992,[6,7] this technique has become an important part of management of equine orthopaedic infections.[8-10]

The reported survival rate of foals with osteomyelitis, of which 70% had concurrent septic arthritis, was 81%.[27] In another study 77% of foals with septic arthritis, of which 22% had concurrent osteomyelitis, survived to discharge.[8] Multiple joint involvement, systemic illness and increased duration of treatment were negatively associated with survival.[8,27,28] Single joint infection, initiation of treatment within 24 hours and the use of multi-modality treatment, including antimicrobial IVRLP, arthrotomy and arthroscopic lavage and debridement, were positively associated with survival, compared to foals receiving systemic antimicrobials and through-and through-needle-lavage only.[8] Of foals treated for septic arthritis that survived, 29% raced as adults,[28] whereas 60% of survivor-foals treated for osteomyelitis raced as adults.[27]

Survival-to-discharge rates for adult horses treated for septic synovitis are 72%,[22] 84%[23] and 90%.[24] Factors associated with a negative outcome were positive bacterial culture,[24] involvement of tendon or bone,[23] involvement of multiple synovial

structures[22] and osteomyelitis.[29] Increased duration of clinical signs before initiation of treatment has also been negatively associated with survival,[30] although this has not been observed in more recent studies.[22,24] Rates of return to athletic function of survivors were 48%,[22] 54%[23] and 69%.[24]

Horses undergoing internal fixation of long bone fractures or arthrodeses that did not develop post-operative surgical site infection were 7.25 times more likely to be discharged than those developing infection, with the latter having a survival-to-discharge rate of 59%.[9] In that study, patients treated for post-operative surgical site infection with antimicrobial RLP showed a weak positive trend (P=0.07) for increased survival to discharge versus those not treated with antimicrobial RLP.[9]

2.2 Antimicrobial regional limb perfusion

2.2.1 Regional limb perfusion with antimicrobials

Antimicrobial RLP is the infusion of an antimicrobial agent under pressure into the vascular system of a portion of the limb isolated from the systemic circulation by the use of a proximally placed tourniquet,[5] which is usually kept in place for 30 minutes.[11,13,31] An additional tourniquet can also be applied distal to the area to be perfused, which will effectively decrease the area to be perfused.[21] The antimicrobial agent is commonly injected into a vein (intravenous RLP) or into the medullary cavity (intra-osseous RLP) of the isolated area.[5,11,17] During RLP, injection under pressure leads to retrograde filling of the venous system of the perfused soft tissue as well as the central medullary and cortical venous system of the bone.[5,32] It is believed that the increased intravascular pressure causes dilation of venous capillaries, lymphatic vessels and post-capillary venules.[33] The contacts between adjacent venous endothelial cells relax, which results in the formation of small gaps in the vessel walls. These temporary changes are believed to be due to the rapid change in venous pressure and do not damage the cells; however, it leads to greater diffusion of substances out of the vessels, resulting in high antimicrobial concentrations in the perfused tissues.[33]

Antimicrobial agents which have been used and studied in equine RLP include amikacin, gentamicin, cephazolin, ceftiofur, vancomycin, enrofloxacin, penicillin, cefoxitin, imipenem, meropenem, chloramphenicol, ticarcillin, timentin, and erythromycin.[6,7,13,16-18,34-40]

2.2.2 The importance of high antimicrobial concentrations

Minimum inhibitory concentration (MIC) is the minimum concentration of an antimicrobial agent that is required to inhibit visible growth of a 10^5 colony forming units (CFU) of bacteria per millilitre (CFU/ml) solution after incubation for 18 – 24 hours *in vitro*.[41] Antimicrobial concentrations higher than MIC, demonstrated by a high peak concentration-to-MIC ratio, may lead to greater effectiveness of the antimicrobial agent,[42] as well as the prevention of emergence of antimicrobial-resistant bacterial strains,[41] especially when referring to concentration-dependent antimicrobials. This group of antimicrobials exerts greatest efficacy by achieving a transient peak concentration, without requiring the maintenance of antimicrobial concentration above MIC during the interdosing interval.[42] This is also explained by the post-antibiotic effect (PAE) of concentration-dependent antimicrobials. The PAE is defined as the delay of logarithmic growth of a microorganism after the antimicrobial concentration has decreased below MIC following the last administration.[43] The magnitude of PAE increased with higher peak concentration of concentration-dependent antimicrobials such as amikacin when evaluated in *vitro* for equine isolates of Methicillin-Resistant *Staphylococcus aureus.*[43]

The concentration of an antimicrobial agent required to prevent the proliferation of selective resistant organisms, usually susceptible to an antimicrobial agent, is termed the mutant protection concentration (MPC). This is defined as the antimicrobial concentration required to inhibit the growth of the least susceptible cell in a high density bacterial population, i.e. ≥ 10^9 CFU/ml of bacterium.[41] Achievement of the MPC is therefore desirable; however, whereas the MIC for amikacin against *E.coli* is 4 µg/ml, the MPC is ≥ 32 µg/ml.[41] These high concentrations can be obtained with RLP[12] without the use of high systemic doses, which may be expensive and detrimental to the animal.[41]

Intrasynovial antimicrobial concentrations of 50 times the MIC for most susceptible pathogens have been achieved after IVRLP without high systemic concentrations.[12] Also, antimicrobial concentrations in synovial cavities remained above MIC for longer than 24 hours after RLP.[44] This may be beneficial with the use of time-dependent antimicrobial agents, which require the maintenance of antimicrobial concentration above MIC during most of the interdosing interval for optimal antibacterial effcts.[41]

Ischaemic areas are a major concern in chronic orthopaedic infections due to inadequate penetration of systemically administered antimicrobial agents. However, these ischaemic areas may be reached with RLP.[5] Also, antimicrobial efficacy in a purulent environment may be decreased due to low pH, high protein binding and enzymatic degradation.[45] The high antimicrobial concentrations achieved in purulent material with RLP may increase the antibacterial efficacy.[5]

It should be noted that controversy exists regarding the use of local antimicrobial agents. Once the anitmicrobial concentration falls below MIC, these low concentrations could lead to the development of local and systemic resistant bacterial infections.[46,47] This risk should be weighed up against the benefits of obtaining high local antimicrobial concentrations without high systemic concentrations.

2.2.3 Amikacin in regional limb perfusion

Amikacin is an aminoglycoside antimicrobial, and has been shown to be the most effective antimicrobial agent against common bacterial isolates from equine musculoskeletal infections.[19] Aminoglycosides are concentration-dependent antimicrobial agents, and therefore their greatest efficacy is seen at maximal peak concentrations.[42] Conditions decreasing the effectiveness of aminoglycosides include anaerobic conditions, reduction in pH and hyperosmolarity, as these may inhibit or block the transportation of the aminoglycosides across the cytoplasmic membranes of the bacteria.[48]

Various MICs have been reported for amikacin, ranging from 4 µg/mL to 16 µg/mL.[12,15,18,49,50] A maximum concentration versus minimum inhibitory concentration ratio, termed the pharmacokinetic-pharmacodynamic breakpoint, of 8-10 is needed for aminoglycosides to maximize their bactericidal effects.[51,52] This makes aminoglycosides suitable for treatment with RLP.[31] Gentamicin is another widely used aminoglycoside in equine practice,[53] but bacterial resistance to gentamicin is common.[54] Resistance against amikacin is less commonly encountered, making it the aminoglycoside of choice in treating equine musculoskeletal infections.[54] Amikacin is widely administered by RLP in the clinical setting. The first study to evaluate the pharmacokinetic properties of amikacin after administration by RLP in the horse, demonstrated amikacin intrasynovial concentrations above 50 times MIC.[12] In a recent study on the use of antimicrobial IVRLP for treatment of injuries to distal limb synovial structures in horses, 2 g of amikacin were used in 30 out of 44 of the cases.[40] In another recent study, amikacin was used in 81% of cases receiving antimicrobial RLP for treatment of established synovial infections.[22]

2.3 Tourniquets used for regional limb perfusion in horses

With IVRLP, a tourniquet is used to isolate an area of the distal limb from its venous and arterial supply.[4,5] A second tourniquet can also be applied distally to decrease the area to be perfused.[21] After intravenous or intraosseous injection of the perfusate into the isolated area, the venous pressure rises.[5,17,55] This is believed to cause dilation of venous capillaries and post-capillary venules, resulting in increased diffusion of antimicrobial molecules into the interstitium.[33] The maximum venous pressure is the highest achievable intravenous pressure distal to the tourniquet before leakage of perfusate into the systemic venous system occurs and it is a reflection of tourniquet efficacy.[55] Human studies have shown that the tourniquet pressure required to maintain vascular isolation is inversely proportional to tourniquet width.[56,57] Therefore wider tourniquets should be more efficient in providing vascular occlusion and it is generally recommended that a tourniquet width of at least the diameter of the extremity be used.[56,57]

