Comparison of pharmacokinetics of marbofloxacin after subcutaneous administration of various multiple-dose regimens to water buffalo calves (*Bubalus bubalis*)

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Objective—To determine pharmacokinetics of marbofloxacin in water buffalo calves (*Bubalus bubalis*) after multiple SC administrations and to assess differences in regimen efficacy.

Animals—18 healthy buffalo calves.

Procedures—Calves (n = 6 calves/group) were assigned to receive marbofloxacin SC in the neck at 1 of 3 dosages (2 mg/kg, q 24 h for 6 days [regimen 1]; 4 mg/kg, q 48 h for 6 days [regimen 2]; and 4 mg/kg, q 24 h for 3 days [regimen 3]). Serum marbofloxacin concentrations were analyzed. Efficacy predictors were estimated on the basis of minimum inhibitory concentration and mutant prevention concentration reported for *Pasteurella multocida* and *Mannheimia haemolytica*.

Results—Mean ± SD area under the concentration-time curve was 5.92 ± 0.40 µg•h/mL for regimen 1, which differed significantly from that for regimens 2 (14.26 ± 0.92 µg•h/mL) and 3 (14.17 ± 0.51 µg•h/mL). Mean residence time and mean elimination half-life for regimen 2 (9.93 ± 0.20 hours and 8.77 ± 0.71 hours) both differed significantly from those for regimens 1 (7.21 ± 0.11 hours and 5.71 ± 0.38 hours) and 3 (7.59 ± 0.13 hours and 7.37 ± 1.19 hours). Values obtained from indices for *P multocida* and *M haemolytica* had an excessively wide range because of the various degrees of antimicrobial susceptibility (low, medium, and high) of the strains.

Conclusions and Clinical Relevance—Regimen 3 had the most favorable indices, and it would be conducive for owner compliance and require less handling of animals. (*Am J Vet Res* 2014;75:1049–1055)

Water buffalo (*Bubalus bubalis*) are of great economic importance in various areas of the world, such as in South America, principally in marginally tropical or subtropical areas. In young buffalo (eg, nursing and weaning calves), the most frequent clinical problems are diarrhea, pneumonia, and pneumoenteritis.[1–3]

Marbofloxacin is a fluoroquinolone with broad-spectrum antimicrobial activity. The pharmacokinetic profile of marbofloxacin makes it a suitable treatment for animals with conditions (especially intestinal tract and pulmonary infections) that have proven to be refractory to initial treatment with an approved antimicrobial. High doses of fluoroquinolones may cause chondrotoxicosis in young animals. Antimicrobial resistance is an issue of concern. Use of fluoroquinolones is associated with widespread antimicrobial resistance. Drug residues in animal-derived food pose threats to human health. For these reasons, both the European Medicines Agency and US FDA recommend rational and prudent use of fluoroquinolones. The European

Abbreviations

AI	Accumulation index
AUC	Area under the concentration-time curve
AUC_{0-24}	Area under the concentration-time curve from 0 to 24 hours
$AUC_{0-\infty}$	Area under the concentration-time curve extrapolated to infinity
AUC_{0-last}	Area under the concentration-time curve from time 0 to the time of the last measurable concentration
Cmax	Maximum concentration
MIC	Minimum inhibitory concentration
MIC_{90}	Minimum inhibitory concentration required to inhibit growth of 90% of isolates
MPC	Mutant prevention concentration
MRT	Mean residence time
MSW	Mutant selection window
Tmax	Time of maximum concentration

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Medicines Agency classifies fluoroquinolones as an antimicrobial of second choice. The FDA prohibits extralabel use of fluoroquinolones under AMDUCA in all food-producing animals.

Dosage considerations include the fact that marbofloxacin, similar to other fluoroquinolones, is concentration dependent[4] and that MPCs are needed to prevent selection for antimicrobial resistance.[5,6] Selection for drug-resistant mutants is most pronounced within the range of antimicrobial plasma concentrations between the MIC of wild-type bacterial populations and the MPC. This range is also called the MSW.[5]

During treatment, if serum and tissue drug concentrations are maintained above the MPC, few or no resistant mutants will be selectively amplified. Limiting the amount of time drug concentrations are within the MSW lessens the selective pressure. Therefore, optimal treatment should rapidly reach concentrations above the MPC and then rapidly decrease to concentrations below the MIC.[7]

The purpose of the study reported here was to determine the pharmacokinetics of marbofloxacin in buffalo calves after multiple SC administrations. A second objective was to assess differences in efficacy of dosage regimens, as determined on the basis of pharmacokinetic and pharmacodynamic efficacy predictors.

