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Pharmacokinetics of marbofloxacin administered via intravenous regional limb perfusion in dairy cows: evaluation of two different tourniquets

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ABSTRACT

Objectives This study evaluated synovial and systemic plasma pharmacokinetic variables of marbofloxacin after a single intravenous regional limb perfusion (IVRLP) performed using two different tourniquets in clinically healthy, standing, non-sedated dairy cows. The authors hypothesised that the type of tourniquet used for IVRLP would influence the synovial fluid concentration of marbofloxacin in the perfused distal limb.

Design The study had a randomised parallel-group design.

Methods Ten adult dairy cows were included. Unilateral hindlimb IVRLP through the dorsal common digital III vein was performed in two groups of five cows (group 1: wide rubber tourniquet; group 2: manual pneumatic tourniquet) using 0.67 mg/kg of marbofloxacin. The tourniquet was applied proximal to the tarsus and maintained for 30 minutes. Samples of jugular blood and synovial fluid from the tibiotarsal joints of the perfused limb were obtained before and at intervals after IVRLP. All samples were analysed for drug concentrations using liquid chromatography tandem mass spectrometry. Pharmacokinetic parameters were determined to establish the influence of tourniquet types. Differences were considered significant at $P \leq 0.05$.

Results No adverse effects from the procedure or marbofloxacin were observed in any animal. Significant differences in synovial concentrations and pharmacokinetic parameters were measured. The mean ± sd areas under the concentration versus time curve from time 0 to 24 hours were 178.98±58.08 µg hour/ml for group 2 and 21.11±9.93 µg hour/ml for group 1. The mean ± sd maximum marbofloxacin concentrations were 75.50±10.19 µg/ml for group 2 and 6.35±1.47 µg/ml for group 1.

Conclusions Performing IVRLP using the dorsal common digital III vein and a manual pneumatic tourniquet set at 300 mmHg above the tarsus in standing cows resulted in significantly higher marbofloxacin concentrations in the tibiotarsal joint compared with those with the wide rubber tourniquet.

Trial registration Local ethical committee (number 41/2012/CEISA).

INTRODUCTION

Antimicrobial intravenous regional limb perfusion (IVRLP) is a well-established technique for the treatment or prevention of the development of orthopaedic infections of equine distal limbs.[1 2] This local method of antimicrobial delivery offers many advantages over systemic administration. Local administration provides particularly high antimicrobial concentrations in the site of infection, and it minimises systemic diffusion and potential side effects.[1 2] A reduction in total dose compared with animal bodyweight and minimal systemic plasma concentrations of antimicrobial agents are suitable to decrease milk levels in lactating dairy cattle.[3]

The first report of IVRLP was published in 1964,[4] and it described the use for local analgesia in bovine digital surgical procedures. IVRLP with antimicrobials was first reported in cattle in 1974,[5] and several clinical studies investigated its use for the treatment of digital septic lesions.[6] The pharmacokinetics of cefazolin,[7] ceftiofur,[8] florfenicol,[9] tetracycline hydrochloride[3] and ampicillin-sulbactam[10] after IVRLP were defined previously in cattle.

Different types of tourniquets were examined previously in standing sedated horses. The results suggested that the ability to maintain vascular isolation in a selected portion of the limb and avoid perfusate leakage into the systemic circulation was essential to perform IVRLP efficaciously. Alkabes and others[11] demonstrated that the Esmarch tourniquet was more effective than the pneumatic tourniquet when placed over the third metacarpal bone. Levine and others[12] revealed that a pneumatic tourniquet proximal to the carpus was more efficient than the Esmarch tourniquet, and a narrow rubber tourniquet was not

sufficient. Therefore, experimental and clinical research of IVRLP in field settings using different types of tourniquets in dairy cow is recommended.

