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Pharmacokinetic/pharmacodynamic modeling in plasma and milk and Monte Carlo simulations of marbofloxacin against *Staphylococcus aureus* and *Mycoplasma agalactiae* in lactating sheep

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ABSTRACT

In livestock ruminants such as sheep, different infectious diseases such as mastitis or contagious agalactia are originated from pathogens as *Staphylococcus aureus* and *Mycoplasma agalactiae*. Fluoroquinolones are authorized in dairy animals, including their extralabel use, as an alternative when other treatment failed in the European Union (EU), however, in the United States, are prohibited from extralabel drug use in food-producing animals. Marbofloxacin, a well-known fluoroquinolone is commonly used in dairy cattle in the EU at 10 mg/kg. However, their off-label use in sheep also has been described. Nevertheless, the dose extrapolations from dairy cows should include pharmacokinetic (PK) studies because of interspecies differences and the potential risks of antimicrobial resistance or toxicity. In this regard, the aims of this research were to (1) describe the i.v. and i.m. PK analysis of marbofloxacin in plasma and milk of lactating sheep at 10 mg/kg, (2) determine the MIC and calculate the tentative epidemiological cutoff values (TECOFF) for *Mycoplasma agalactiae* and *Staphylococcus aureus* wild-type isolates from sheep, and (3) conduct a pharmacokinetic and pharmacodynamic (PK/PD) analysis with the Monte Carlo simulation to obtain the probability of target attainment for different MIC values, known as the PK/PD cutoff values. The results of this study could help to establish the efficacy of a 10 mg/kg dosage regimen of marbofloxacin in lactating sheep. Plasma and milk concentrations were described with a nonlinear mixed effects model. The intramuscular bioavailability was 88%, and the volume of distribution was 1.31 L/kg with a clearance value of 0.38 L/h/kg. Half-lives after i.v. and i.m. dosing were 6.53 and 7.09 h in plasma, and 6.62 and 6.65 h in milk, respectively. High concentrations were determined in milk with area under the curve (AUC) milk/plasma ratios close to 1.28. The MIC values for *Staphylococcus aureus* and *Mycoplasma agalactiae* were obtained, and TECOFF values of 1.0 and 2.0 µg/mL, respectively, were determined. The Monte Carlo simulations predicted that the dosage regimen of 10 mg/kg per 24 h in lactating sheep can be adequate for intermediate and high MIC values of 0.5 and 1.0 µg/mL, respectively, and could be useful for populations with a target AUC/MIC ratio ≤48 for *Staphylococcus aureus*, but not for *Mycoplasma agalactiae*. Results derived for this study could be taken as previous tentative points for further studies of marbofloxacin in lactating and nonlactating sheep in a clinical context.

Key words: marbofloxacin, sheep, Monte Carlo simulations, pharmacokinetics, milk

INTRODUCTION

The current use of antibacterials in livestock animals is one of the main therapeutic tools in the European Union (**EU**), where infectious diseases such as mastitis or contagious agalactia in small ruminants are the cause of significant economic losses, as well as a public health problem (Contreras et al., 2007; Mavrogianni et al., 2011). In countries of the Mediterranean region, such as France or Spain, the main pathogens responsible for these infections are *Staphylococcus aureus* and *Mycoplasma agalactiae* (Fernández-Varón et al., 2021). Typical treatments against these bacteria include the use of macrolides or β-lactams, following the indications of the

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European Medicines Agency (**EMA**) in relation to the categorization of antibiotics (EMA, 2020). However, it is also established that the use of these antimicrobials must be based on previous antibiotic susceptibility tests (**AST**) whenever possible, to minimize resistance, a problem that reduces the effectiveness of these drugs, making necessary the use of alternatives (EMA, 2017; Nelli et al., 2022). Following the EMA regulations, there are 4 categories of antibiotics: avoid (A), restrict (B), caution (C), and prudence (D). It is at this point where category B plays an important role, with fluoroquinolones being an interesting alternative given the high resistance that these pathogens have against more conventional treatments typical of categories D and C (Gautier-Bouchardon, 2018; EMA, 2020). Consequently, the extralabel use of these drugs is authorized in the EU. In the United States, extralabel use of fluoroquinolones is prohibited in by the Food and Drug Administration (**FDA**). Therefore, the indications referred to in this study fall under EMA regulations (FDA, 1997; EMA, 2024).

Marbofloxacin is one of the main fluoroquinolones used in the EU for the treatment of intestinal and respiratory infections, mastitis, or other diseases of the udder, and its use at 8 mg/kg and 10 mg/kg has been approved in pigs and cattle for these diseases (Petracca et al., 1993; Aliabadi and Lees, 2002; EMA, 2024). The extralabel use and pharmacokinetics (**PK**) at lower doses of fluoroquinolones in other species, such as goats, sheep, and horses, have been described (Coskun et al., 2020; Bousquet-Mélou et a., 2021; Lorenzutti et al., 2021).

Different PK studies of marbofloxacin in sheep after i.v. and i.m. administration with doses from 2 to 2.5 mg/kg have been published (Sidhu et al., 2010; Mahmood et al., 2015; Munawar et al., 2017), as well as the PK and effects of i.m. administration at 3 mg/kg combined with albendazole in sheep infected with *Mannheimia haemolytica* (Altan et al., 2019). Finally, the endotoxin-induced fever's effect after i.v. administration also has been evaluated (Coskun et al., 2020). But, to the knowledge of the authors of this manuscript, only one study has reported the PK in plasma and milk at 2.5 mg/kg after i.v. and i.m. dosing in lactating sheep (Shem-Tov et al., 1997), and there are no PK studies of marbofloxacin at 10 mg/kg in lactating sheep that include pharmacokinetic and pharmacodynamic (**PK/PD**) analysis against *Staphylococcus aureus* and *Mycoplasma agalactiae*, despite their emergence as important pathogens in ruminants (Poumarat et al., 2016).