Materials and Methods

Animals—Eighteen healthy water buffalo calves (7 to 15 days old; mean ± SD body weight, 53.8 ± 11.09 kg) were used in the study. The study was approved by the Animal Experimentation Ethics Committee of the Universidad Nacional del Litoral School of Veterinary Medicine (authorization No. 16/2008).

A parallel design was used. Six calves were assigned by use of a stratified randomization procedure to each of 3 groups; each group received marbofloxacin[a] SC in the neck at 1 of 3 dosages (2 mg/kg, q 24 h for 6 days [regimen 1]; 4 mg/kg, q 48 h for 6 days [regimen 2]; or 4 mg/kg, q 24 h for 3 days [regimen 3]). Time immediately before administration of the first dose of marbofloxacin was designated as time 0.

Blood samples (4 mL) were collected from the left jugular vein of each calf at various times. For calves administered marbofloxacin in accordance with regimen 1, blood samples were collected 0, 10, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours after the first, third, and sixth injections; 1, 2, 12, and 24 hours after the second, fourth, and fifth injections; and 28, 32, 36, and 48 hours after the sixth injection. For calves administered marbofloxacin in accordance with regimen 2, blood samples were collected 0, 10, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 28, 32, 36, and 48 hours after the first and second injections as well as 0, 10, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 28, 32, 36, 48, 52, 56, 60, and 72 hours after the third injection. For calves administered marbofloxacin in accordance with regimen 3, blood samples were collected 0, 10, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours after the first and second injections as well as 0, 10, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 28, 32, 36, 48, 52, 56, 60, and 72 hours after the third injection. Samples were allowed to clot at ambient temperature (approx 25°C) for 30 minutes after collection; samples then were centrifuged at 1,800 $\times$ *g* for 20 minutes. Serum aliquots were frozen (–80°C) until assayed. Analyses were performed within 4 weeks after sample collection.

Analytic technique—Serum marbofloxacin concentrations were quantified by means of high-performance liquid chromatography as described elsewhere.[8] Ofloxacin[b] (5 µg/mL) in 0.1N formic acid was used as an internal standard. A volume of 300 µL of serum was placed into a 15-mL screwcap tube, and 75 µL of the internal standard and 4.5 mL of trichloromethane were added. Tubes were mixed for 10 minutes in a horizontal agitator; samples then were centrifuged at 3,200 $\times$ *g* for 7 minutes at 10°C. The organic layer was aspirated and transferred to another tube, which was evaporated under a stream of nitrogen gas at 40°C. Samples were reconstituted in 150 µL of mobile phase; the mobile phase consisted of buffer solution, methanol, acetonitrile, acetic acid, and triethylamine (74:20:4:1:1 [vol/vol/vol/vol/vol]). The buffer (pH, 2.7) was a 0.4% aqueous solution of tetrabutylammonium hydrogen sulfate and diammonium hydrogen phosphate. A 20-µL aliquot of each reconstituted sample was injected into the high-performance liquid chromatography system. Separation was achieved with a C18 reverse-phase column[c] (particle size, 5 µm; length $\times$ diameter, 150 $\times$ 4.6 mm; mean pore diameter, 100 Å) and C18 guard column[c] (particle size, 5 µm; length $\times$ diameter, 30 $\times$ 4.0 mm; mean pore diameter, 100Å) at 23°C. The UV detection wavelength was 295 nm, and flow rate was 0.6 mL/min. Limit of quantification was 0.025 µg/mL, and results were linear between 0.025 and 15 µg/mL. Mean ± SD precision and accuracy of the limit of quantification were 7.89 ± 0.03% and 93.28 ± 11.53%, respectively. Mean ± SD interassay and intra-assay reproducibility were 5.2 ± 1.9% and 3.71 ± 1.63%, respectively.