Lameness is a major concern in the dairy cattle industry because it results in significant economic losses.[13] Therefore, lameness is one of the most important animal welfare issues.[14] Deep digital septic conditions and soft-tissue infections of the bovine foot, such as interdigital phlegmon, are responsible for severe lameness, a consequent decrease in milk production and possible premature culling.[15 16] Frequently, the septic process associated with distal limb infections in cattle involves a mixed population of Gram-positive/Gram-negative microorganisms. The most common bacterial agents isolated in these disorders are *Trueperella (Actinomyces/Arcanobacterium) pyogenes, Fusobacterium (Fusiformis) necrophorum, Escherichia coli, Streptococcus* species, *Moraxella osloensis* and *Salmonella* species.[17 18] Typically, in calves the most common bacterium isolated from infected joints is *Mycoplasma bovis* while in older cattle is *T pyogenes*.[19]

Marbofloxacin is a synthetic antibacterial agent in the fluoroquinolone class, and it has been approved for use in veterinary medicine since 1995 in Europe and the USA to treat respiratory, urinary and dermatological diseases that affect companion animals.[20–22] Marbofloxacin has been approved for use in food-producing animals (cattle and pigs) only in Europe for respiratory, soft tissue and infective gastroenteric diseases since 1997, and it has been registered in the UK for *E coli* acute mastitis in dairy cattle since 2000.[23 24]

Marbofloxacin exhibits a broad spectrum of activity, and it is effective against many Gram-negative strains, some Gram-positive strains and *Mycoplasma* species. However, it exhibits weak activity against most anaerobes.[25] Actually, previous studies in veterinary as well as in human medicine showed a broad-spectrum anti-anaerobe in vitro activity of marbofloxacin.[26 27] The bactericidal activity of fluoroquinolones, including marbofloxacin, is concentration-dependent against Gram-negative bacteria and time-dependent against Gram-positive bacteria.[28 29] These drugs also exert a prolonged post-antibiotic effect on some bacterial species. Marbofloxacin is an advanced, third-generation, veterinary fluoroquinolone whose broad spectrum includes bacteria regularly cultured from natural occurring septic joint in calves: *M bovis, T pyogenes, E coli, Haemophilus somnus and Streptococcaceae*.[30]

There are no antimicrobial drugs approved for use as IVRLP in food animals. The off-label use of fluoroquinolones and cephalosporins in food-producing animal species is illegal in the USA, which is similar to the prohibition of the use of tetracycline hydrochloride. Florfenicol is not labelled for use in lactating dairy cows. The off-label use of marbofloxacin is not prohibited in Europe, and withdrawal times are established for meat and milk production. However, its use as a first-line treatment is restricted, and it should only be used when supported by antimicrobial susceptibility test results.

Nevertheless, because of the broad spectrum and the concentration-dependent bactericidal activity,[28 29] the optimal diffusion within tissues and the prolonged post-administration effect,[25] marbofloxacin could be a valuable option for treating septic conditions of bovine distal limb with IVRLP, especially when a polymicrobic infection is suspected or diagnosed. The present study determined the efficacy of two different tourniquet types after a single IVRLP with marbofloxacin in clinically healthy, standing non-sedated dairy cows and evaluated the pharmacokinetic parameters for plasma and synovial fluid.

We hypothesised that the type of tourniquet used for IVRLP would influence the synovial fluid concentration of marbofloxacin in the perfused distal limb.

MATERIALS AND METHODS

Animals

Ten lactating adult dairy cows (mean bodyweight, 557.9 kg, range 534–598 kg; mean age 4.1 years, range 3–7 years; breed, Italian Friesian; daily milk production range 14–20 l) were included in the study. All experimental procedures were performed with the approval of the local ethical committee. All animals were healthy on physical examination and were free of lameness and mammary gland diseases. None of the cows had received any medication for at least 180 days before the study.

Experimental protocol

Standing non-sedated animals were restrained in a chute for claw trimming (figure 1). One pelvic limb of each cow was randomly selected and assigned to one of two experimental groups (five limbs/group). The same clinician applied the tourniquet proximal to the tarsus, around the distal portion of the tibia. Rolled gauze pads were placed on the depression between tibia and the gastrocnemius muscle tendon on both sides of the leg before tourniquet application. Group 1 had a manually applied wide rubber elastic tourniquet (10×500 cm, 6–8 full circumferential turns as tight as possible; Esmarch Bandage), and group 2 had applied a manual pneumatic tourniquet (11×76 cm cuff set at 300 mmHg of pressure; VBM Germany). Distal to the tourniquet, the dorsal common digital III vein was clipped, aseptically prepared and used to perfuse the pelvic limb. A 19-g butterfly needle was introduced into the vein, and 0.67 mg/kg of marbofloxacin (Marbocyl 10%, Vétoquinol) diluted to 60 ml with sterile water for injections was infused manually via a slow bolus injection over 60–90 seconds. The tourniquet was released 30 minute after the beginning of the infusion. Blood samples (3 ml) were collected from the left jugular vein at the following times: 0 (before injection), 0.08, 0.25, 0.5 (immediately after the tourniquet was released), 1, 2, 4, 8, 12, 24 and 48 hours after the injection. Blood samples were immediately centrifuged to obtain the plasma. Synovial samples (0.5–1 ml) were aseptically collected via serial arthrocentesis of the tibiotarsal joint dorsal recess at the following times: 0, 0.5