From a pharmacodynamic (**PD**) point of view, the clinical break points (**CBP**) of marbofloxacin for the interpretation of AST against pathogens as *Staphylococcus aureus* and *Mycoplasma agalactiae* are unknown in lactating sheep. Following the recommendations of European subcommittee for Veterinary Antimicrobial Susceptibility Testing (**VETCAST**) for establishment a CBP, different issues are required: modeling the distribution of MIC of wild-type strains to determine a tentative epidemiological cutoff (**TECOFF**), calculating a target PK/PD index based on previous PK modeling, and subsequently, determining the probability of target attainment (**PTA**, %) for various MIC values, known as PK/PD cutoff (**PK/PDco**) values using the Monte Carlo simulation (Toutain et al., 2017; Bousquet-Mélou et al., 2021). Finally, for each given dose regimen simulated, the PTA endpoint value used to PK/PDco calculation is 90% (Toutain et al., 2021).

The typical PK/PD index for fluoroquinolones is the area under the curve (**AUC**)/MIC ratio, which represents the area under the drug concentration–time curve divided by MIC (Toutain et al., 2021). However, is most commonly used as the f_uAUC/MIC ratio, where f_uAUC indicates the area of the free drug concentrations under the curve over time (Toutain et al., 2023). The free or unbound concentration to proteins of an antimicrobial determines its clinical efficacy, because it can transfer from plasma to different tissues (Toutain et al., 2023). A systemic drug can access to the milk by passive diffusion influenced by the pH changes between plasma and milk and by the action of transports (Fleishaker, 2003; Pulido et al., 2006). The free or unbound concentration to milk proteins can be used for evaluating the infection site exposure for mastitis (Fleishaker, 2003; Pyörälä, 2009; Toutain et al., 2017). Values of f_uAUC/MIC of 30 to 55 and 100 to 125 for gram-positive and gram-negative bacteria, respectively, have been recommended from human studies (Papich, 2014). However, in veterinary medicine, different animal species could require other values determined by their drug-bacteria interactions and may be different with the targets used in human medicine (Aliabadi and Lees, 2002; Paulin et al., 2018; Serrano-Rodríguez et al., 2017). This is particularly important in the case of udder infections where antimicrobial concentrations in the mammary gland or in milk are critical for the clinical cure of the animals (Toutain et al., 2017). In this context, different authors have proposed the milk concentrations after intramammary or systemic administration and the derived AUC/MIC ratios as surrogate markers of clinical efficacy for the treatment of mastitis in ruminants (Schneider et al., 2004; Toutain et al., 2017). However, there are no established PK/PD targets for antimicrobials in milk, but AUC/MIC ratios from 48 to 65 for marbofloxacin against staphylococci and *Mycoplasma agalactiae*, as well as from 50 to 67 for cefquinome against *Escherichia coli* have been proposed in milk from goats and cows, respectively (Lorenzutti et al., 2021; Xiao et al., 2022; Serrano-Rodríguez et al., 2023). Moreover, these are very specific studies and new assays are needed to evaluate these drug-bacteria interac-

tions. Nevertheless, and regardless of the biological fluid used, the PK/PD relationships of antimicrobials need robust PK models that also include Monte Carlo simulation as a predictive method, as recommended by the EMA (2016). In fact, the Monte Carlo methods are algorithms based in simulations and computations routinely used to predict the distribution of clinical outcomes, such as the probability of therapeutic success, based on PK and PD properties of an antimicrobial (Toutain et al., 2021). This approach optimizes dosing regimens to maximize efficacy while minimizing resistance and supports clinical decisions tailored to different clinical scenarios (de Velde et al., 2018).

In veterinary pharmacology, concentration–time curves from PK data are commonly analyzed using compartmental or noncompartmental methods with the classic 2-state approach (Riviere et al., 2016). However, in recent years, different authors have recommended the use of nonlinear mixed effect models (**NLMEM**) as a tool for analysis, simulation, and prediction of concentrations and PK/PD data from animal studies (Bon et al., 2018). The NLMEM approach offers advantages such as explaining the interindividual variability (**IIV**) of PK parameters and their correlation, description, and quantification of the effect of covariates such as weight, sex, age, or breed. In addition, they can also be used with unbalanced data and crossover design to obtain robust simulations (Schoemaker and Cohen, 1996; Mould and Upton, 2012; Schenk et al., 2021). These models, combined with Monte Carlo simulation, are particularly relevant for antimicrobials (de Velde et al., 2018). According to EMA and VETCAST recommendations, NLMEM models should be used for more robust and efficient analysis of antibiotics (EMA, 2016; Toutain et al., 2017).

The current research aims to (1) perform a PK analysis in plasma and milk with marbofloxacin in lactating sheep by i.v. and i.m. administration at 10 mg/kg using the NLMEM approach; (2) determine the MIC and the TECOFF values for *Mycoplasma agalactiae* and *Staphylococcus aureus* wild-type isolates from sheep; (3) calculate the PTA and the PK/PDco values by Monte Carlo simulations from different PK/PD targets; and (4) compare the PK and PK/PD relationships with MIC and TECOFF data to assess the efficacy of a dose of 10 mg/kg of marbofloxacin in lactating sheep.

MATERIALS AND METHODS

Animals, Treatment, and Sample Collection

Twelve clinically healthy, female Lacaune sheep (84.3 ± 8.6 kg; mean ± SD) from a private sheep farm in Albacete, Spain, were used. The average age of the animals was 33.5 mo. The number of lactations was between 2 and 3. The number of days in lactation was 82 (80–84; median [range]). The daily milk production was 3,430 ± 749 mL (median ± SD). The number of animals was chosen using the equation resource approach and following the recommendations for preclinical PK trials in veterinary medicine (Chittenden, 2011; Charan and Kanthari, 2013). Each animal received marbofloxacin (Forcyl) at a dose of 10 mg/kg either i.v. or i.m. A crossover design was used in 2 phases with a 15-d washout period.