Different types of tourniquets are used clinically when performing IVRLP in horses. An elastic tourniquet (Esmarch bandage) is commonly used because of its availability, low cost and ease of application.[37,58,59] A pneumatic tourniquet can also be used and yields more constant and controlled pressure.[55] Two recent equine studies compared the use of different tourniquets during IVRLP on standing horses. In 2010, Levine *et al.* reported that pneumatic and wide elastic tourniquets placed proximal to the carpus were not statistically different in achieving high synovial fluid amikacin concentrations when performing amikacin IVRLP via the cephalic vein.[18] Both the pneumatic and the wide elastic tourniquets were superior to the use of a narrow elastic tourniquet.[18] In 2011, Alkabes *et al.* reported that a wide elastic tourniquet placed distal to the carpus was superior to a pneumatic tourniquet in achieving high synovial fluid amikacin concentrations when performing IVRLP via the palmar digital vein.[16] Thus the use of a wide elastic tourniquet was statistically similar when palced above the carpus and superior to a pneumatic tourniquet when palced distal to the carpus for IVRLP when used in standing horses. Combined with its lower cost and ease of application the wide elastic tourniquet is a common choice for the equine practitioner.

2.4 Use of anaesthesia in antimicrobial regional limb perfusion

Antimicrobial regional limb perfusion can be performed with the horse under general anaesthesia[11-15] or standing sedation.[16-18] Treatments performed on standing sedated horses are normally preferred as they eliminate the extra costs and risks associated with general anaesthesia.[60] Although antimicrobial RLP is considered clinically effective on the standing horse as an adjunct for the treatment of distal limb infections,[3,22,40] some authors question the efficacy and repeatability of pressure when applying a tourniquet on the standing horse.[37] Although both a scintigraphic study[58] and contrast radiological study[3] have demonstrated adequate vascular occlusion in the standing horse with an elastic rubber tourniquet, movement of the conscious animal is believed to cause inadvertent leakage of the perfusate from the perfused area due to failure of vascular occlusion by the tourniquet.[61,62] Sudden shifting of weight from one limb to another in the standing horse can double the venous pressure in the perfused area, which might overpass the maximal venous

pressure and therefore cause leakage.[17] Therefore, regional anaesthesia (perineural anaesthesia or addition of local anaesthetic agent to the perfusate) is often performed concurrently on standing sedated horses undergoing antimicrobial RLP to increase their comfort and to decrease their movement.[16,17,31,35] However, its effects on the comfort of the animal and the regional pharmacokinetic parameters of the antimicrobial (e.g. peak concentration, duration of concentration above MIC) have not been evaluated.

Local anaesthetic agents are cocaine derivatives that consist of an aromatic region and a basic amine side, joined by an ester or amide bond. The local anaesthetic agents block the propagation of the action potential, due to inhibition of the voltage gated sodium channels.[63,64] Local anaesthetic angents commomly used in equine practice include lignocaine, mepivacaine and bupivacaine . Lignocaine has a rapid onset (~3 minutes) and a medium duration of anaesthesia (60 - 120 minutes) and is widely used for local anaesthesia.[63,64] Mepivacaine has similar pharmacocological properties to lignocaine, has a similar onset of action and a slighty longer duration (± 20%) of anesthesia.[64] Mepivacaine is also less irritating to tissue than other local anaesthetics.[65] Bupivacaine has similar structural properties than lignocaine, exept that the amine-containing group is a butyl piperidine. It is a very potent local anaesthetic agent with a slower onset of action (~15 minutes) and cabable of producing prolonged local anaesthesia (400 - 450 minutes).[64] Local anaesthetics commonly used with RLP are mepivacaine and lignocaine.[35,59]

CHAPTER 3: OBJECTIVES AND HYPOTHESES

3.1 Objectives

The objectives of the study were to evaluate the effects of:

- Regional anaesthesia on the comfort level of standing, sedated horses undergoing IVRLP with amikacin.
- Regional or general anaesthesia on the synovial pharmacokinetic parameters of amikacin administered by IVRLP to horses.

3.2 Hypotheses

- It was hypothesised that the addition of regional anaesthesia (intravenous or perineural) during IVRLP would cause a significant increase in the horses' comfort level as well as in the synovial fluid amikacin concentrations compared to IVRLP without regional anaesthesia in standing, sedated horses.
- It was further hypothesised that amikacin concentrations in synovial fluid after IVRLP on standing, sedated horses with the concurrent use of intravenous or perineural regional anaesthesia would be comparable to those obtained after IVRLP under general anaesthesia.

CHAPTER 4: MATERIALS AND METHODS

4.1 Animals

4.1.1 Sample size

By convention, at least 15 degrees of freedom were required for the error term in the appropriate analysis variance (Appendix 1). In this study a sample size of 8 animals met this requirement.[66]

4.1.2 Inclusion criteria and care of the animals

Eight clinically healthy adult Nooitgedacht mares were used in the study. Inclusion criteria were normal physical examination, absence of lameness at walk and trot, absence of palpable musculoskeletal abnormalities on the front limbs and no history of any drug administration for at least eight weeks prior to the study. The study was approved by the Animal Use and Care Committee and the Research Committee of the University of Pretoria (Project number V046-10).

The mares were housed in outdoor sheltered pens with free access to hay and water, except for the last 6 hours prior to general anaesthesia when food was withheld. Clinical examinations were performed daily from the day before treatment until 24 hours after collection of the last sample. In between treatments, the mares returned to the herd and had free access to pasture grazing, hay and water.

4.2 Study design

A prospective, blinded, four-period, cross-over study was conducted. Each mare received 4 treatments with a wash-out period of one week between treatments. The order of treatments was randomly allocated by the consulting statistician (Appendix 2). The first treatment was assigned to either the left or right front limb by the flip of a coin with subsequent treatments alternated between the front limbs.

4.3 IVRLP treatments

Amikacin (Amikacin Fresenius, Bodene (Pty Ltd., Port Elizabeth, South Africa) IVRLP was administered to all horses on 4 different occasions:

- Under standing sedation without regional anaesthesia (**CNT**);
- Under standing sedation with addition of 20 mL of lignocaine (Lignocaine 2%, Bayer, Isando, South Africa) to the perfusate (**IVA**);
- Under standing sedation 20 minutes after performing perineural anaesthesia, consisting of an ulnar, median and musculocutaneous nerve block with 35 mL of lignocaine[67] (**PNA**);
- Under general anaesthesia (**GA**).

The same person (LMRM) performed the nerve blocks (Figure 1) 20 minutes prior to IVRLP on all horses for treatment PNA and placed a light bandage over the injection sites in all horses undergoing treatments CNT, IVA and PNA to ensure blinding of the primary author (Figure 2).

Figure 1: The ulnar (shown in the picture), median and musculocutaneous nerves were blocked with 35 mL 2% lignocaine in Perineural Anaesthesia (PNA) treatment.

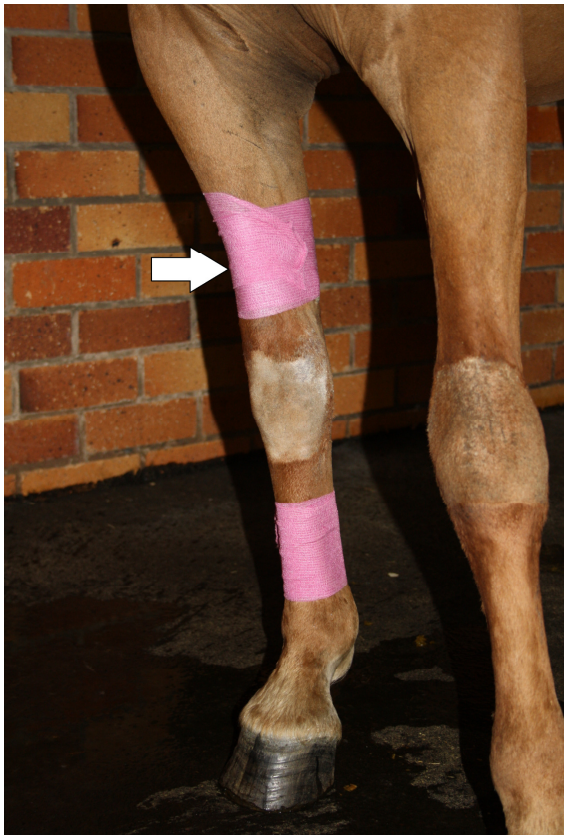


Figure 2: The distal antebrachium was covered with a light bandage (arrow) in all horses in the standing treatments to conceal whether a median, ulnar and musculocutaneous nerve block had been performed or not. The bandage around the mid-metacarpus indicated the limb to be treated.