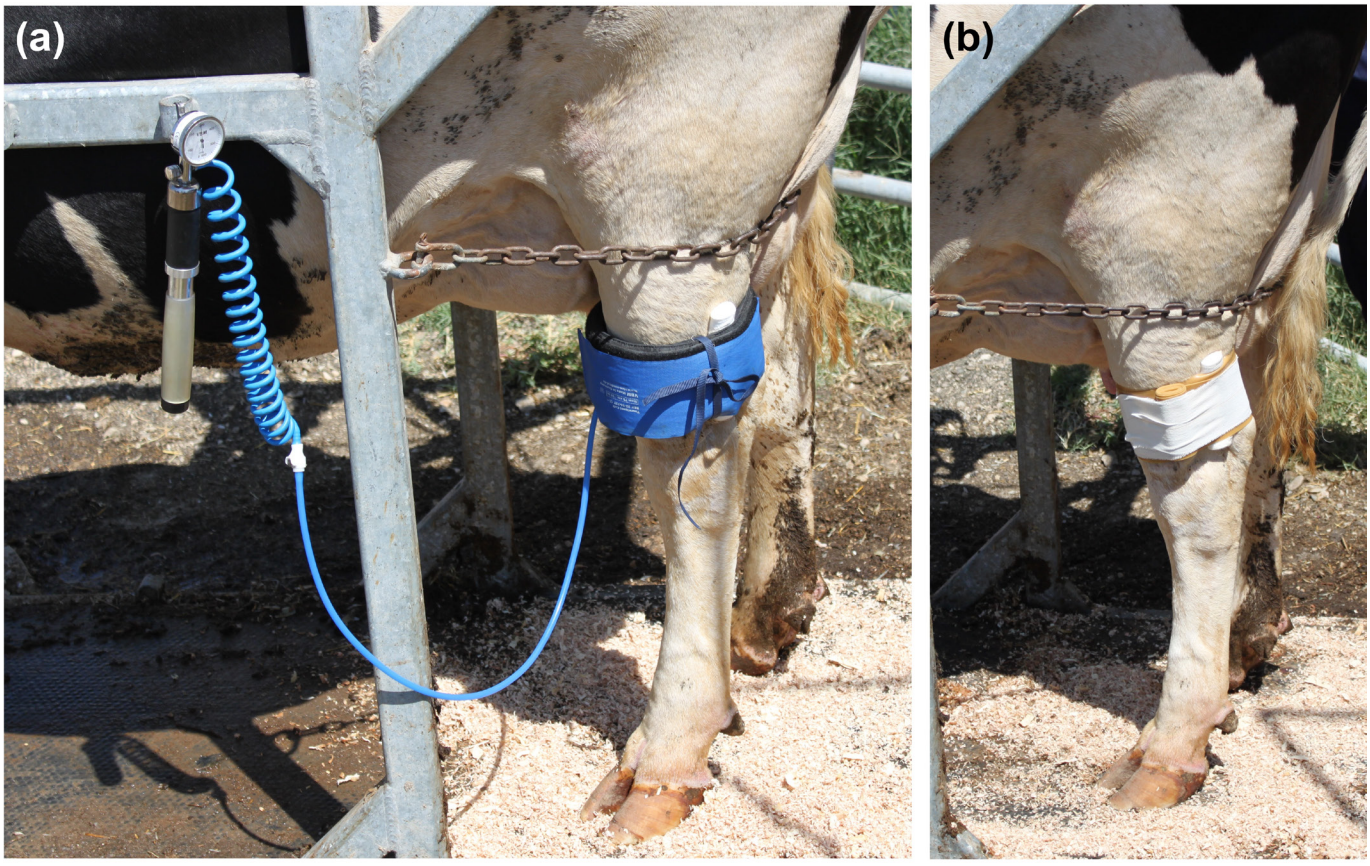

Figure 1 Standing non-sedated dairy cow restrained in a chute for claw trimming: intravenous regional limb perfusion using the manual pneumatic (a) or the rubber elastic tourniquet (b).