Animals were under semi-intensive regimen in which the animals graze during part of the day, and they receive additional feed (salt and dietary supplements). Animals were observed daily for general health and clinical observation. Local damage was also evaluated by the veterinarian through visual inspection for possible lesions including necrosis, bleeding, abscesses, cysts, and other abnormal pathological findings.

The absence of mastitis was assessed using the California Mastitis Test and the absence of disease was determined through physical examination and blood analysis. The study was approved by the Bioethics Committee of the University of Murcia.

Blood samples were collected at 0 (before dosing), 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 3 2, 48 and 72 h after dosing using a Venoject (Terumo) jugular stick system. Samples were centrifuged at 1,500 × *g* for 15 min, and the plasma was obtained and stored at –45°C until analysis. In the same way, milk samples were collected from homogenized milking yields collected immediately before dosing on the day of treatment administration (time 0) and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 32, 48, and 72 h after administration. The milk samples were collected once the milking was completed and the sample container was shaken. The milking was done using an individual milking sheep machine. Total amount of milk extracted were stored at 4 to 6°C before taking final samples and stored at –45°C. Local damage after i.m. administration were evaluated (pain, temperature at the point of injection, inflammatory reactions, and so on).

Analytical Method and Protein Binding Analysis

Concentrations of marbofloxacin in plasma and milk were measured using a HPLC method with fluorescence detection (Siefert et al., 1999). The system was a Jasco model PU-1585 HPLC pump, with a PU-2080-04 high-pressure quaternary gradient pump, a FP-920 Fluorescence Detector, and an AS-950 autoinjector (Jasco, Tokyo, Japan), connected to a computer with Jasco Chrompass Chromatography data system (Jasco, Tokyo, Japan).

Each sample was analyzed following this methodology: 200 µL of plasma or milk were mixed with 20 µL of internal standard solution (enrofloxacin) and 200 µL

of acetonitrile were added, the mix was shaken and the proteins were precipitated in an ultrasonic bath. Finally, were centrifuged at 1,600 × *g* for 10 min at 25°C. The supernatant obtained was diluted 4-fold with buffer solution of disodium hydrogen phosphate 67 m*M* at pH 7.5, and was transferred to autosampler vials. For each sample, 30 µL was injected into the system, and the separation was developed using a reverse-phase column (Ascentis C18, Supelco Scientists; 150 × 4.6 mm, 5 µm). Both autosampler vials and column temperature were maintained at 24°C. The mobile phase was a mix of acetonitrile (20%) and trifluoroacetic acid (1 g/L; 80%) in an isocratic method with a flow rate of 1.0 mL/min. The times of retention of marbofloxacin and enrofloxacin were ~6.7 and 12.6 min, respectively. The fluorescence detection was performed with an excitation wavelength of 297 nm and an emission wavelength of 515 nm.

Quality controls were prepared from a pool of blank sheep plasma or milk spiked with 9 concentrations of marbofloxacin and enrofloxacin between 0.005 and 6 µg/mL. Plasma intraday precision was estimated from 6 replicates of 3 standard samples used for calibration curves (relative SD <1.9%). Milk intraday precision was relative SD <9.5%. Interday precision was estimated from the analysis of standard samples (plasma or milk) on 3 separate days. Plasma interday obtained a relative SD <3.58%. Milk interday assay obtained a relative SD <8.45%. The limit of quantification and the limit of detection were 0.005 and 0.004 µg/mL, respectively, for both serum and milk.

The protein binding of marbofloxacin in plasma and milk was evaluated by in vitro equilibrium dialysis. Plasma and milk samples obtained from the animals before drug administration were used. They were spiked with stock solutions of marbofloxacin to achieve final concentrations of 0.025, 0.05, 0.25, 0.50, and 1.00 µg/mL. Dialysis was conducted using a semipermeable membrane (Spectra/Por, molecular weight cutoff: 12–14 kDa) against sodium phosphate buffer at 64 m*M* at pH of 7.4 for plasma samples. For the milk samples, a pH of 6.6 was measured and selected for dialysis. Subsequently, samples were shaken at 100 rpm for 24 h at 37°C. Samples from both sides of each membrane were taken and analyzed by the HPLC method. The unbound fraction (***fu***) was calculated as the ratio of the concentration in the buffer respect to plasma or milk samples.

Pharmacokinetic and Statistical Analysis

The concentrations of marbofloxacin in plasma and milk measured by the HPLC method were corrected by unbound fraction f_u, and only free concentrations were used to PK analysis. Consequently, all parameters obtained and PK/PD relationships calculated in this research include this correction, as have been recommended (Toutain et al., 2023).

Both free plasma and milk concentrations were simultaneously modeled with a NLMEM approach using the Monolix 2023R1 Suite software (Simulations Plus/Lixoft Ltd., Lancaster, CA). After previous evaluations of structural models including 1, 2, or 3 compartments and multiple and single absorption rates, the final model was selected according to a reduction of the variability and following different statistical criteria, such as the reduction of likelihood ratio tests as −2·log-likelihood, the Akaike information criterion, and the Bayesian information criterion (Mould and Upton, 2013; Weisskopf et al., 2020). In addition, the goodness-of-fit plots of the final model were also checked after visual inspection of the scatter plots of population and individual predicted versus observed concentrations, the population and individual weighted residuals versus predictions over time, and, finally, the visual predictive check plots (**VPC**; Bousquet-Mélou et al., 2021).