Pharmacokinetic analysis—Marbofloxacin serum concentration–time curves were processed with a commercial program.[d] The Cmax and Tmax for each calf were obtained directly from the concentration data. Noncompartmental pharmacokinetic parameters included the elimination rate constant λ (calculated as the slope of the terminal phase of the plasma concentration curve that included a minimum of 4 points), elimination half-life (calculated as 0.693/λ), AUC (calculated by use of the logarithmic trapezoidal rule), total AUC (sum of $AUC_{0–last}$ for each of the doses), area under the first moment curve, and MRT (calculated as area under the first moment curve/AUC). The AI of Cmax or AUC was calculated as the value after the last dose divided by the value after the first dose. All values were reported as mean ± SD.

Pharmacokinetic and pharmacodynamic indices—The Cmax and $AUC_{0–24}$ were used in the calculation of predictors of efficacy for concentration-dependent antimicrobials (ie, Cmax/MIC and $AUC_{0–24}$/MIC). Also, the indices $AUC_{0–24}$/MPC and the percentage of time the concentration was above the MIC and MPC and within the MSW were calculated. All indices were calculated as the mean of

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individual values for the various numbers of doses (ie, 3 or 6) per calf and the mean of all calves by regimen doses against each MIC_{90} and MPC.

The authors were not aware of any published data concerning antimicrobial activity of marbofloxacin against isolates obtained from buffalo. Therefore, published data[9] of mean MIC_{90} and MPC for isolates obtained from cattle with respiratory tract infections were used.

Statistical analysis—Normal distribution of data was confirmed with a Shapiro-Wilk test. Differences among pharmacokinetic parameters of treatment groups were determined by use of an ANOVA with a post hoc Duncan test. Values of $P \leq 0.05$ were considered significant.

Results

Evaluation of serum marbofloxacin concentration–time curves and pharmacokinetic parameters obtained after 3 SC administrations revealed differences among the regimens (**Figure 1**; **Tables 1–4**). Mean Cmax for the first and last dose after administration of 4 mg/kg differed significantly ($P < 0.001$) from the Cmax for the first and last dose after administration of 2 mg/kg. Similarly, mean $AUC_{0\text{–last}}$ differed significantly ($P < 0.001$) between doses administered at 48-hour intervals and doses administered at 24-hour intervals. There were no significant differences among regimens for total $AUC_{0\text{–last}}$ ($P = 0.190$) or total $AUC_{0\text{–}24}$ ($P = 0.242$). There was a slight albeit nonsignificant ($P = 0.237$) accumulation with regimen 3 (mean ± SD AI for AUC, 1.18 ± 0.26 μg•h/mL). There were no significant ($P = 0.472$) differences in AI for AUC. Mean elimination half-life ($P = 0.003$) and MRT ($P = 0.002$) were significantly different at 24 and 48 hours.

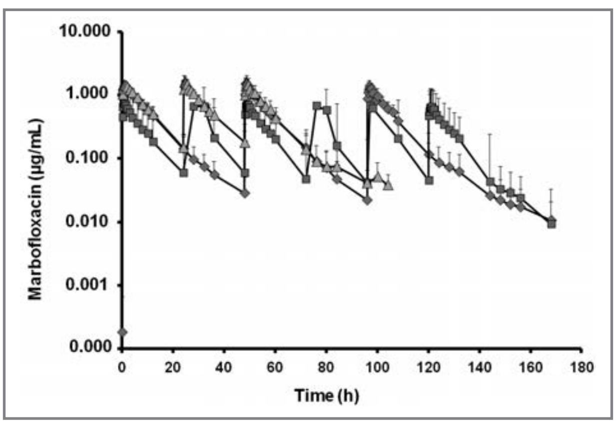

Figure 1—Mean ± SD serum marbofloxacin concentration–time profile in 18 water buffalo calves (n = 6 buffalo/group) assigned to receive marbofloxacin SC in the neck at 1 of 3 dosages (2 mg/kg, q 24 h for 6 days [regimen 1; squares]; 4 mg/kg, q 48 h for 6 days [regimen 2; diamonds]; or 4 mg/kg, q 24 h for 3 days [regimen 3; triangles]). Time immediately before administration of the first dose of marbofloxacin was designated as time 0.

The efficacy predictors Cmax/MIC, $AUC_{0\text{–}24}$/MIC, $AUC_{0\text{–}24}$/MPC, and the percentage of time the concentration was above the MIC and MPC and within the MSW were highly variable (**Tables 5 and 6**). Antimicrobial susceptibility of the strains was highly variable. The most favorable pharmacokinetic and pharmacodynamic indices were obtained with regimens 2 and 3.