(immediately after the tourniquet was released), 1, 2, 4, 8, 12, 24 and 48 hours after infusion. The supernatant was collected after centrifugation. All samples were stored at –80°C until analysis. The cows were evaluated daily for five days after the IVRLP procedure to monitor evidence of lameness, distal limb swelling, local reaction at the injection site and possible major complications.

Sample analysis and analytical method

All plasma and synovial samples were analysed for marbofloxacin concentrations using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using rapid sample preparation, which only requires an ultrafiltration step with centrifugal filter devices.[31] Ofloxacin (another fluoroquinolone antibacterial agent) was used as the Internal Standard (IS). Briefly, 175 µl of each sample were mixed with 85 µl of methanol containing IS, at a final concentration of 200 ng/ml in an Eppendorf tube. The solution obtained was vortexed for two minutes and transferred to an ultrafiltering device. Capped tubes were centrifuged at 13,500 rpm for 15 minutes. The filtrate solution was transferred to a glass vial and submitted for LC-MS/MS analysis. The injection volume was 2 µl. The HPLC equipment consisted of a Series 200 Micro-LC Pump system with autosampler from Perkin Elmer (Norwalk, Connecticut, USA). The HPLC system was coupled to a triple quadrupole mass spectrometer API 2000 from AB-Sciex (Toronto, ON, Canada) equipped with a Turbo Ion-Spray source. An ultrafast chromatographic separation was obtained using a 20-mm column to maximise the speed of the analysis. Linearity was assessed in the range of 5–2500 ng/ml. The Limit of Detection (LOD) and the Limit of Quantification (LOQ) were 1 ng/ml and 5 ng/ml, respectively. Plasma and synovial concentrations versus time profiles of marbofloxacin are presented as the mean ± sd.

Pharmacokinetic evaluation

Pharmacokinetic parameters were evaluated for measured plasma and synovial concentrations using PKsolver V.2.0 and an add-in program for pharmacokinetic and pharmacodynamic data analyses in Microsoft Excel. Pharmacokinetic parameters of plasmatic concentrations were calculated using non-compartmental analysis (NCA) for intravenous infusion using the linear trapezoidal rule. Pharmacokinetic parameters for synovial fluid concentrations were calculated using the linear trapezoidal rule with NCA for extravascular compartments. The following pharmacokinetic parameters were calculated for plasma and synovial concentrations: area under the concentration–time curve from time 0 to 24 hours ($AUC_{0\text{-}24}$), area under the concentration–time curve from time zero to infinity ($AUC_{0\text{-}inf}$), maximum drug concentration (C_{max}), half-life ($T_{1/2}$), clearance of the drug (CL), apparent volume of distribution during terminal phase (V_z) and mean residence time extrapolated to infinity (MRT_{inf}).

The apparent volume of distribution at steady state (V_{ss}) was evaluated only with NAC for plasmatic concentrations. The results are presented as the mean ± sd for each of the pharmacokinetic parameters evaluated in groups 1 and 2.

Statistical analysis

Statistical analysis was performed on plasma and synovial pharmacokinetic parameters using the SPSS V.20.0 software package with the aim of verifying significant differences in pharmacokinetic behaviour of marbofloxacin using two different tourniquets for IVRLP administration. Considering that the data distribute in an asymmetric manner the non-parametric Mann-Whitney U test was used to compare plasmatic and synovial pharmacokinetic variables calculated for groups 1 and 2. A value of P≤0.05 was considered significant.

RESULTS

All animals tolerated the IVRLP procedure with marbofloxacin well. Mild discomfort was displayed (eg, shifting weight and occasionally lifting the foot from the ground) during the first 30 minutes after the tourniquet was applied, which ceased several minutes after the tourniquet was released.