The final PK model was built using 3 compartments. A schematic diagram is showed in Figure 1. For plasma concentrations, the absorption was described by the first-order absorption rate ($\mathbf{K_a}$) and the bioavailability ($\mathbf{F_{IM}}$) after i.m. route, respectively. The distribution of central and peripheral compartments was described by the volume of central compartment ($\mathbf{V_c}$) and the volume of peripheral compartment ($\mathbf{V_p}$), respectively. The clearance processes were described by the clearance of the central compartment (**Cl**), and the intercompartmental clearance between central and peripheral compartment Q. For milk concentrations, the intercompartmental clearance between central and milk compartment was Q_{milk}, and the volume of milk compartment was $V_{milking}$. A log-normal distribution was assumed for all parameters of the model, whereas F_{IM} was assumed to have a logit-normal distribution (Wang et al., 2019). Additionally, for the milk compartment, the milking of the udder every 24 h was described by an emptying effect, as has been recommended in these models (Woodward and Whittem, 2019). Accordingly, the volume of the milk compartment was fixed at 0.12 L, which corresponds to the average amount of milk that can be collected between 2 consecutive milkings (Salman et al., 2011; Serrano-Rodríguez et al., 2023). The model was described by as ordinary differential equations system from Equation 1 to Equation 4 (see supplemental materials, linked in the Notes, for more information):

$$\frac{dA_0}{dt} = -K_a \cdot A_0, \quad [1]$$

$$\frac{dA_1}{dt} = K_a \cdot A_0 - \frac{Cl}{V_c} \cdot A_1 - \frac{Q_{milk}}{V_c} \cdot A_1 + \frac{Q_{milk}}{V_{milking}} \cdot A_2 - \frac{Q}{V_c} \cdot A_1 + \frac{Q}{V_p} \cdot A_3, \quad [2]$$

$$\frac{dA_2}{dt} = \frac{Q_{milk}}{V_c} \cdot A_1 - \frac{Q_{milk}}{V_{milking}} \cdot A_2, \quad [3]$$

and

$$\frac{dA_3}{dt} = \frac{Q}{V_c} \cdot A_1 - \frac{Q}{V_p} \cdot A_3, \quad [4]$$

where A_0, A_1, A_2, and A_3 are the marbofloxacin amounts in the depot, central, milk, and peripheral compartments, respectively, and d over dt for each term is the respective elimination rate. Plasma, milk, and peripheral concentrations were calculated as $C_1 = A_1/V_c$, $C_2 = A_2/V_{milking}$, and $C_3 = A_3/V_p$, respectively.

The residual variability of the model for the predicted concentrations was defined using a combined error model with the following equation: $CONC_{obs} = CONC_{pred} + (a + b \cdot CONC_{pred}) \cdot \varepsilon$, where $CONC_{obs}$ is the observed plasma or milk concentration, $CONC_{pred}$ is the predicted concentration by the PK model, and a and b are components for the residual error (Mould and Upton, 2013).

Each parameter of the structural model was described as $\theta_i = \theta_{pop} \cdot e^{\eta\theta\omega}$, where θ_i is the parameter estimate for each ith animal from the set of i = n animals, which is defined by the product of the typical population value estimated by the model named θ_{pop}, and $e^{\eta\theta\omega}$, where $\eta\theta\omega$ is the IIV associated with each ith animal from the corresponding typical value θ_{pop}. Moreover, in our dataset, 2 continuous covariates were studied to evaluate its influence on pharmacokinetics of marbofloxacin—weight of animals and volume of milk—and were included into the previous equation as $\theta_i = \theta_{pop} \cdot (COV_{\theta i}/COV_{mean})^{\beta} \cdot e^{\eta\theta\omega}$, where $COV_{\theta i}$ is the covariate value estimate or each animal, COV_{mean} is the mean covariate value of the population, and β is the regression coefficient (Mould and Upton, 2013).

The robustness of the model was evaluated in Monolix using a convergence assessment with 500 replicates, as well as the shrinkage values for each parameter and a nonparametric bootstrap analysis with a 95% CI. This analysis was performed in RStudio using the package Rsmlx (R Speaks Monolix; Goutelle et al., 2020).

Other parameters obtained were the AUC calculated directly by integration of the model, such as AUC for plasma and milk concentrations ($\mathbf{AUC_{milk}}$ and $\mathbf{AUC_{plasma}}$, respectively), as well as AUC from 0 to 24 h ($\mathbf{AUC_{24}}$) for plasma and milk concentrations (Serrano-Rodríguez et al., 2023). Volume of distribution at steady state, plasma and milk elimination half-lives, maximum plasma and milk concentrations observed ($\mathbf{C_{max}}$) and the time to reach C_{max} ($\mathbf{T_{max}}$) were also obtained. Subsequently, the AUC_{milk}/AUC_{plasma} ratios were calculated. Specific milk parameters, such as the volume of milk at each time interval ($\mathbf{V_{milk}}$) and the percentage of recovery of marbofloxacin in milk and the amount excreted were also determined (Fernández-Varón et al., 2021).

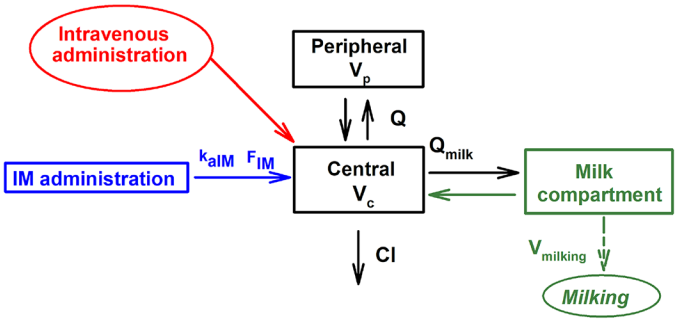

Figure 1. Structure of the 3-compartment model selected to simultaneously describe plasma and milk concentrations of marbofloxacin after i.v. and i.m. administration.

Descriptive statistics and distribution tests were performed for all PK parameters obtained. The distribution of the data was normal and parametric tests were used. The significance level was set at $P < 0.05$. For the comparison of each parameter between routes the unpaired pairwise *t*-test was used, and for each fluid (plasma or milk) the paired pairwise *t*-test was used. This analysis was performed with R version 4.3.2 and RStudio version 2023.12.0-369.