Discussion

Marbofloxacin is a concentration-dependent antimicrobial. Three multiple-dose regimens (same total dose of marbofloxacin) were used to determine the regimen that was most suitable from the standpoint of efficacy and for management of water buffalo calves. Young (7 to 15 days old) calves were selected because they have a high prevalence of diarrhea, pneumonia, and pneumoenteritis.

Extralabel use of fluoroquinolones, including marbofloxacin, is prohibited in food-producing animals in the United States. Marbofloxacin administration at the same dosage as for regimen 1 in the present study (2 mg/kg, SC, q 24 h for 3 to 5 days) has been authorized in Europe for the treatment of infections in cattle that have proven to be refractory to initial treatment with an approved antimicrobial. Marbofloxacin is reserved for conditions that have responded poorly to other classes of antimicrobials; there is a strict withdrawal time of ≥ 28 days for meat, fat, and offal, which is under the responsibility of the attending veterinarian. Regimens 2 and 3 in the present study provided the same total dose as regimen 1 but were chosen to allow for comparison with results reported by other authors who used similar doses. The regimens in the study reported here also avoided fluoroquinolone toxicosis in neonates[4] that is possible at a higher dose (10 mg/kg).[7]

Pharmacokinetic values obtained with regimen 1 agreed with those reported for a single dose (2 mg/kg)

Table 1—Pharmacokinetic values for marbofloxacin in water buffalo calves (n = 6) after administration at 2 mg/kg, SC, every 24 hours for 6 days (regimen 1).

	Dose						
Variable	1	2	3	4	5	6*	6†
Tmax (h)	0.65 ± 0.27	1.33 ± 0.52	0.64 ± 0.51	1.17 ± 0.41	1.17 ± 0.41	1.07 ± 0.63	1.07 ± 0.63
Cmax (μg/mL)	0.82 ± 0.13	0.71 ± 0.15	0.76 ± 0.15	0.69 ± 0.12	0.69 ± 0.14	0.68 ± 0.08	0.68 ± 0.08
$AUC_{0\text{-}24}$ (μg•h/mL)	6.02 ± 1.04	6.42 ± 1.68	6.05 ± 1.58	5.23 ± 1.15	6.04 ± 1.23	5.71 ± 0.98	6.28 ± 1.28
$AUC_{0\text{–}\infty}$ (μg•h/mL)	6.56 ± 1.35	—	6.49 ± 1.90	—	—	6.06 ± 1.24	6.39 ± 1.33
λ (h^{-1})	0.12 ± 0.03	—	0.12 ± 0.02	—	—	0.13 ± 0.02	0.09 ± 0.03
$t_{1/2}\,\lambda$ (h)	6.06 ± 1.59	—	5.77 ± 1.12	—	—	5.31 ± 0.86	8.48 ± 2.49
$MRT_{0\text{–}24}$ (h)	7.24 ± 1.14	—	7.09 ± 0.94	—	—	7.29 ± 0.74	9.49 ± 1.43

Values reported are mean ± SD.
*Value determined at 24 hours after dose 6. †Value determined at the last measurable concentration after dose 6.
λ = Elimination rate constant calculated by use of linear regression with the last measured concentration–time values. $MRT_{0\text{–}24}$ = The MRT from 0 to 24 hours. $t_{1/2}\,\lambda$ = Elimination half-life. — = Not determined.

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in this species.[10–12] An accumulation process was not observed with regimen 1, as determined on the basis of Cmax (AI, 0.85 ± 0.17 µg/mL) and AUC (AI, 0.96 ± 0.17 µg•h/mL). An accumulation process was not observed with regimens 2 and 3, and there were no significant differences for the AI of Cmax ($P = 0.237$) and AUC ($P = 0.472$). The absence of accumulation despite use of a dose of 4 mg/kg could have been attributable to the clearance capacity of the calves. Although the animals were young, they had developed an elimination capacity (specifically, renal excretion as the principal means for excretion of marbofloxacin). In ruminants, the development of glomerular filtration is completed 1 to 3 days after birth, whereas tubular secretion processes require up to 1 or 2 weeks after birth.[13] In addition, this capacity can be evaluated with the parameters that measure the permanence or elimination of a drug in an animal. The half-life and MRT were < 12 hours, which would justify the absence of accumulation, given that the interval between doses was ≥ 24 hours. The elimination half-life and MRT were similar for the various regimens. Mean ± SD elimination half-life was 5.71 ± 0.38 hours, 8.78 ± 0.71 hours, and 7.37 ± 1.19 hours for regimens 1, 2, and 3, respectively. Mean residence time was 7.21 ± 0.11 hours, 9.93 ± 0.20 hours, and 7.59 ± 0.13 hours for regimens 1, 2, and 3, respectively. Elimination half-life was significantly ($P = 0.003$) different for dosing intervals of 24 and 48 hours. The MRT also was significantly ($P = 0.002$) different for dosing intervals of 24 and 48 hours. These values were in accordance with those obtained in buffalo by other investigators.[11,12] On the other hand, the lack of significant differences among total $AUC_{0–last}$ ($P = 0.190$) confirmed that there was no accumulation process.