Synovial fluid samples were collected at all time points without evident blood contamination. After repeated aseptic arthrocentesis in two limbs, a minimal subcutaneous swelling of the dorsomedial aspect of the tarsus was detected, which resolved spontaneously within 24 hours. Despite serial arthrocentesis, no major complication was observed in any of the animals.

No lameness or distal oedema of the perfused limb was observed in any of the groups during the five-day observation period after IVRLP, and no local irritation was noted at the drug injection site.

No marbofloxacin was detected in plasma or synovial fluid samples at 0 hours.

The calibration curves in plasma and synovial fluid were linear from 5 to 2500 ng/ml, and correlation coefficients were ≥0.99. The accuracy and precision ranges were 87–114 per cent and 15–20 per cent, respectively, for plasma and synovial fluid.

Plasma and synovial concentrations versus time profiles of marbofloxacin were evaluated, and table 1 shows the mean ± sd peak concentrations. Plasmatic and synovial pharmacokinetic parameters were calculated, and table 2 shows the mean ± sd values. Marbofloxacin was detected in all venous samples collected before tourniquet release. No significant difference in plasmatic pharmacokinetic variables was found between groups 1 and 2. In contrast, significant dissimilarities were observed between the two tourniquet groups in synovial concentrations and pharmacokinetic parameters (P≤0.05). Higher concentrations of marbofloxacin in synovial fluid were reached in group 2, before and after the tourniquet was released. The synovial mean concentration ± sd peak was 4.86±1.88 µg/ml for group 1 and 51.97±15.07 µg/ml for group 2 (P=0.01208) just after the tourniquet release (0.5 hours). The maximum synovial concentration of marbofloxacin was reached at 1 hour in groups 1 and 2, with a mean ± sd peak of 6.35±1.47 µg/ml and 75.50±10.19 µg/ml, respectively (P=0.01208). The mean ± sd $AUC_{0\text{-}24}$ and $AUC_{0\text{-}inf}$ values in group 1 were 21.11±9.93 µg hour/ml and 21.28±9.91 µg hour/ml, respectively. Group 2 exhibited higher values of $AUC_{0\text{-}24}$ and $AUC_{0\text{-}inf}$ in synovial fluid, with a mean ± sd peak of 178.98±58.08 µg hour/ml and 180.95±57.33 µg hour/ml, respectively. The mean half-life ($T_{1/2}$)± sd value was 10.16±2.38 hours in group 2, which was significantly higher (P=0.02852) than the mean ± sd synovial $T_{1/2}$ calculated in group 1 (5.48±1.83 hours).

TABLE 1 Mean ± sd peak of marbofloxacin concentrations (µg/ml) in groups 1 and 2 in plasma and synovial fluid at different times

	Group 1		Group 2	
Time (hours)	Plasma	Synovial fluid	Plasma	Synovial fluid
0.08	0.13±0.05		0.05±0.05	
0.25	0.24±0.10		0.14±0.03	
0.5	0.88±0.50	4.86±1.88	0.45±0.16	51.97±15.08
1	1.99±0.80	6.35±1.47	2.77±0.38	75.50±10.19
2	1.42±0.39	3.37±1.88	1.73±0.27	33.47±13.01
4	0.80±0.30	0.47±0.11	1.12±0.29	8.48±5.25
8	0.56±0.27	0.34±0.15	0.72±0.20	2.67±3.24
12	0.30±0.13	0.21±0.18	0.42±0.25	0.63±0.17
24	0.10±0.07	0.12±0.09	0.16±0.08	0.29±0.08
48	0.00±0.00	0.03±0.07	0.01±0.04	0.10±0.07

DISCUSSION

There are no peer-review clinical data on marbofloxacin chondrotoxicity and tenotoxicity in animals. Previously, in vitro adverse effects of fluoroquinolones on equine chondrocytes and tenocytes were demonstrated.[32 33] Enrofloxacin induced arthropathy or tendinopathy in growing animals.[34] However, the fluoroquinolones-associated tendonitis or tendon ruptures observed in human beings were not documented definitively in horses. Local irritant properties of fluoroquinolones were described previously,[35-36] but no clinical signs of phlebitis or thrombophlebitis after a single IVLP administration of marbofloxacin were observed in any of the animals, as reported in horses.[37]