MIC Measurements and Epidemiological Cutoff Determination

Data from *Staph. aureus* (n = 21) were obtained from Lacaune sheep with mastitis from different farms from Spain and were generously supplied by Exopol laboratory S.L. (Zaragoza, Spain). Strains were isolated (n = 21) by incubation into agar plates and identified by PCR methods. The MIC was obtained by microdilution following the Clinical and Laboratory Standards Institute standard (CLSI, 2017). An initial inoculum of $5 \cdot 10^5$ cfu/mL was incubated at increasing concentrations of marbofloxacin from 0.0625 to 32.0 μg/mL in Mueller-Hinton broth (Fluka Analytical), and were incubated for 24 h at 37°C. The next day, the MIC was defined as the lowest antimicrobial concentration that prevented visible growth

of each isolate. *Mycoplasma agalactiae* (n = 15) isolates recovered from individual mastitis samples in Lacaune sheep from different farms in Spain were obtained, identified by PCR methods, and analyzed. The MIC values were obtained by the methodologies described by Hannan (2000) and Fernández-Varón et al. (2021). A stationary-phase culture of each mycoplasma isolate from sheep was carried out in mycoplasma medium without antimicrobials, supplemented with sodium pyruvate (0.5%) and phenol red (0.005%) in 96-well round-bottomed plates. Subsequently, marbofloxacin was added to achieve final concentrations from 0.0625 µg/mL to 32.0 µg/mL. After incubation at 37°C for 48 h, MIC was described as the lowest concentration of marbofloxacin at which no growth (no color change) was observed (Albers and Fletcher, 1982).

For the knowledge of the authors, no epidemiological cutoff (**ECOFF**) of marbofloxacin have been reported for these pathogens in sheep, consequently, TECOFF were calculated with ECOFFinder version 2.0 (Turnidge et al., 2006). This software identifies subpopulations based on the MIC distributions of the isolates for each drug-bacteria interaction, then fits the population data and calculates the cutoff point and defines it as ECOFF or TECOFF values for tentative data. The calculation of these values combined with a PK/PDco determination could be used as a tentative CBP break point of marbofloxacin for sheep (Toutain et al., 2017).

Monte Carlo Simulations and PK/PD Relationships

The parameters estimated and concentrations predicted by the model were used to perform 2 different Monte Carlo simulations. First, simulated dose regimens of marbofloxacin were built in plasma and milk in a hypothetical scenario of 5 d of treatment at 10 mg/kg every 24 h by i.v. and i.m. routes (n = 5,000 per group). Values obtained for AUC_{24} and AUC from 96 to 120 h ($\mathbf{AUC_{96–120}}$) were used to calculate the accumulation index and milk penetration (Li et al., 2013). Second, the simulated AUC_{24} values were used to calculate the AUC/MIC ratios in plasma and milk for a MIC range from 0.0125 µg/mL to 8.0 µg/mL. Considering that fluoroquinolones as well as many antimicrobials in food-producing animals are administered in multiple doses every 24 h, these PK/PD ratios were calculated as different increments of time periods every 24 h for each duration of activity with AUC/MIC values of 24, 48, 72, 96, and 120 in plasma and milk (Toutain et al., 2017). Finally, the PTA was obtained and the highest probability selected was 90% (Asín-Prieto et a., 2015; Paulin et al., 2018; Serrano-Rodríguez et al., 2023).

RESULTS

Pharmacokinetic and Statistical Analysis

The PK model for i.v. and i.m. administrations was able to describe the concentrations in plasma and milk adequately. The plots with the mean and SD of the observed values and the VPC plots for each route in both fluids are displayed in Figure 2, and it can be shown that most observed concentration values fell within the prediction intervals and were centered around the median (50%). The model parameters are presented in Table 1, and the precision of the estimates was good (CV ≤35% in most parameters), with shrinkage values from −13.9% to 26.0%, indicating adequate distribution of individual parameters throughout the population (Savic and Karlsson, 2009). Additionally, the plots of observations versus predictions, residuals, and bootstrap analysis suggested a good description of the observed data (see supplemental material). None of the evaluated covariates (weight and milk volume) influenced the estimation of the model parameters and were excluded. Secondary parameters are shown in Table 2. Plots of simulated plasma and milk levels after multiple marbofloxacin administrations at 10 mg/kg every 24 h by i.v. or i.m. route for 5 d are shown in Figure 3.

Marbofloxacin exhibited a high bioavailability by i.m. route of 88% and a fast absorption of 2.49 L/h. The distribution of the drug was high where the sum of volume compartments exceeded unity (1.31 L/kg). Protein binding values described as an unbound fraction of marbofloxacin were 0.77 ± 0.11 for plasma and 0.68 ± 0.11 for milk, respectively. Plasma clearance was medium to low, with an overall extraction ratio of 0.093, close to the value of 0.1 obtained in nonlactating sheep (Sidhu et al., 2010), indicating that this antimicrobial can be classified as a drug with low clearance in sheep, based on previously established veterinary break points (Toutain and Bousquet-Mélou, 2004a).

For i.v. and i.m. routes, the marbofloxacin half-life was 6.53 and 7.09 h in plasma and 6.62 and 6.55 h in milk, respectively. Milk production per day was 1.39 and 1.55 L for i.v. and i.m. administrations. Protein bindings values were described as an unbound fraction (mean [CV]), with values of 0.77 (14.25%) in plasma and 0.68 (15.49%) in milk.