A proportional response was observed for regimens 2 and 3. Mean Cmax and AUC were dose dependent. This is important for the efficacy indices (eg, AUC/MIC), especially for fluoroquinolones. Total $AUC_{0–last}$ had no importance from the standpoint of efficacy indices because it is not used to calculate those indices. For these reasons, mean $AUC_{0–24}$ was the most relevant parameter regarding pharmacokinetic and pharmacodynamic correlation. In this case, the principal factor

Table 2—Pharmacokinetic values for marbofloxacin in buffalo calves (n = 6) after administration at 2 mg/kg, SC, every 24 hours for 6 days (regimen 1).

Variable	24 hours*	Infinity†	AI
Mean Cmax (µg/mL)	0.72 ± 0.06	0.72 ± 0.06	0.84 ± 0.17
Mean $AUC_{0–24}$ (µg•h/mL)	5.92 ± 0.40	6.48 ± 0.09	—
Total AUC (µg•h/mL)‡	35.49	36.05	0.96 ± 0.17
Mean $t_{1/2}$ λ (h)	5.71 ± 0.38	—	—
Mean $MRT_{0–24}$ (h)	7.21 ± 0.11	—	—

Values reported are mean ± SD.

*Represents the mean of each variable for doses 1 to 6 at 24 hours. †Represents value extrapolated to infinity. ‡Sum of the means for each of the 6 doses.

— = Not determined.

Table 3—Pharmacokinetic values for marbofloxacin in buffalo calves (n = 6) after administration at 4 mg/kg, SC, every 48 hours for 6 days (regimen 2).

Variable	Dose 1	Dose 2	Dose 3*	Dose 3†	Total‡	Mean ± SD§	AI
Tmax (h)	0.87 ± 0.36	0.53 ± 0.26	0.89 ± 0.45	0.89 ± 0.45	—	—	—
Cmax (µg/mL)	1.41 ± 0.26	1.63 ± 0.28	1.45 ± 0.36	1.45 ± 0.36	—	1.49 ± 0.12	1.01 ± 0.24
$AUC_{24\ truncated}$ (µg•h/mL)	14.55 ± 2.84	12.84 ± 3.26	12.01 ± 3.58	—	39.40	—	—
$AUC_{0–48}$ (µg•h/mL)	15.19 ± 4.29	14.34 ± 4.22	13.35 ± 4.36	13.76 ± 4.72	42.88	14.29 ± 0.92	0.93 ± 0.10
$AUC_{0–\infty}$ (µg•h/mL)	15.50 ± 4.49	14.63 ± 4.64	13.73 ± 4.71	13.85 ± 4.85	43.29	—	—
λ (h^{-1})	0.09 ± 0.02	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	—	—	—
$t_{1/2}$ λ (h)	8.46 ± 1.78	8.28 ± 2.30	9.59 ± 3.09	9.05 ± 1.60	—	8.78 ± 0.71	—
$MRT_{0–48}$ (h)	10.02 ± 1.59	9.70 ± 1.76	10.06 ± 1.74	11.32 ± 2.22	—	9.93 ± 0.20	—

Values reported are mean ± SD.

*Value determined at 48 hours after dose 3. †Value determined at the last measurable concentration after dose 3. ‡Represents sum of the means for each of the 3 doses. §Represents mean ± SD of the means for each of the 3 doses determined at 48 hours.

$AUC_{24\ truncated}$ = The AUC truncated at 24 hours. $AUC_{0–48}$ = The AUC from 0 to 48 hours.

See Table 1 for remainder of key.