In treatments CNT, IVA and PNA, horses were sedated with romifidine (Sedivet Boehringer Ingelheim, Ingelheim Pharmaceuticals (Pty) Ltd., Randburg, South Africa) (0.03 mg/kg bwt i.v.) and butorphanol (Torbugesic, Fort Dodge Animal Health, Iowa, USA) (0.01 mg/kg bwt i.v.) 5 minutes prior to the placement of the tourniquet. Additional sedation (romifidine [0.015 mg/kg bwt i.v.] and butorphanol [0.01 mg/kg bwt i.v.]) was administered to standing horses if during the treatment period the primary author (ATM) considered that the procedure could not continue any longer without additional sedation due to discomfort of the horse. The number of additional sedations administered was recorded.

For treatment GA, a 16-gauge, 2-inch over-the-needle catheter (Nipro Safelet Cath, Niporo Corporation, Osaka, Japan) was aseptically placed into the left or right jugular vein. Horses were sedated with romifidine (0.09 mg/kg bwt i.v.) and 5 minutes later

anaesthesia was induced with ketamine (Ketamine-Fresenius, Safeline Pharmaceuticals, Johannesburg, South Africa) (2 mg/kg bwt i.v.) and diazepam (A-Lennon Diazepam, Pharmacare Limited, Woodmead, South Africa) (0.025 mg/kg bwt i.v.). General anaesthesia was maintained with an intravenous continuous rate infusion of a combination of 45 mg romifidine, 50 g guaifenesin (GGE Powder, Kyron Laboratories (Pty) Ltd., Benrose, South Africa) and 1 g ketamine in 1 L of Lactated Ringer's solution (Bodene (Pty) Ltd., Port Elizabeth, South Africa), which was administered to effect for the duration of the treatment. Oxygen (15 L/min) was administered intra-nasally for the duration of general anaesthesia, which ended with removal of the tourniquet. Recovery from anaesthesia was unassisted.

For all treatments, a 12-cm wide elastic tourniquet (Esmarch bandage, Surgimed, Pretoria, South Africa) was placed by the primary author (ATM) in the distal aspect of the antebrachium in the same manner in all horses (Figure 3). The primary author injected the perfusate over 60-90 seconds into the cephalic vein at the palmaro-medial aspect of the carpus using a 23 gauge butterfly needle directed distad (Scalp vein set, Shandong Zibo Shanchuan Medical Instrument Co. Ltd., Zibo, China) (Figure 4). The tourniquet was kept in place for 30 minutes following the end of the injection in all treatments.

The total volume of the perfusate was 50 mL in all treatments and was divided into three syringes, which were administered always following the same sequential order:

- For treatments GA, PNA and CNT the first syringe contained 20 mL of Lactated Ringer's solution; for treatment IVA, the first syringe contained 20 mL of 2% lignocaine. The primary author was blinded to the contents of the syringe.
- In all groups the second syringe contained 1 g of amikacin diluted in Lactated Ringer's solution to a total volume of 20 mL.
- In all groups the third syringe contained 10 mL of Lactated Ringer's solution.

4.4 Sample collection

Prior to commencement of amikacin IVRLP, the dorsal aspect of the carpus was clipped with an electrical clipper with a number 40 blade. Before arthrocentesis, the

area was scrubbed three times with chlorhexidine soap (Hibiscrub, Medical and Hospital Supplies, Pretoria West, South Africa), after which it was sprayed three times with chlorhexidine in alcohol (Hibitane, Medical and Hospital Supplies, Pretoria West, South Africa) and wiped in between sprays with sterile gauze swabs. A fourth spray with chlorhexidine in alcohol was left on the area. Samples of synovial fluid (approximately 0.6 mL) were aseptically collected from the middle carpal joint via dorsal arthrocentesis[67] using a 22-gauge, 1.5-inch needle (Figure 5) and placed in heparinised tubes (Becton Dickinson (Pty) Ltd., Woodmead, South Africa). Synovial samples were collected before IVRLP and tourniquet placement (T0), 30 minutes after perfusate administration and before tourniquet release (T0.5), and 1.5 (T1.5), 6 (T6), 12 (T12) and 24 (T24) hours post-administration of perfusate. Blood samples were collected by puncture of the jugular vein into heparinised tubes at T0 and T0.5. All samples were centrifuged (1.207 *g*, 8 minutes) and the supernatant was frozen at -80 ºC for amikacin concentration analysis.

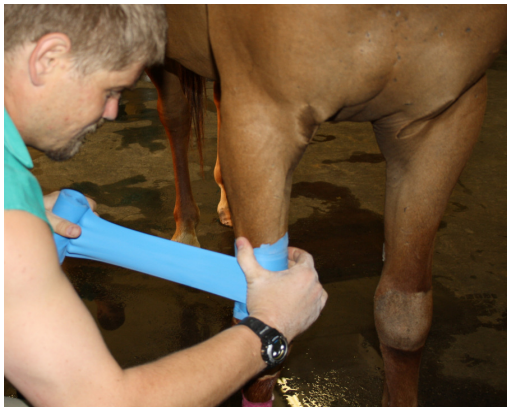


Figure 3: A 12-cm wide elastic tourniquet was applied to the distal aspect of the antebrachium.

Figure 4: The cephalic vein was used for injection of perfusate.

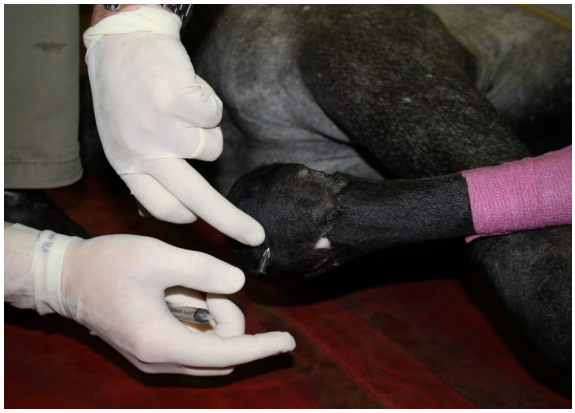


Figure 5: Samples of synovial fluid (approximately 0.6 mL) were aseptically collected from the middle carpal joint via dorsal arthrocentesis using a 22-gauge, 1.5-inch needle.

4.5 Clinical assessment

Assessment of discomfort of the standing, sedated horses undergoing amikacin IVRLP (CNT, IVA and PNA) was performed by using a visual analogue scale of discomfort (VAS).[68] The VAS scale consisted of a horizontal line of 100 mm with no markings, with 0 mm representing no discomfort and 100 mm representing severe discomfort (horse becoming aggressive, pawing intensively and/or rearing) (Appendix 3). The 30-minute period of IVRLP was subdivided into two 15-minute periods (0-15 minutes and 15-30 minutes) and a VAS score was subjectively assigned to each period by the primary author who was blinded to the treatment group. If a horse received additional sedation during the first 15-minute period, a VAS score of 100 was assigned to both the first and second periods. If the first additional sedation was administered during the second period, only the second period was assigned a score of 100. The average of the scores assigned to both periods was calculated and used in the statistical analysis. The number of times the horse lifted the treated limb (LIFT) during the procedure was also recorded. Heart rate (HR) and respiratory rate (RR) of each horse were recorded 5 minutes after sedation before tourniquet placement (baseline) and 15, 30 and 40 minutes after perfusate administration in treatments CNT, IVA and PNA (See Appendix 4 for data capture sheet).

4.6 Amikacin measurement

Amikacin concentrations were measured using fluorescence polarization immunoassay[18] (Integra 400 Plus, Roche Products (Pty) Ltd., Randburg, South Africa). In-house performance of the assay was assessed measuring 4 independent, 5 serial dilutions from a mother solution of amikacin in synovial fluid (500 µg/mL) and using diluent from the calibration kit. The coefficient of variation, limit of quantification and coefficient of determination were calculated based on standard curves.