Statistical comparison of PK parameters showed that AUC and AUC_{24} values were higher in milk ($P < 0.05$) with AUC_{milk}/AUC_{plasma} ratios close to 1.28, in the same form as T_{max} values. These results suggest a higher milk penetration of marbofloxacin, as has been previously described in other ruminants (Fernández-Varón et al.,

Table 1. Pharmacokinetic parameters in plasma and milk for marbofloxacin in sheep after i.v. and i.m. administrations at 10 mg/kg; nonparametric bootstrap is included[1]

Parameter	Estimate	IIV (%CV)	Bootstrap estimate (95% CI)	Shrinkage (%)
F_{IM}	0.88	8.67	0.92 (0.86–0.97)	14.5
K_a (L/h)	2.49	58.77	2.37 (1.86–2.86)	8.76
Cl (L/h/kg)	0.38	17.47	0.38 (0.37–0.41)	−7.52
V_c (L/kg)	0.81	27.97	0.86 (0.82–0.89)	−1.08
Q (L/h/kg)	0.054	34.37	0.05 (0.05–0.06)	−13.9
V_p (L/kg)	0.58	13.46	0.60 (0.56–0.63)	26.6
Q_{milk} (L/h/kg)	0.13	64.85	0.14 (0.13–0.16)	−6.33
corr_Q_Cl		0.82	0.80 (0.62–0.84)	
a_2		0.0071	0.007 (0.006–0.007)	
b_2		0.19	0.19 (0.18–0.19)	
b_3		0.29	0.30 (0.29–0.31)	

[1]F_{IM} is the bioavailability of i.m. route; K_a is the absorption rate constant for i.m. route; V_c and V_p are the volumes of central compartment and peripheral compartments, respectively; Cl represents the clearance of the central compartment; Q is the intercompartmental clearance between central and peripheral compartment; Q_{milk} is the intercompartmental clearance between central and milk compartment. The correlation between random effects is denoted as corr_Q_Cl. The residual variability between observed and predicted concentrations is described by the components a and b of the error model, which are represented by a_2 and b_2 for plasma concentrations, and by b_3 for milk concentrations. The interindividual variability (IIV) is expressed as the coefficient of variation of the random effects. Note: parameters obtained with free plasma and free milk concentrations, respectively.

2021). Differences between routes and fluids were not found in other parameters, such as C_{max}, V_{milk}, and recovery.

MIC Susceptibility and TECOFF

The MIC distribution values, MIC_{50}, MIC_{90}, and TECOFF values for each pathogen are shown in Table 3. The TECOFF values for *Staph. aureus* and *Mycoplasma agalactiae* calculated from the distribution fitted curves were 1.0 and 2.0 µg/mL, respectively, and the observed and fitted distributions are shown in Figure 4.

Monte Carlo Simulations and PK/PD Relationships

The accumulation ratios from the simulated AUC for plasma and milk after multiple administrations of marbofloxacin (mean [CV]) were 1.15 (25.13%) and 1.03 (1.15%) in plasma and milk for the i.v. route, and 0.92 (26.21%) and 1.15 (8.30%) for plasma and milk for the

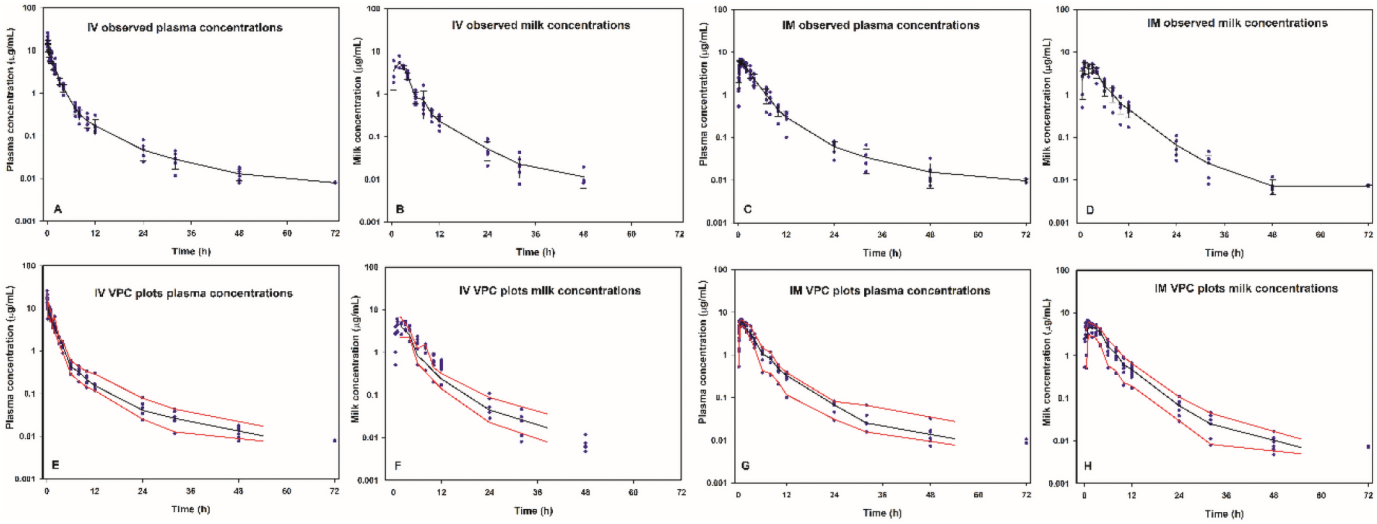

Figure 2. Concentration-time curves of marbofloxacin after i.v. and i.m. administration at 10 mg/kg in plasma and milk. Top row: mean ± SD plots of marbofloxacin in plasma after i.v. administration (A), in milk after i.v. administration (B), in plasma after i.m. administration (C), and in milk after i.m. administration (D). Bottom row: visual predictive check plots of marbofloxacin in plasma after i.v. administration (E), in milk after i.v. administration (F), in plasma after i.m. administration (G), and in milk after i.m. administration (H). The observed data (plasma and milk concentrations) are shown in blue for each plot. The empirical percentiles are shown in red. Observed data are free plasma and milk concentrations, respectively.