Despite the minimum inhibitory concentration (MIC) of marbofloxacin not established for the main pathogens responsible for bovine limb infections, the breakpoint MIC (MICBP) of marbofloxacin was established and validated by the Clinical and Laboratory Standards Institute[38] for resistant bacteria (MICBP ≥4 µg/ml), and the present study has shown a synovial concentration above this limit after IVRLP (table 1). Synovial fluid

TABLE 2 Mean ± sd peak of pharmacokinetic parameters in groups 1 and 2 for the plasmatic and synovial fluid concentrations of marbofloxacin

	Group 1		Group 2	
Pharmacokinetic parameters	**Plasma**	**Synovial fluid**	**Plasma**	**Synovial fluid**
AUC_{0-24} (µg hour/ml)	12.44±4.80	21.11±9.93	16.31±5.15	178.98±58.08
AUC_{0-inf} (µg hour/ml)	12.62±4.69	21.28±9.91	16.76±5.06	180.95±57.33
C_{max} (µg/ml)	1.99±0.76	6.35±1.47	2.77±0.38	75.50±10.19
$T_{1/2}$ (hours)	5.66±1.10	5.48±1.83	5.74±0.49	10.16±2.38
CL (l/hour kg)	33.25±12.63	20.87±9.46	23.51±5.47	2.24±0.73
V_{ss}(l/kg)	0.67±0.15		0.52±0.09	
V_z (l/kg)	0.74±0.38	0.27±0.07	0.52±0.13	32.70±13.45
MRT_{inf} (hours)	7.99±1.72	5.33±1.89	8.43±0.83	3.93±1.15

AUC_{0-24}, area under the concentration–time curve from time 0 to 24 hours; AUC_{0-inf}, area under the concentration–time curve from time zero to infinity; C_{max}, maximum drug concentration; CL, clearance of the drug; MRT_{inf} mean residence time extrapolated to infinity; $T_{1/2}$, half-life; V_{ss},apparent volume of distribution at steady state; V_z,apparent volume of distribution during terminal phase.

concentrations measured in group 2 exceeded the CLSI limit value of resistance by approximately 10–20 times for a period of 2 hours and remained above this value for 4 hours (8 hours for one of the sampled animals). The synovial concentrations attained were higher than the MIC against 90 per cent of the population (MIC_{90}), which is notable for the pathogens that are secondarily involved in distal limb infections, such as *E coli* or *Staphylococcus aureus*.

Many studies reported wide variations in synovial fluid antimicrobial concentrations after IVRLP in different individuals, limbs and procedures.[1 11 12 36 39 40] This individual variability may be due to limb motion, different tourniquet application modality and pressure, subcutaneous perfusate leakage, anatomical differences, the fixed dose regardless of the bodyweight and laboratory errors. The cows in this study were restrained in the same manner in a chute, and the same investigator performed all the IVRLP procedures to reduce individual variability. The antimicrobial dose was corrected to the cow bodyweight.

Limb movement was noted in the standing non-sedated cows of this study, but it was not objectively evaluated. Limb movements were responsible for pressure fluctuations in the pneumatic tourniquet cuff. The manual or electronic system of pressure control in the pneumatic tourniquets allows better control and standardisation of the applied pressure during the IVRLP period.

The pressure under the Esmarch tourniquet was not measured in this study, but it presumably underwent analogous variations. The anatomical features above the tarsus and the udder size in dairy cows render difficult a consistent elastic tourniquet application, which may affect its performance.

Some investigations of the role of different tourniquets to improve the efficacy of antimicrobials administered by IVRLP in horses suggested that a higher haemostatic pressure, and a consequently reduced leakage of the drug during its administration, permitted higher antimicrobial concentrations in the distal joints.[9 12 41]

Significantly higher values of the synovial pharmacokinetic parameters C_{max}, AUC_{0-24} and AUC_{0-inf} (P=0.01208) and $T_{1/2}$ (P=0.02852) were observed in group 2 in this study, which suggests that higher haemostatic pressure is associated with the pneumatic tourniquet.