4.7 Pharmacokinetic analysis

Regional synovial pharmacokinetic parameters were estimated by non-compartmental analysis using a software package (SummitPK Solutions, SAS institute, Cary, USA). The area under the concentration-time curve over 24 hours post-administration (AUC_{0-24}), area under the concentration-first-moment-time curve over 24 hours post-administration ($AUMC_{0-24}$), mean residence time (MRT) and terminal elimination half-life ($T_{1/2}$) were estimated. The AUC_{0-24} was calculated by log-linear trapezoidal rule and Lamda-Z (λ_z) was estimated with uniform weighting. The observed peak raw mean concentration (C_{max}) was obtained from inspection of the raw data. A fixed theoretical minimum inhibitory concentration (MIC) of 4 µg/mL was adopted for pharmacokinetic analysis.[12,15,50]

4.8 Statistical analysis

All the statistical analyses were performed by Statistical Analysis System (SAS, SAS Institute, Cary, USA). A linear mixed model analysis of variance (ANOVA) cross-over and cross-over repeated measures was used. Analysis of variance was implemented for the statistical comparison of clinical parameters, including VAS, LIFT, HR and RR. The potential effect of LIFT or VAS on systemic and synovial fluid amikacin concentrations at T0.5 was evaluated by correlation analysis. Drug concentrations, AUC_{0-24}, C_{max}, and HR at 15 min were transformed logarithmically before analysis to meet normality assumptions. Post-hoc Tukey-Kramer mean separation was used to identify differences among least squared means. Significant difference was set at $P < 0.05$.

CHAPTER 5: RESULTS

5.1 Animals

The mares had a mean (range) weight and age of 390 (557 - 430) kg and 7 (5.5 - 10) years, respectively. All horses received all treatments. Recovery from general anaesthesia was uneventful in all cases. One mare (horse 7) became recumbent during CNT 29 minutes after perfusate administration and stood up 10 minutes after removal of the tourniquet. The same mare also became recumbent during IVA at 5 minutes and stood up at 26 minutes after perfusate administration. In both instances the mare's clinical parameters were within normal limits at 40 minutes after perfusate administration and T1.5. This mare's rPkp and clinical parameters are included in Appendix 5 and Appendix 6 respectively. However, due to the fact that the mare was not standing during the procedures, the data from this mare was excluded from all the treatment groups for statistical analysis.

5.2 Clinical assessment

The clinical parameters for horses 1-6 and 8 are shown in Appendices 7 - 13. Results of VAS scores, LIFT, HR and RR are summarized in Table 1. Significantly lower VAS scores (mean ± SD) were observed with PNA (19 ± 15) compared to CNT (81 ± 13) ($P < 0.001$) or IVA (69 ± 36) ($P < 0.001$). Significantly lower LIFT (mean ± SD) was observed with PNA (20 ± 20) compared to CNT (54 ± 22) ($P < 0.036$). No additional sedations were necessary with PNA in comparison with treatments CNT and IVA, where median (range) of 1.5 (0-3) and 1 (0-4) additional sedations were administered, respectively. Median (range) time to first additional sedation were 17 (8 - 28.5) and 17.75 (9 - 25.3) minutes in CNT and IVA, respectively. No significant differences in HR and RR were observed among any of the standing treatments at any time.

Table 1. Mean ± SD results of number of times the limb was lifted (LIFT), visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR) in standing, sedated horses undergoing amikacin intravenous regional limb perfusion without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) or with perineural anaesthesia (PNA). HR and RR were measured 5 minutes after sedation before tourniquet placement (baseline) and 15, 30 and 40 minutes after perfusate administration. Significant differences (P < 0.05) between groups are depicted with different superscript letters for the specific outcome.

Parameter	CNT	IVA	PNA
LIFT (number of times)	54 ± 22^{a}	53 ± 33^{ab}	20 ± 20^{b}
VAS	81 ± 13^{a}	69 ± 36^{a}	19 ± 15^{b}
HR (beats/min)			
Baseline	28 ± 5^{a}	32 ± 8^{a}	29 ± 4^{a}
15 min	31 ± 3^{a}	35 ± 5^{a}	33 ± 3^{a}
30 min	35 ± 9^{a}	34 ± 4^{a}	33 ± 5^{a}
40 min	30 ± 4^{a}	29 ± 3^{a}	33 ± 4^{a}
RR (breaths/min)			
Baseline	11 ± 3^{a}	12 ± 6^{a}	11 ± 6^{a}
15 min	10 ± 2^{a}	13 ± 7^{a}	9 ± 1^{a}
30 min	12 ± 4^{a}	15 ± 7^{a}	9 ± 4^{a}
40 min	10 ± 3^{a}	10 ± 4^{a}	9 ± 3^{a}

5.3 In-house performance of the amikacin concentration measurement assay

The results of the 4 independent serial dilutions are summarised in Appendix 14. The in-house performance of the amikacin measurement kit revealed a coefficient of variation of <10%, a limit of quantification of 31.25 µg/mL and a coefficient of determination > 0.99.

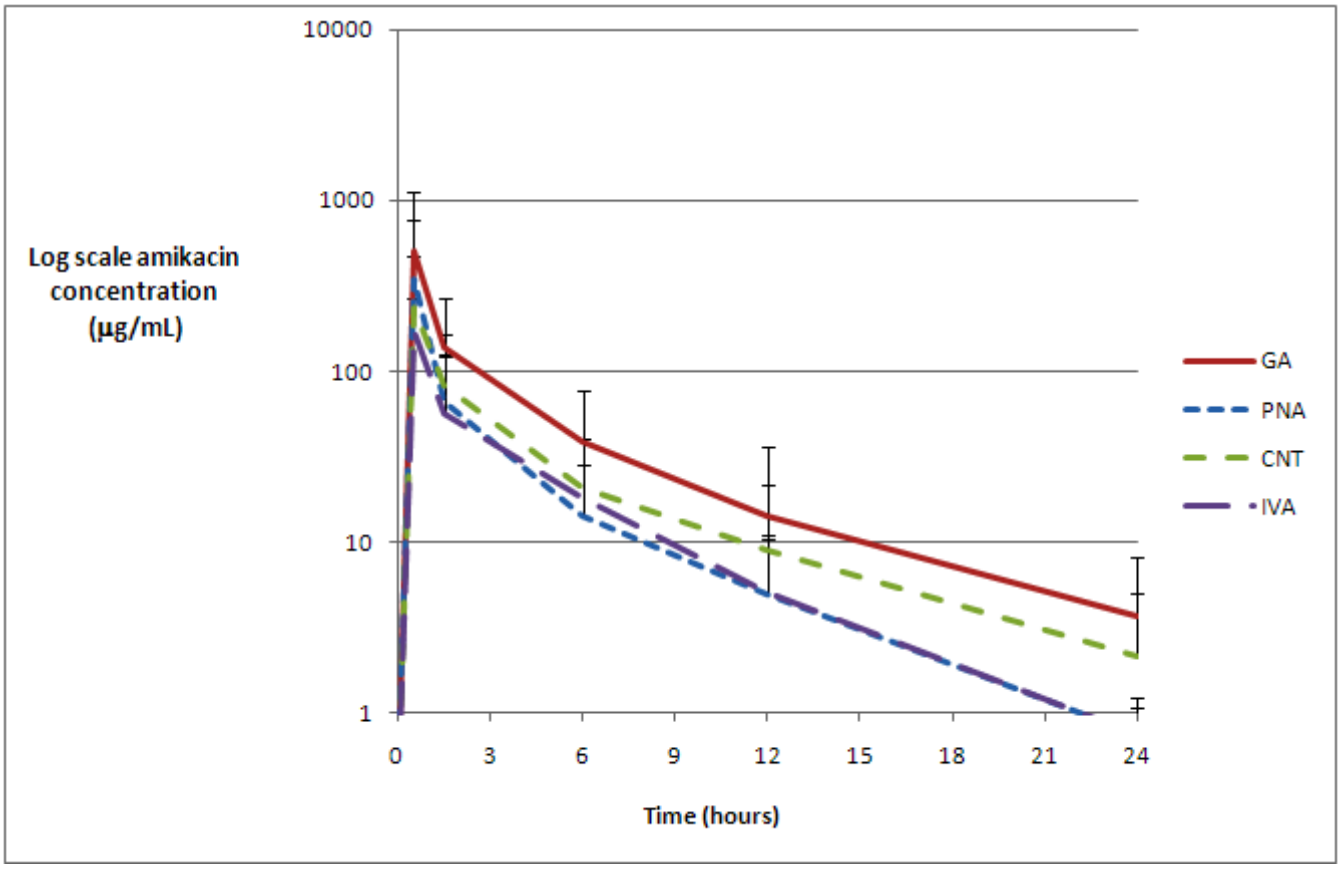
5.4 Amikacin measurements and rPkp parameters

5.4.1 Synovial concentrations

A synovial sample obtained at T0.5 in a horse from treatment GA was excluded from the analysis due to measurement error.