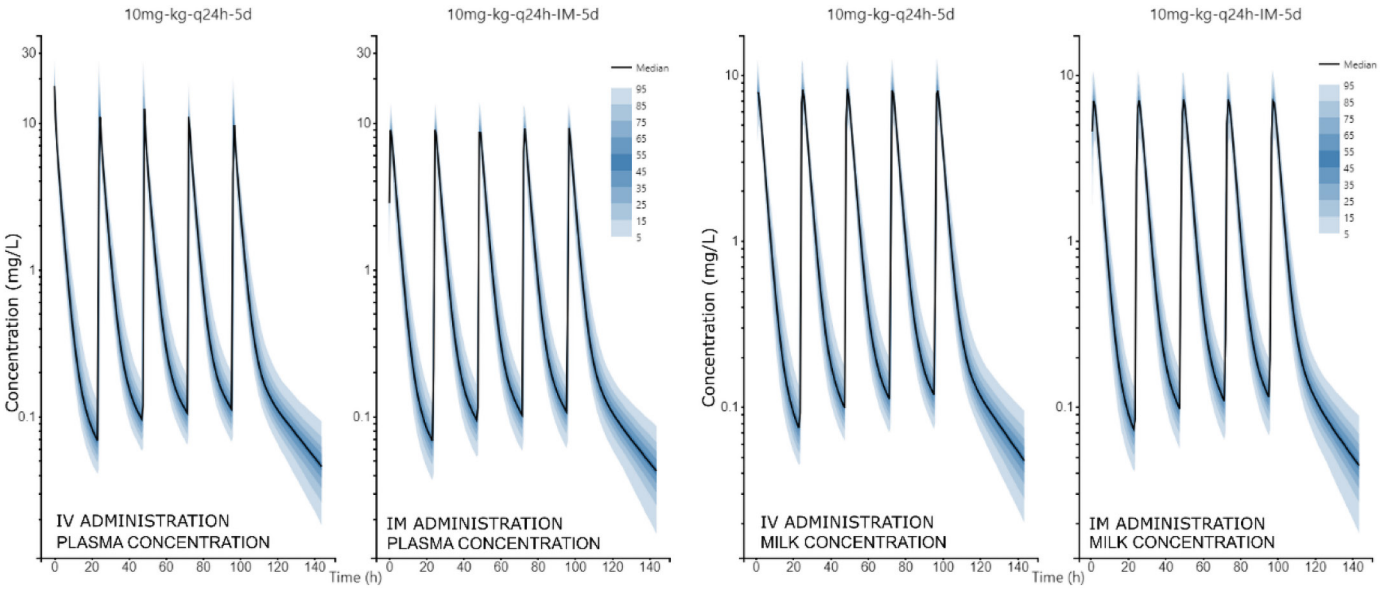

Figure 3. Simulated plots of marbofloxacin concentrations in plasma and milk after i.v. or i.m. administration at a dose of 10 mg/kg every 24 h (q24h) for 5 d of treatment. Data expressed as percentiles in different shades of blue, and the median is shown black.

i.m. route, respectively. Statistical comparisons between simulated AUC_{24} and $AUC_{96–120}$ showed no difference between these values.

Simulated AUC_{24} were used to calculate the AUC/MIC ratios, and the PK/PDco values of marbofloxacin at 10 mg/kg per 24 h were obtained after i.v. and i.m. administrations in plasma and milk. Data were expressed as MIC (mg/L) to achieve an AUC/MIC ratio in the target population greater than a PTA = 90% and were described in Table 4 and Figure 5. The highest PK/PDco values were observed for AUC/MIC ratios between 24 and 48, and lower values between 72 and 120, respectively. This trend was observed in plasma and milk, suggesting no apparent influence between fluids. (Fernández-Varón et al., 2021).

DISCUSSION

In this research, a PK analysis of marbofloxacin at 10 mg/kg in lactating sheep was conducted by the NLMEM approach, combining 2 administration routes and concentrations in plasma and milk.

A very fast absorption and high bioavailability of marbofloxacin by i.m. route at 10 mg/kg was observed,

Table 2. Secondary parameters in plasma and milk for marbofloxacin in sheep after i.v. and i.m. administrations at 10 mg/kg. Data presented as estimate (CV %)[1]

	Plasma		Milk	
Parameter	i.v.	i.m.	i.v.	i.m.
AUC (mg/L·h)	32.92 (14.64)	36.28 (17.63)	42.48 (21.24)	45.55 (27.84)
AUC_{24} (mg/L·h)	31.71 (14.16)	34.69 (17.92)	41.60 (20.74)	44.58 (27.34)
$t_{1/2}$ (h)	6.53 (17.35)	7.09 (11.72)	6.62 (31.55)	6.55 (29.39)
C_{max} (mg/L)		10.11 (44.21)	11.98 (31.73)	9.85 (37.26)
T_{max} (h)		0.79 (31.04)	1.66 (58.44)	2.0 (44.72)
AUC_{milk}/AUC_{plasma}			1.28 (14.85)	1.27 (26.35)
V_{milk} (L)			1.39 (24.51)	1.55 (22.13)
Recovery in milk (%)			0.42 (51.00)	0.37 (61.91)
Amount excreted (mg)			3.12 (21.09)	2.67 (24.81)

[1]C_{max} and T_{max} are the maximum plasma or milk concentration following extravascular administration and the time to reach this peak concentration, respectively. $t_{1/2}$ is the half-life associated with the elimination phase. AUC and AUC_{24} are the areas under the concentration–time curve from zero to infinity and from zero to 24 h, respectively. AUC_{milk}/AUC_{plasma} are the milk/plasma maximum AUC ratios from zero to infinity. V_{milk} is the milk volume at 24 h. Recovery is the percentage of marbofloxacin excreted in milk and the amount excreted are the mg of marbofloxacin recovered in milk. Note: parameters obtained with free plasma and free milk concentrations, respectively.