Table 4—Pharmacokinetic values for marbofloxacin in buffalo calves (n = 6) after SC administration at 4 mg/kg, SC, every 24 hours for 3 days (regimen 3).

Variable	Dose 1	Dose 2	Dose 3*	Dose 3†	Total‡	Mean ± SD§	AI
Tmax (h)	0.89 ± 0.39	0.54 ± 0.28	0.96 ± 0.46	0.96 ± 0.46	—	—	—
Cmax (µg/mL)	1.50 ± 0.13	1.76 ± 0.37	1.64 ± 0.50	1.64 ± 0.50	—	1.63 ± 0.13	1.18 ± 0.26
$AUC_{0–24}$ (µg•h/mL)	14.55 ± 2.84	14.36 ± 4.06	13.60 ± 5.08	15.28 ± 6.83	42.51	14.17 ± 0.51	1.03 ± 0.13
$AUC_{0–\infty}$ (µg•h/mL)	16.10 ± 3.90	17.78 ± 9.71	15.08 ± 6.38	15.52 ± 6.89	44.20	—	—
λ (h^{-1})	0.11 ± 0.02	0.10 ± 0.03	0.11 ± 0.02	0.10 ± 0.02	—	—	—
$t_{1/2}$ λ (h)	6.67 ± 1.40	8.74 ± 5.60	6.69 ± 1.30	7.67 ± 2.19	—	7.37 ± 1.19	—
$MRT_{24–last}$ (h)	7.66 ± 0.82	7.67 ± 0.96	7.44 ± 0.70	9.84 ± 2.60	—	7.59 ± 0.13	—

Values reported are mean ± SD.

*Value determined at 24 hours after dose 3. †Value determined at the last measurable concentration after dose 3. ‡Represents sum of the means for each of the 3 doses. §Represents mean ± SD of the means for each of the 3 doses determined at 24 hours.

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Table 5—Efficacy predictors estimated for marbofloxacin against bacteria with high, medium, and low antimicrobial susceptibility* isolated from respiratory infections of cattle.

	High			Medium			Low		
Variable	Cmax/MIC	AUC_{0-24}/MIC	AUC_{0-24}/MPC	Cmax/MIC	AUC_{0-24}/MIC	AUC_{0-24}/MPC	Cmax/MIC	AUC_{0-24}/MIC	AUC_{0-24}/MPC
Pasteurella multocida									
Regimen 1	48.25 ± 3.73	394.22 ± 26.76	197.11 ± 13.38	24.13 ± 1.86	197.11 ± 13.38	98.56 ± 6.69	0.72 ± 0.06	5.91 ± 0.40	2.96 ± 0.20
Regimen 2†	102.80 ± 4.63	875.53 ± 86.49	437.76 ± 43.25	51.40 ± 2.32	437.76 ± 43.25	218.88 ± 21.62	1.54 ± 0.07	13.13 ± 1.30	6.57 ± 0.65
Regimen 3	108.85 ± 5.66	944.62 ± 33.77	472.31 ± 16.88	54.46 ± 4.33	472.31 ± 16.88	236.15 ± 8.44	1.63 ± 0.13	14.17 ± 0.51	7.08 ± 0.25
Mannheimia haemolytica									
Regimen 1	24.13 ± 1.86	197.11 ± 13.38	48.28 ± 3.35	2.90 ± 0.22	23.65 ± 1.61	11.83 ± 0.80	0.72 ± 0.06	5.91 ± 0.40	2.96 ± 0.20
Regimen 2†	51.04 ± 2.32	437.76 ± 43.25	109.44 ± 10.81	6.17 ± 0.28	52.53 ± 5.19	26.26 ± 2.59	1.54 ± 0.07	13.13 ± 1.30	6.56 ± 0.66
Regimen 3	54.43 ± 4.33	472.31 ± 16.88	118.05 ± 4.22	6.53 ± 0.52	56.68 ± 2.03	28.34 ± 1.01	1.63 ± 0.13	14.17 ± 0.51	7.08 ± 0.25

Values reported are mean ± SD.