Results of measured amikacin concentrations in synovial fluid from middle carpal joint are illustrated in Graph 1 and summarised in Appendix 15 for GA, Appendix 16 for PNA, Appendix 17 for IVA and Appendix 18 for CNT. No significant differences in amikacin concentrations were found among treatments at any of the time points. Maximum amikacin concentrations (mean ± SD; µg/mL) in middle carpal joint synovial fluid were 239 ± 231, 172 ± 94, 344 ± 431 and 503 ± 634 for CNT, IVA, PNA and GA groups, respectively. (Although high, these standard deviations are correct. In the rest of the results, the mean regional pharmacokinetic parameters and amikacin concentrations are presented with the coefficient of variance, rather than standard deviation.)

Graph 1: Log scale middle carpal synovial fluid amikacin concentration (µg/ml) plotted against time (hours) over 24 hours after amikacin intravenous regional limb perfusion in standing, sedated horses without regional anaesthesia (CNT), with intravenous regional anaesthesia (IVA) or with perineural regional anaesthesia (PNA) and in horses under general anaesthesia (GA). The graph was plotted from the raw data. Vertical bars indicate standard deviation.



5.4.2 Pharmacokinetic parameters

The estimated rPkp for middle carpal synovial fluid amikacin concentrations are summarised in Table 2. No significant differences were found among treatments for any of the rPkp. Mean synovial concentrations exceeded the theoretical MIC of 4 µg/mL for at least 12 hours in all treatment groups.

Table 2: Results of the regional pharmacokinetic parameters (rPkp) (mean ± coefficient of variation) [range] of amikacin in middle carpal synovial fluid after amikacin intravenous regional limb perfusion in standing, sedated horses without regional anaesthesia (CNT), with intravenous regional anaesthesia (IVA) or with perineural regional anaesthesia (PNA) and in horses under general anaesthesia (GA). No significant differences were found among groups. CV = coefficient of variation.

PK parameter	CNT	IVA	PNA	GA
AUC_{0-24} (h*µg/mL)	907 ± 1.15 [33.1 - 2942]	518 ± 0.83 [173 - 1289]	462 ± 0.98 [132 - 1526]	1075 ± 1.23 [165 - 3703]
$AUMC_{0-24}$ (h*h*µg/mL)	3974 ± 1.11 [105 - 8070]	1575 ± 0.89 [526 - 5179]	2100 ± 0.92 [491 - 3894]	4933 ± 1.14 [723 - 14877]
C_{max} (µg/mL)	239 ± 0.97 [6.66 - 615]	172 ± 0.55 [63.7 - 311]	344 ± 1.25 [28.3 - 1039]	503 ± 1.26 [76.6 - 1752]
MRT (h)	4.48 ± 0.55 [2.32 - 4.17]	3.79 ± 0.16 [2.71 - 6.47]	3.60 ± 0.26 [2.39 - 9.25]	4.88 ± 0.42 [2.67 - 5.16]
$T_{½}$ (h)	4.24 ± 0.35 [2.76 - 7.12]	4.42 ± 0.34 [3.01 - 3.89]	3.91 ± 0.17 [2.62 - 4.53]	4.30 ± 0.13 [3.07 - 4.21]
T_{max} (h)	0.5 ± 0 [0.5 - 0.5]	0.5 ± 0 [0.5 - 0.5]	0.5 ± 0 [0.5 - 0.5]	0.5 ± 0 [0.5 - 0.5]

5.4.3 Systemic amikacin concentrations

Systemic venous blood amikacin concentrations are summarised in Appendix for GA, Appendix for PNA, Appendix for IVA and Appendix for CNT. All systemic amikacin concentrations at T0 were below the limit of quantification. Mean ± CV systemic amikacin concentrations (µg/mL) at T0.5 were 1.5 ± 1.7 for GA, 4.5 ± 0.8 for IVA, 6.2 ± 0.9 for PNA, and 9.4 ± 0.5 for CNT. No significant differences were found among treatments. Also, no significant association between LIFT or VAS on systemic amikacin concentrations at T0.5 or synovial fluid C_{max} was observed.

CHAPTER 6: DISCUSSION

6.1 Clinical effects of regional anaesthesia on standing horses undergoing IVRLP

In the present study, the use of perineural regional anaesthesia increased the comfort level of standing, sedated horses undergoing IVRLP; however, the use of intravenous regional anaesthesia in standing, sedated horses did not have any beneficial clinical effect compared with IVRLP without regional anaesthesia.

The failure of intravenous regional anaesthesia to reduce the discomfort of horses is unclear but might be attributed to pain caused by the tourniquet being located proximal to the desensitized area. In humans undergoing intravenous regional anaesthesia, a double cuff tourniquet is used,[69,70] where the proximal cuff is initially inflated to perform the intravenous injection of the local anaesthetic, which desensitizes the appendage distal to the cuff within 5 minutes. Thereafter, the distal cuff, situated immediately distal to the proximal cuff and on the desensitised area, is inflated, which will maintain vascular isolation of the distal appendage for the duration of the procedure. The proximal cuff, which is located outside the desensitized area, is deflated, thus not contributing to pain or discomfort. To the authors' knowledge, no information is available on the use of double cuff tourniquets in horses. When perineural regional anaesthesia was performed in the present study, the tourniquet was placed on the area presumed to be desensitized by the nerve block. This might explain the increase in comfort levels in standing, sedated horses with the addition of perineural regional anaesthesia in this study.

The reported volume of local anaesthetic agent included in the perfusate of horses undergoing RLP varies, ranging from 1 to 45 mL.[22,35] In humans, when intravenous regional anaesthesia (also named the Bier block) is performed, the conventional dosage of lignocaine is 3 mg/kg.[69,70] This dose is decreased in paediatric emergency medicine to 1.5 mg/kg to reduce the risk of local anaesthetic toxicity developing at the time of tourniquet release, which can result in a sudden peak of systemic local anaesthetic concentration.[71,72,73] In the canine patient a dosage of 3mg/kg has been

reported.[74] If these dosages (1.5 – 3 mg/kg) are extrapolated directly to the 450-kg adult horse, a volume of 34 – 77 mL would be needed. However, in bovine patients volumes of 15-30 mL of 2% lignocaine are considered effective in providing distal limb anaesthesia with intravenous regional anaesthesia.[75,76] Considering the ample difference in dosages and volumes used for intravenous regional anaesthesia among dogs, humans and bovines, it is not known whether the volume of local anaesthetic administered in the current study was sufficient to provide anaesthesia of the limb. Further studies would be necessary to determine the volume of local anaesthetic necessary for effective intravenous regional anaesthesia of the equine limb.

Although mepivacine is less irritating to tissue than lignocaine,[65] the latter was used in the study due to unavailability of a registered 2% mepivacine product in South Africa licensed for animal use.

6.2 Effects of regional or general anaesthesia for IVRLP on regional antimicrobial pharmacokinetics

This study showed that synovial fluid amikacin concentrations after IVRLP on standing, sedated horses with the concurrent use of regional anaesthesia were similar to those obtained after IVRLP under general anaesthesia, although mean concentrations and rPkp were numerically higher in GA. Similarly, synovial fluid amikacin concentrations after standing IVRLP with the use of regional anaesthesia were similar to those after standing IVRLP without regional anaesthesia, although mean synovial amikacin concentrations and rPkp were numerically higher in PNA treatment than in CNT, which were higher than IVA. A possible explanatory reason for the lack of statistical differences between groups is the high variability in the synovial amikacin concentrations as observed in previous studies.[12,15,18] Some of the factors which could have contributed to this variability include antimicrobial dose, perfusate volume and speed of injection, use of local anaesthetics, blood contamination of synovial fluid samples and administration of IVRLP to standing versus recumbent animals. These factors are discussed below.

Despite the high variability in synovial amikacin concentrations, in our study the mean synovial fluid C_{max}/MIC exceeded the pharmacokinetic-pharmacodynamic breakpoint of aminoglycosides of 8-10 in all treatments which may maximize their bactericidal effects.[51,52] These high concentrations obtained in the synovial fluid might help decrease the development of or treat resistant bacterial infections,[41,43] although local antimicrobial use have been associated with the development of resistant local and systemic bacterial infections, as discussed in the literature review.[46,47] Due to the high variability of synovial fluid C_{max}, accurate prediction of individual amikacin concentrations in the middle carpal joint after IVRLP is difficult in clinical cases.