Similar investigations that compared pneumatic and elastic tourniquets, proximal and distal to the equine carpus, suggested equal or more efficient haemostatic pressure of the elastic cuffs. However, limb motion in standing animals leads to variations in tourniquet pressure, which results in a leakage of antimicrobials into the systemic circulation.[1] Errico and others[42] reported that sudden weight shifts in the front limbs of a horse doubled intravascular pressure distal to the tourniquet, but a recent study did not demonstrate a consistent effect of limb movement on synovial or systemic drug concentrations.[12 40] One study used an electronic pneumatic tourniquet and demonstrated that this tourniquet type was more reliable because it constantly reset to the selected pressure when limb motion occurred.[43] Instead, the measured haemostatic pressure depends on the ability of the operator using an elastic tourniquet, and the pressure cannot be reset if some variations occur.

A kinetics problem of non-linear distribution was detected when the tourniquet was released due to the alteration of the first-time equilibrium between the systemic circulation and the distal limb area and between the vascular and joint compartments in the distal limb area. Significantly higher values of synovial V_z were observed in group 2 (P=0.01208), which confirmed that a higher volume of the drug moved from the joint into the plasma at the moment of release.

Synovial C_{max} exhibited higher values in group 2 (P<0.05), which was reached 30 minutes after tourniquet release (the 1 hour time point). The increased plasmatic

concentrations resulted in increased synovial concentrations after tourniquet release in both groups.

The observed second flip-flop phenomenon after tourniquet release reflected a recirculation of the drug among the above-mentioned compartments until concentrations reached a new equilibrium phase. Some studies of IVRLP reported that synovial concentration peaks were reached before tourniquet release,[37] but other studies demonstrated a parallel relationship of tissue levels and systemic blood concentration.[3 9 44] However, studies of the use of different types of tourniquets for IVRLP demonstrated no differences in the time of C_{max}.

Complications following antimicrobial IVRLP in cattle are infrequent and often include minor complications such as haematoma and abscess formation at the site of injection; however, repeated IVRLP with high doses of benzylpenicillin in cows suffering from septic claw disorder were associated with the development of generalised distal limb vessel thrombosis.[45 46]

The primary limitation of the present study is that it was performed on healthy dairy cows without signs of distal limb infection, which could modify the efficacy of the IVRLP in clinical settings. Nevertheless, the study is an initial step in the evaluation of the potential application of marbofloxacin for the treatment of deep digital septic conditions in dairy cattle by IVRLP.

The observations of this study and the optimal tolerance of the procedure support the use of IVRLP with a single administration of marbofloxacin via the dorsal common digital III vein in standing cows using a manual pneumatic tourniquet set at 300 mmHg above the tarsus. These data could represent the basis for an optimal treatment of distal limb infection in cows, achieving a local rational therapeutic dose, while avoiding subtherapeutic or toxic levels, bacterial resistance and possible risks of unacceptable residues in edible tissues. However, more pharmacodynamic studies of marbofloxacin and other antimicrobials in different animal species and for specific infections are necessary to designate the values of surrogate markers of antimicrobial drug efficacy (eg, C_{max}/MIC ratio, time above the MIC_{90}).

In conclusion, this study suggests that the use of a manual pneumatic tourniquet above the tarsus in standing, non-sedated dairy cows results in a superior outcome during IVRLP compared with the rubber tourniquet.

Contributors Substantial contributions to conception and design, acquisition of data or analysis and interpretation of data: all the authors have been involved equally in this study. On the basis of their competence and specialisation each one has done an important and uniform contribution for: conception and design of this study and acquisition data (in particular the surgeons: GC, VV, CMM and LP); samples analysis (in particular the chemists: CM, MS and DC); pharmacokinetic evaluation, statistical analysis and interpretation of data (in particular the pharmacologists and toxicologists: SMRT, DZ and MA). Drafting the article or revising it critically for important intellectual content: all the authors have been involved equally in this study. It has been drafted especially by the first author and the corresponding authors (GC and SMRT), but it has been constantly revised by all authors involved (VV, CMM and LP, CM, MS DC, DZ and MA). Final approval of the version to be published: all the authors. All the authors agree to be accountable for all aspects of the work.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Local ethical committee (number 41/2012/CEISA).

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Data sharing statement All authors agree to the publication of these data that have not been published yet.

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