*Antimicrobial susceptibility for *P multocida* was defined as follows[9]: high (MIC, 0.015 µg/mL; MPC, 0.03 µg/mL), medium (MIC, 0.03 µg/mL; MPC, 0.06 µg/mL), or low (MIC, 1 µg/mL; MPC, 2 µg/mL). Antimicrobial susceptibility for *M haemolytica* was defined as follows[9]: high (MIC, 0.03 µg/mL; MPC, 0.12 µg/mL), medium (MIC, 0.25 µg/mL; MPC, 0.5 µg/mL), or low (MIC, 1 µg/mL; MPC, 2 µg/mL). †The AUCs for regimen 2 are truncated at 24 hours.

Table 6—Efficacy predictors estimated for marbofloxacin on the basis of MIC and MPC for bacteria with high, medium, and low antimicrobial susceptibility* reported elsewhere.[9]

	High			Medium			Low		
Variable	Time above MIC† (%)	Time above MPC† (%)	Time within MSW§ (%)	Time above MIC† (%)	Time above MPC† (%)	Time within MSW§ (%)	Time above MIC† (%)	Time above MPC† (%)	Time within MSW§ (%)
Pasteurella multocida									
Regimen 1	100	100	0	100	96.32 ± 3.09	3.68	0	0	0
Regimen 2	100	96.14 ± 3.69	3.86	96.14 ± 3.69	72.25 ± 2.73	22.89	6.77 ± 2.23	0	6.77
Regimen 3	100	100	0	100	100	0	19.53 ± 1.38	0	19.53
Mannheimia haemolytica									
Regimen 1	100	78.89 ± 2.25	21.11	46.64 ± 5.73	17.54 ± 4.41	29.1	0	0	0
Regimen 2	96.14 ± 3.69	52.73 ± 2.85	43.41	40.88 ± 1.47	22.56 ± 1.99	18.32	6.77 ± 2.23	0	6.77
Regimen 3	100	100	0	85.9 ± 2.97	51.12 ± 4.49	34.78	19.53 ± 1.38	0	19.53

Values reported are mean ± SD.

†When the mean value is 0, it is not shown because all data were above 100% or values were above the MIC or MPC. §The time within MSW is calculated as the difference between the mean time above the MIC and mean time above the MPC.

See Table 5 for remainder of key.

that determined this value was the dose (2 vs 4 mg/kg), which was independent of the dosing interval (24 or 48 hours) because there was no accumulation.

A high-dose, short-term regimen of fluoroquinolones has been developed with the purpose of early resolution of clinical signs, enhancement of concentration-dependent bactericidal activity, and reduction in the potential for the emergence of antimicrobial resistance.[14] The postantibiotic effect associated with fluoroquinolones allows for the use of high doses of marbofloxacin to be administered less frequently, which supports administration in accordance with a high-dose, short-term regimen every 24 or 48 hours. This regimen should be more conducive to client compliance because of the shorter duration of treatment and the need for animals to be handled less often.

Resistance develops mainly through mutation of the *parC* and *gyrA* genes. As the efficacy index AUC/MIC increases, bactericidal activity is likely to become more potent, which lowers the potential for first- and second-step mutations (and thus antimicrobial resistance).

Antimicrobial susceptibility of selected strains is extremely broad. Consequently, the efficacy indices Cmax/MIC and AUC/MIC are extremely broad. On the basis of the effectiveness criteria for pharmacokinetic and pharmacodynamic indices described by various authors,[4,15,16] the doses used in the study reported here would only be effective against bacteria of high and medium antimicrobial susceptibility. It would be necessary to use higher doses to affect strains with low antimicrobial susceptibility (Table 5). Regimen 1 was sufficient only for the treatment of infections with an MIC_{90} < 0.048 µg/mL, as determined on the basis of the following equation proposed in another study[17]: Dose = ([AUC/MIC]•clearance•MIC)/bioavailability, where the target AUC/MIC is 125 hours. However, with regimens 2 and 3, the antimicrobial susceptibility was slightly higher (0.108 µg/mL for regimen 2 and 0.116 µg/mL for regimen 3) in buffalo calves.

Therefore, SC administration of marbofloxacin at a dose of 2 or 4 mg/kg to buffalo calves may be adequate for the treatment of infections caused by highly susceptible bacteria (some strains of *Escherichia coli* or *Salmonella* spp), which are sometimes involved in the diarrheic syndrome in buffalo neonates.[1,18] Doses of 2 and 4 mg/kg do not appear to be sufficient for the treatment of infections with an MIC > 0.048 or 0.1 µg/mL, respectively. In the present study, the chosen regimen would only be adequate for treatment of strains of *Pasteurella multocida* with medium (MIC, 0.03 µg/mL) or high (MIC, 0.015 µg/mL) antimicrobial susceptibility and *Mannheimia haemolytica* with high (MIC, 0.03 µg/mL) antimicrobial susceptibility.