A possible source of variability in the synovial amikacin concentrations is different degree of blood contamination among the synovial fluid samples.[17] The degree of blood contamination of each sample was not recorded. Arthrocentesis of the middle carpal joint is regarded as easy[67] and all arthrocentesises were performed by the primary author in a similar manner, after which all samples were centrifuged before storage in an attempt to minimize the effect of blood contamination. However, blood contamination of the synovial samples as a souce of variability in the synovial fluid concentrations cannot be completely exldued.

Sudden weight shifts on the front limbs of a horse can double intravascular pressure distal to the tourniquet,[17] which might exceed the maximum venous pressure and cause leakage of the perfusate from the isolated area. This has been suggested as possible cause of perfusate leakage.[18,61,62] However, in the present study no significant effect of limb movement on synovial or systemic amikacin concentrations could be demonstrated. It is possible that quantifying the number of times the limb was lifted was not sensitive enough for detecting an effect of limb movement on synovial or systemic amikacin concentrations, as LIFT could have been either a gentle lift of the limb or an aggressive pawing.

Intravenous pressure in the perfused area is also affected by the injected perfusate volume and the speed of injection.[55] Reported volumes of perfusate for antimicrobial IVRLP with a tourniquet placed proximal to the carpus are 50[18] and 100 mL[40,59] in the adult horse respectively.[11,16-18,40] In the current study the total perfusate volume was

50mL. However, the ideal volume of perfusate, which should allow the highest possible intravenous pressure in the perfused area without exceeding the maximum venous pressure, is not known for equine carpus and distal limb perfusion. Further studies are needed to determine the ideal volume of perfusate with or without prior exsanguination.[55]

Local anaesthetic agents are cocaine derivatives that consist of an aromatic region and a basic amine side, joined by an ester or amide bond. The local anaesthetic agents block the propagation of the action potential, due to inhibition of the voltage-gated sodium channels.[63] A side effect of lignocaine is dilation of arterioles, partly due to relaxation of the vascular smooth muscle and partly due to inhibition of the sympathic nervous system.[63,77] It is possible that addition of intra-perfusate lignocaine during IVRLP can cause a decrease in the regional intravascular pressure due to arteriolar dilation and therefore lead to a lower diffusion of antimicrobial molecules into the interstitium. Further studies will be needed to evaluate this.

Although some authors advocate the use of a third of the systemic dose of the applicable antimicrobial agent for RLP,[5] a set dose of amikacin is generally used, both in clinical practice and research studies.[4] A fixed dose was therefore used in this study without adjustment for to the animal's weight. This could contribute to variability in synovial fluid amikacin concentrations.[17] Doses of amikacin used for IVRLP varies from 0.25 to 2.5 g of amikacin in experimental studies and up to 4 g in clinical cases.[10-12,15-18,37,59,78] In the current study 1 g of amikacin was used, with a resultant mean ± SD middle carpal synovial fluid amikacin concentrations (C_{max}; µg/mL) of 239 ± 231, 172 ± 94, 344 ± 431 and 503 ± 634 for CNT, IVA, PNA and GA, respectively. In a study using 1 g of amikacin, a C_{max} (mean ± SD) of 542 ± 173 µg/mL was reported in the distal interphalangeal joint after IVRLP using the palmar vein in standing horses.[17] Likewise, after IVRLP with 1 g of amikacin, a C_{max} (mean ± SD) of 701.9 ± 366.8 µg/mL was reported in the tibiotarsal joint, after IVRLP using the saphenous vein in horses under general anaesthesia.[15] The C_{max} obtained in the current study is difficult to compare to other studies, due to different methodologies used, including amikacin dose, injected vein, joint sampled, duration of perfusion and type of tourniquet used.

6.3 Recumbency of horse 7 during IVA and CNT

The reason for recumbency of horse 7 during CNT and IVA is uncertain, but it was interpreted as severe discomfort. This behaviour was not seen in any of the other horses and was regarded as an abnormal response. To the authors' knowledge, recumbency of horses as a sign of discomfort during RLP has not been reported before and it has not been encountered in a clinical setting by the authors. Of interest is that the mare did not become recumbent during PNA. We can speculate that the discomfort from the tourniquet was alleviated by the proximal perineural anaesthesia and not in the IVA and CNT groups. The data obtained from this horse was removed from the analysis since the CNT and IVA were treatments to be performed under standing sedation, which excludes recumbency. We believe that the fact that the mare became recumbent during the IVRLP could have affected the tourniquet efficacy (hence the rPkp results) as well as inference with the assessment of LIFT and VAS.

6.4 Limitations of the study

This study was performed on healthy horses. In inflamed joints, increased amikacin delivery and clearance from the synovial fluid after antimicrobial IVRLP have been demonstrated when compared to healthy joints;[78] therefore, the rPkp obtained with the different treatments in the present study cannot be directly extrapolated to horses with orthopaedic infections. In septic synovitis, the pH in the synovial cavity decreases,[79,80] which may decrease the efficacy of some antimicrobial agents.[45] This may be overcome with high intrasynovial concentrations exceeding the pharmacokinetic-pharmacodynamic breakpoint.[51,52]

The number of horses used in the study was based on the type of analysis to be performed and did not take variability of synovial fluid amikacin concentrations into account. Based on our results, the power of the study to detect differences of 40 µg/mL in C_{max} was approximately 10%. Based on the variability observed in this study, a sample size higher than 100 horses would be required to demonstrate

possible differences between groups, which were both economically and logistically not feasible. The low power is mostly due to the high variability observed in the synovial fluid amikacin concentrations, which is consistent with previous studies.[12,15,18]

Injection of the perfusate was performed over 60-90 seconds[55] in all cases, which should have minimized variability due to injection rate. However this could have been injected with an injection pump at a fixed rate and time.

The use of VAS to assess the discomfort of horses receiving standing antimicrobial IVRLP is acknowledged to be subjective. Assessment of pain in animals is by nature subjective due to the inability to verbally communicate their level of pain and no gold standard scale is available for this assessment. However, a visual analogue scale is commonly used in horses to evaluate pain[68,81] and has been shown to have good intra- and inter-observer agreement.[82] The blinded use of this scale proved to be useful to evaluate discomfort of horses in this study and revealed differences in discomfort among different treatments on standing, sedated horses. The results obtained with VAS were supported by LIFT and the number of additional sedations administered, which showed that treatment PNA provided superior comfort to the horses in this study. In order to improve the objectivity of VAS, each treatment could have been recorded and blindly evaluated by three or more people. This could have decreased the subjectivity of VAS.

It is possible that PNA had an advantage over IVA, because the perineural anaesthesia was performed 20 minutes prior to IVRLP in PNA, versus injection of the local anaesthetic only at the time of IVRLP in IVA. This resulted in more time for the local anaesthetic agent to work before placement of the tourniquet in PNA. The different times for LIFT were not recorded; however, the VAS was less in all groups during the first 15 minutes than during the last.

6.5 Possible further studies

The use of a double cuff tourniquet in horses undergoing IVRLP, with the addition of intraperfusate lignocaine, could be evaluated. These tourniquets are routinely used in humans undergoing intravenous regional anaesthesia[69,70] (see discussion). It would be of interest whether this practice would decrease the discomfort of horses during IVRLP.

Of interest would also be to compare antimicrobial levels after IVRLP under general anaesthesia with and without the addition of intraperfusate lignocaine, in order to determine if the vasodilatory properties of intravenous lignocaine will affect the delivery of the antimicrobial agent.

CHAPTER 7: CONCLUSIONS

Based on the results obtained in this study, we can conclude that:

- The use of perineural regional anaesthesia proximal to the tourniquet alleviates the discomfort of horses when performing antimicrobial IVRLP on the standing, sedated horse. Therefore, perineural regional anaesthesia can be recommended as an adjuvant technique when performing IVRLP treatments in standing horses.
- The use of regional anaesthesia (either as perineural or intravenous) or general anaesthesia during IVRLP with amikacin does not affect the regional antimicrobial concentrations and pharmacokinetic parameters. Therefore, the use of general anaesthesia for administration of antimicrobial IVRLP to horses is not justified.

APPENDICES

Appendix 1: Anova table, indicating the sources of variation and degrees of freedom.[66]

Source of Variation	Degrees of freedom
Horses	7
Periods	3
Treatments	3
Carry-over	3
Residual error	15
Total	**31**

Appendix 2: Randomisation of treatments for IVRLP in standing, sedated horses without regional anaesthesia (A), with perineural regional anaesthesia (B), with intravenous regional anaesthesia (C) and in horses under general anaesthesia (D). The asterisk (*) indicates the treatment orders selected randomly for this study.

Permutation Number	Permutation	Sample
1	ABCD	*
2	ABDC	
3	ACBD	
4	ACDB	
5	ADBC	
6	ADCB	*
7	BACD	
8	BADC	*
9	BCAD	
10	BCDA	
11	BDAC	*
12	BDCA	*
13	CABD	
14	CADB	
15	CBAD	
16	CBDA	
17	CDAB	*
18	CDBA	
19	DABC	*
20	DACB	
21	DBAC	
22	DBCA	*
23	DCAB	
24	DCBA	

Appendix 3: Visual analogue scale (VAS) of discomfort.

A score of 0 to 100 is indicated on the line, with 0 indicating no discomfort, and 100 indicating severe discomfort.[68]

_____

0 mm **100 mm**

Appendix 4: Data capture sheet that was used during intravenous regional limb perfusion on each occasion. Temp = temperature, HR = heart rate, RR = respiratory rate, No = number and LIFT = number of times the limb was lifted.

Name of horse:					**Treatment No:**	
Date:		**Horse No:**			**Weight:**	
Temp:		**Initial sedation:**			**Volume:**	
HR:		**Romifidine** (0.03mg/kg):				
RR:		**Butorphanol** (0.01mg/kg)**:**				
		Bolus if needed:			Volume:	Time of bolus:
Time IVRLP injected:		**Romifidine** (0.015mg/kg)				
		Butorphanol (0.01mg/kg)				
	Real Time	**Joint samples**	**HR/RR**	**Blood sample**	**LIFT:**	
Baseline:						
After sedation:						
15 min:						
30 min:						
40 min:						
1.5 hr:						
6hr:						
12hr:						
24hr:						
			24 hrs	**48 hrs**		
		Temp				
		HR:				
		RR:				

Appendix 5: Results of the regional pharmacokinetic parameters (rPkp) (mean) of amikacin in middle carpal synovial fluid after amikacin intravenous regional limb perfusion in horse 7 under standing sedation without regional anaesthesia (CNT), with intravenous regional anaesthesia (IVA) or with perineural regional anaesthesia (PNA) and under general anaesthesia (GA).

rPkp	CNT	IVA	PNA	GA
AUC_{0-24} (h.µg/mL)	114	975	134	1097
$AUMC_{0-24}$ (h.h.µg/mL)	770	3134	1334	7219
C_{max} (µg/mL)	17.4	505	13.1	160
C_{max}/MIC_4	4	126	3	40
MRT_{inf} (h)	6.5	3.17	9.25	6.33
$T_{½}$ (h)	4.61	4.31	5.68	5.14

Appendix 6: Raw clinical data for horse 7, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated.

Horse number 7				
Limb	Left	Right	Left	Right
Weight (kg)	408	400	396	390
Treatment Group	PNA	GA	CNT	IVA
LIFT (number of times)	19		51	5
Number of extra sedations	0		2	0
Time to first sedation (min)			15.25	
VAS 0-15 min	0		80	100
VAS 16-30 min	19		100	24
VAS (average)	9		90	62
HR before IVRLP (beats per min)	Not recorded		32	28
HR at 15min (beats per min)	40		40	32
HR at 30 min (beats per min)	36		Not recorded - recumbent	32
HR at 40 min (beats per min)	40		32	32
RR before IVRLP (breaths per min)	Not recorded		12	8
RR at 15 min (breaths per min)	16		16	24
RR at 30 min (breaths per min)	12		Not recorded - recumbent	8
RR at 40 min (breaths per min)	12		12	8

Appendix 7: Raw clinical data for horse 1, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated.

Horse number 1				
Limb	Left	Right	Left	Right
Weight (kg)	383	370	375	357
Treatment Group	CNT	PNA	IVA	GA
LIFT (number of times)	48	14	94	
Number of extra sedations	3	0	2	
Time to first sedation (min)	13		17.25	
VAS 0-15 min	100	11	78	
VAS 16-30 min	100	40	100	
VAS (average)	100	26	89	
HR before IVRLP (beats per min)	24	28	32	
HR at 15min (beats per min)	28	32	40	
HR at 30 min (beats per min)	30	30	42	
HR at 40 min (beats per min)	32	32	28	
RR before IVRLP (breaths per min)	18	16	12	
RR at 15 min (breaths per min)	12	8	28	
RR at 30 min (breaths per min)	12	8	28	
RR at 40 min (breaths per min)	12	6	16	

Appendix 8: Raw clinical data for horse 2, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated. Values which were not recorded due to human error are indicated with an asterisk (*).

Horse number 2				
Limb	Left	Right	Left	Right
Weight (kg)	390	390	385	370
Treatment Group	CNT	GA	IVA	PNA
LIFT (number of times)	55		67	64
Number of extra sedations	2		4	0
Time to first sedation (min)	8		9	
VAS 0-15 min	100		100	2
VAS 16-30 min	100		100	93
VAS (average)	100		100	48
HR before IVRLP (beats per min)	28		32	32
HR at 15min (beats per min)	28		32	32
HR at 30 min (beats per min)	20		30	*
HR at 40 min (beats per min)	22		24	32
RR before IVRLP (breaths per min)	8		24	8
RR at 15 min (breaths per min)	12		12	8
RR at 30 min (breaths per min)	20		20	*
RR at 30 min (breaths per min)	12		12	6

Appendix 9: Raw clinical data for horse 3, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated.

Horse number 3				
Limb	R	L	R	L
Weight (kg)	393	372	370	365
Treatment Group	GA	CNT	PNA	IVA
LIFT (number of times)		79	8	18
Number of extra sedations		1	0	0
Time to first sedation (min)		28.5		
VAS 0-15 min		62	5	9
VAS 16-30 min		100	10	63
VAS (average)		81	8	36
HR before IVRLP (beats per min)		36	24	32
HR at 15min (beats per min)		32	32	28
HR at 30 min (beats per min)		36	28	28
HR at 40 min (beats per min)		28	28	32
RR before IVRLP (breaths per min)		10	8	12
RR at 15 min (breaths per min)		6	8	8
RR at 30 min (breaths per min)		12	8	8
RR at 40 min (breaths per min)		6	8	8

Appendix 10: Raw clinical data for horse 4, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated.

Horse number 4				
Limb	L	R	L	R
Weight (kg)	430	420		412
Treatment Group	IVA	GA	CNT	PNA
LIFT (number of times)	4		15	4
Number of extra sedations	0		1	0
Time to first sedation (min)			20	
VAS 0-15 min	0		35	0
VAS 16-30 min	7		100	11
VAS (average)	4		68	6
HR before IVRLP (beats per min)	24		28	24
HR at 15min (beats per min)	32		32	28
HR at 30 min (beats per min)	32		32	32
HR at 40 min (beats per min)	32		32	28
RR before IVRLP (breaths per min)	6		10	8
RR at 15 min (breaths per min)	12		8	8
RR at 30 min (breaths per min)	18		8	4
RR at 40 min (breaths per min)	12		8	8

Appendix 11: Raw clinical data for horse 5, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated.

Horse number 5				
Limb	Right	Left	Right	Left
Weight (kg)	385	369	375	357
Treatment Group	PNA	CNT	GA	IVA
LIFT (number of times)	5	85		50
Number of extra sedations	0	3		1
Time to first sedation (min)		17		17.75
VAS 0-15 min	0	57		72
VAS 16-30 min	8	100		100
VAS (average)	4	79		86
HR before IVRLP (beats per min)	36	32		32
HR at 15min (beats per min)	36	36		40
HR at 30 min (beats per min)	36	36		36
HR at 40 min (beats per min)	40	3		28
RR before IVRLP (breaths per min)	10	12		8
RR at 15 min (breaths per min)	8	12		8
RR at 30 min (breaths per min)	8	8		12
RR at 40 min (breaths per min)	12	12		8

Appendix 12: Raw clinical data for horse 6, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated. Values which were not recorded due to human error are indicated with an asterisk(*).