On the other hand, MIC is related to drug-susceptible populations and is not relevant to the prevention

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of the development of antimicrobial-resistant mutants. Mutant prevention concentration may be useful in selecting an antimicrobial dosage to reduce the emergence of antimicrobial-resistant bacteria. It has been suggested[19] that AUC/MPC is the single pharmacodynamic index with the least variation and therefore best predicts prevention of emergence of antimicrobial resistance. An AUC/MPC of 35 was found to be sufficient to prevent the growth of antimicrobial-resistant mutants.[19–21] In the present study, only strains of *P multocida* with high and medium antimicrobial susceptibility and *M haemolytica* with high antimicrobial susceptibility met this requirement.

With regard to other indices, the percentage of time the concentration is above the MPC would need to be at least 33% of the dosing interval and time within the MSW ≤ 20% to 30% (for high inoculum size) to prevent resistance selection.[19–21] With regimens 2 and 3, the percentage of time the marbofloxacin concentration was above the MPC was 0% for *P multocida* and *M haemolytica* strains with low antimicrobial susceptibility.[21] With regimens 2 and 3, the percentage of time the marbofloxacin concentration was above the MPC was > 33% for *P multocida* strains with high antimicrobial susceptibility. With regimen 3, the mean ± SD percentage of time the marbofloxacin concentration was above the MPC was 51.12 ± 4.49% for *P multocida* strains with medium antimicrobial susceptibility (Table 6). Consequently, the percentage of time the marbofloxacin concentration was within the MSW was < 20% to 30% for *P multocida* with medium or high antimicrobial susceptibility and *M haemolytica* with high antimicrobial susceptibility. Thus, regimen 3 resulted in the most favorable indices.

The present study confirmed results of previous pharmacokinetic and pharmacodynamic experiments with calves, which concluded that a multiple-dose regimen of marbofloxacin (2 mg/kg, q 24 h) was optimal for eradication of pathogens with an MIC ≤ 0.04 µg/mL.[22,23] In the study reported here, regimen 1 provided serum marbofloxacin concentrations sufficient to kill bacteria with an MIC < 0.048 µg/mL. In contrast, regimen 3 achieved a longer time during which the marbofloxacin concentration was above the MPC and was within the MSW for pathogens of medium antimicrobial susceptibility. For pathogens of low antimicrobial susceptibility, much higher doses would be required, but it is necessary to know the safety of such a high dose in young animals. Because pathogens of medium and low antimicrobial susceptibility are typically the organisms encountered in second-intention healing that are refractory to initial treatments, they also potentially have the highest risk of antimicrobial resistance.

The clinical antimicrobial susceptibility breakpoint of 1 µg/mL corresponds to the MIC of the family Pasteurellaceae members with 1 or 2 mechanisms of resistance,[18,24] which means that treatment in accordance with a high-dose regimen would likely prevent the emergence of antimicrobial resistance. On the other hand, it is necessary to avoid the possible toxic effects of fluoroquinolones in neonates or young animals.

Given that the pharmacokinetic and pharmacodynamic indices for marbofloxacin in buffalo have not yet been established, we could conclude that our obtained values for the indices are better for a high-dose, short-term regimen. This regimen (4 mg/kg, SC, q 24 h) also would lend itself to better compliance because of the shorter duration of treatment and requirement that animals be handled less often. However, it is necessary to consider that an increase of dosage would entail a modification of withdrawal time, which must be considered by clinicians.

In contrast, it appears that a higher dose would be required for pathogens of low susceptibility, and these regimens, including regimen 3, would not be sufficient to prevent the amplification of preexisting antimicrobial-resistant subpopulations and selective growth of mutants for these strains. Future studies are required to determine the MIC and MPC of pathogenic bacteria isolated from buffalo calves.

a. Marbocyl, Marbofloxacin 10%, Vetoquinol, Lure, France.
b. Ofloxacin, Sigma Chemical Co, St Louis, Mo.
c. Kromasil, Scharlab SL, Barcelona, Spain.
d. PCnonlin, version 4.0, Statistical Consultants Inc, Lexington, Ky.

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