Horse number 6				
Limb	Left	Right	Left	Right
Weight (kg)	415	403	403	406
Treatment Group	GA	PNA	IVA	CNT
LIFT (number of times)		33	55	58
Number of extra sedations		0	1	0
Time to first sedation (min)			25.3	
VAS 0-15 min		5	47	46
VAS 16-30 min		55	100	94
VAS (average)		30	74	70
HR before IVRLP (beats per min)		28/8	24	24
HR at 15min (beats per min)		32/8	36	32
HR at 30 min (beats per min)		*	36	36
HR at 40 min (beats per min)		36	32	32
RR before IVRLP (breaths per min)		8	12	8
RR at 15 min (breaths per min)		8	12	8
RR at 30 min (breaths per min)		*	8	12
RR at 40 min (breaths per min)		12	4	12

Appendix 13: Raw clinical data for horse 8, showing weight, number of times the limb was lifted (LIFT), score for visual analogue scale of discomfort (VAS), heart rate (HR) and respiratory rate (RR), during amikacin intravenous regional limb perfusion under standing sedation without regional anaesthesia (CNT), with intravenous anaesthesia (IVA) and with perineural anaesthesia (PNA). The treatment under general anaesthesia (GA) is also indicated.

Horse number 8				
Limb	Left	Right	Left	Right
Weight (kg)	404	380	384	380
Treatment Group	PNA	GA	IVA	CNT
LIFT (number of times)	11		82	40
Number of extra sedations	0		1	0
Time to first sedation (min)			23	
VAS 0-15 min	0		85	51
VAS 16-30 min	23		100	93
VAS (average)	12		93	72
HR before IVRLP (beats per min)	32		48	24
HR at 15min (beats per min)	36		40	32
HR at 30 min (beats per min)	40		36	52
HR at 40 min (beats per min)	36		28	28
RR before IVRLP (breaths per min)	22		12	12
RR at 15 min (breaths per min)	12		8	12
RR at 30 min (breaths per min)	16		12	12
RR at 40 min (breaths per min)	10		6	8

Appendix 14: Synovial fluid amikacin concentrations obtained in the in-house performance assessment of the amikacin measurement kit. Amikacin concentration was measured in 4 independent, 5 serial dilutions from a mother solution of amikacin (500 µg/mL) in synovial fluid.

Constituted concentration (µg/mL)	Dilution 1 (µg/mL)	Dilution 2 (µg/mL)	Dilution 3 (µg/mL)	Dilution 4 (µg/mL)
500	402	461	458	428
250	226	236	228	217
125	121	117	117	117
63	56	62	63	61
31	30	31	30	30
0	0.5	0.4	0.4	<0.3

Appendix 15: Middle carpal synovial fluid concentrations (µg/mL) of amikacin in horses undergoing amikacin IVRLP under general anaesthesia (GA). Samples were collected before IVRLP and tourniquet placement (T0), 30 minutes after perfusate administration and before tourniquet release (T0.5), and 1.5 (T1.5), 6 (T6), 12 (T12) and 24 (T24) hours post-administration of perfusate. *This value was omitted due to a measurement error. CV = coeffcient of variation.

Horse	Limb	T0	T0.5	T1.5	T6	T12	T24
1	Right	0.4	1753	399	122	63	13
2	Right	0.6	544	230	33	13	6
3	Right	<0.3	173	89	30	6	1
4	Right	0.5	309	101	29	7	3
5	Right	<0.3	68	77	28	7	2
6	Left	<0.3	*	34	8	2	1
8	Right	<0.3	161	44	17	3	0.5
	Mean	0.4	501	139	38	14	3.7
	CV	0.3	0.7	0.9	1.0	1.6	1.2

Appendix 16: Middle carpal synovial fluid concentrations (µg/mL) of amikacin in standing sedated horses undergoing amikacin IVRLP with perineural anaesthesia (PNA). Samples were collected before IVRLP and tourniquet placement (T0), 30 minutes after perfusate administration and before tourniquet release (T0.5), and 1.5 (T1.5), 6 (T6), 12 (T12) and 24 (T24) hours post-administration of perfusate. CV = coeffcient of variation.

Horse	Limb	T0	T0.5	T1.5	T6	T12	T24
1	Right	0.4	28	23	5	2	0.7
2	Right	<0.3	901	145	38	10	0.4
3	Right	<0.3	101	35	7	2	0.7
4	Right	1.0	125	33	7	2	0.6
5	Right	0.4	156	37	10	3	0.9
6	Right	<0.3	1040	161	29	15	1.8
8	Left	0.5	64	33	4	1	<0.3
	Mean	0.5	345	67	14	5	0.7
	CV	0.4	1.2	0.9	1.0	1.0	0.7

Appendix 17: Middle carpal synovial fluid concentrations (µg/mL) of amikacin in standing sedated horses undergoing amikacin IVRLP with intravenous anaesthesia (IVA). Samples were collected before IVRLP and tourniquet placement (T0), 30 minutes after perfusate administration and before tourniquet release (T0.5), and 1.5 (T1.5), 6 (T6), 12 (T12) and 24 (T24) hours post-administration of perfusate. CV = coeffcient of variation.

Horse	Limb	T0	T0.5	T1.5	T6	T12	T24
1	Left	<0.3	95	25	7	1	0.4
2	Left	<0.3	216	65	15	3	0.7
3	Left	<0.3	64	28	6	2	0.4
4	Left	<0.3	115	9	8	2	0.6
5	Left	<0.3	133	67	16	6	1
6	Left	<0.3	269	15	10	3	0.9
8	Left	0.6	312	193	67	18	1.2
	Mean	0.3	172	57	18	5	0.7
	CV	0.3	0.5	1.1	1.2	1.2	0.4

Appendix 18: Middle carpal synovial fluid concentrations (µg/mL) of amikacin in standing sedated horses undergoing amikacin IVRLP without regional anaesthesia (CNT). Samples were collected before IVRLP and tourniquet placement (T0), 30 minutes after perfusate administration and before tourniquet release (T0.5), and 1.5 (T1.5), 6 (T6), 12 (T12) and 24 (T24) hours post-administration of perfusate. CV = coeffcient of variation.

Horse	Limb	T0	T0.5	T1.5	T6	T12	T24
1	Left	0.4	63	32	16	11	3
2	Left	0.4	278	215	61	37	8
3	Left	0.4	49	22	4	1	0.4
4	Left	<0.3	615	166	21	6	1
5	Left	<0.3	5	7	2	1	<0.3
6	Right	<0.3	472	106	31	6	0.9
8	Right	0.4	188	37	12	2	<0.3
	Mean	0.4	239	84	21	9.0	2.1
	CV	0.3	1.0	1.0	1.0	1.4	1.4

Appendix 19: Systemic concentrations (µg/mL) of amikacin IVRLP in horses under general anaesthesia (GA) before tourniquet placement (T0), and 30 minutes after perfusate administration and before tourniquet release (T0.5). CV = coeffcient of variation.

Horse	T0	T0.5
1	<0.3	<0.3
2	<0.3	<0.3
3	<0.3	1.0
4	<0.3	<0.3
5	<0.3	<0.3
6	<0.3	1.5
8	<0.3	7.6
Mean	<0.3	1.5
CV		1.7

Appendix 20: Systemic concentrations (µg/mL) of amikacin IVRLP in standing sedated horses with perineural anaesthesia (PNA) before tourniquet placement (T0), and 30 minutes after perfusate administration and before tourniquet release (T0.5). CV = coeffcient of variation.

Horse	T0	T0.5
1	<0.3	14
2	<0.3	2.2
3	<0.3	0.3
4	<0.3	1.2
5	<0.3	8.8
6	<0.3	1.6
8	<0.3	9.6
Mean	<0.3	6.2
CV		0.9

Appendix 21: Systemic concentrations (µg/mL) of amikacin IVRLP in standing sedated horses with intravenous anaesthesia (IVA) before IVRLP and tourniquet placement (T0), and 30 minutes after perfusate administration and before tourniquet release (T0.5). CV = coeffcient of variation.

Horse	T0	T0.5
1	<0.3	7.2
2	<0.3	1.7
3	<0.3	3.6
4	<0.3	7.1
5	<0.3	1.0
6	<0.3	1.3
8	<0.3	10
Mean	<0.3	4.5
CV		0.8

Appendix 22: Systemic concentrations (µg/mL) of amikacin IVRLP in standing sedated horses without regional anaesthesia (CNT) before IVRLP and tourniquet placement (T0), and 30 minutes after perfusate administration and before tourniquet release (T0.5). CV = coeffcient of variation.

Horse	T0	T0.5
1	<0.3	10.4
2	<0.3	8.2
3	<0.3	13.7
4	<0.3	4.5
5	<0.3	13.5
6	<0.3	2.7
8	<0.3	11.9
Mean	<0.3	9.4
CV		0.5

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