Table 3. Minimum inhibitory concentration distributions and TECOFF values[1]

Item	*Staphylococcus aureus*	*Mycoplasma agalactiae*
Concentration (µg/mL)		
0.0625	—	1
0.125	4	2
0.25	9	3
0.5	7	4
1	1	3
2	—	—
4	—	—
8	—	2
Total	21	15
MIC_{50}	0.25	0.5
MIC_{90}	0.5	1.0
TECCOF (µg/mL)	1.0	2.0

[1]MIC_{50} and MIC_{90} are the antibacterial concentrations that inhibit 50% and 90% bacterial population, respectively. TECOFF is the tentative epidemiological cutoff value obtained after fitted the bacterial distribution of MIC values.

Table 4. PK/PDco values of marbofloxacin at a dose of 10 mg/kg per 24 h in plasma and milk of sheep after i.v. and i.m. administrations[1]

	Plasma		Milk	
AUC/MIC	i.v.	i.m.	i.v.	i.m.
24	1.0	1.0	1.0	0.5
48	0.5	0.5	0.5	0.25
72	0.25	0.25	0.25	0.25
96	0.25	0.25	0.125	0.125
120	0.125	0.125	0.125	0.125

[1]Data expressed as MIC (µg/mL) to achieve an AUC/MIC ratio of marbofloxacin in the target population greater than a PTA = 90%. Note: parameters obtained with free plasma and free milk concentrations, respectively.

with an estimate at 88%, very close to the data of 86% at 2.5 mg/kg reported by Shem-Tov et al. (1997). The volume of distribution was high at 1.39 L/kg, suggesting an extensive tissular penetration and distribution into extravascular tissues. This value was similar to the data reported by Sidhu et al. (2010) but higher than values reported for lactating sheep by Shem-Tov et al. (1997). In this way, plasma clearance observed was lower than that described in nonlactating sheep at 2 mg/kg but higher than that described at 2.5 mg/kg in lactating sheep (Shem-Tov et al., 1997; Sidhu et al., 2010). Consequently, the elimination half-lives obtained in this study were longer than those described in nonlactating and lactating sheep by i.v. route (Shem-Tov et al., 1997; Coskun et al., 2020), but were lower than those described by i.m. in healthy nonlactating sheep (Altan et al., 2019). These results indicate that the half-life is a secondary parameter highly influenced by different factors such as the bioanalytical method used, the animals, or the PK analysis developed as has been previously described (Toutain and Bousquet-Mélou, 2004b).

The levels of marbofloxacin in milk were slightly higher than in plasma (Table 2), with AUC_{milk}/AUC_{plasma} ratios of 1.28 and close related half-lives. Similar findings in sheep, cows, and goats indicate a wide access to the milk compartment for this fluoroquinolone (Shem-Tov et al., 1997; Schneider et al., 2004; Lorenzutti et al., 2017, Serrano-Rodríguez et al., 2023). In this context, it must be highlighted that this drug is amphoteric, with a carboxylic acid and ionizable nitrogen heterocycle and pKa values of 5.8 and 8.2, respectively (Mahmood et al., 2015). Consequently, at pH 6 to 8, marbofloxacin is sufficiently lipid-soluble to penetrate tissues such as the udder (Fernández-Varón et al., 2021). Regarding this, the marbofloxacin milk levels found in our study can be explained by 2 mechanisms well known for fluoroquinolones: the ion trap effect due to the changes in pH between plasma and milk, and the transport and secretion

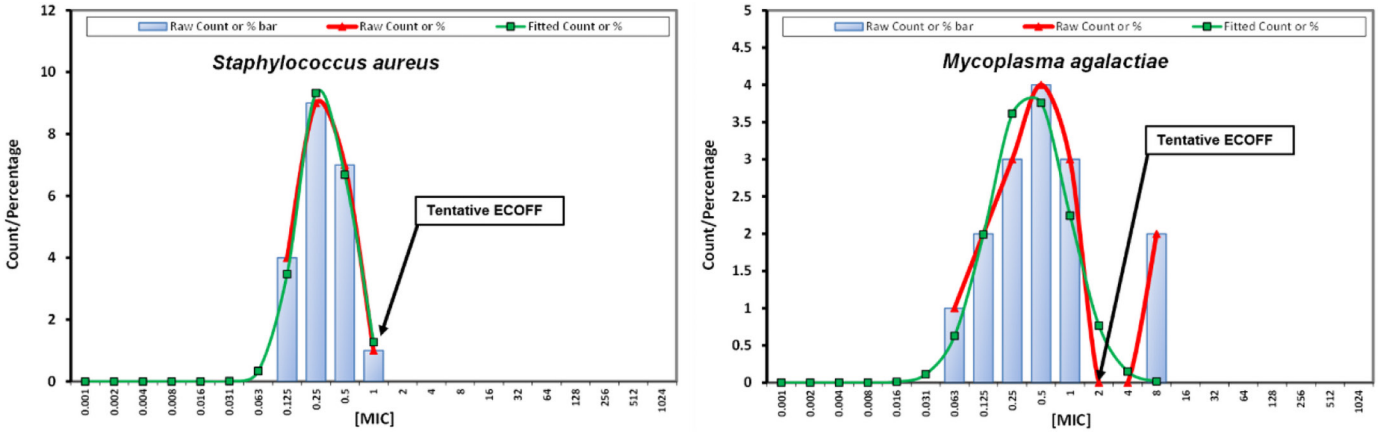

Figure 4. Distribution values of MIC of marbofloxacin against *Staphylococcus aureus* (left panel) and *Mycoplasma agalactiae* (right panel) isolated from sheep with mastitis used in this research. Tentative epidemiological break point (ECOFF) is indicated by a black arrow. Raw count shown with the red line and fitted curve with the green line.

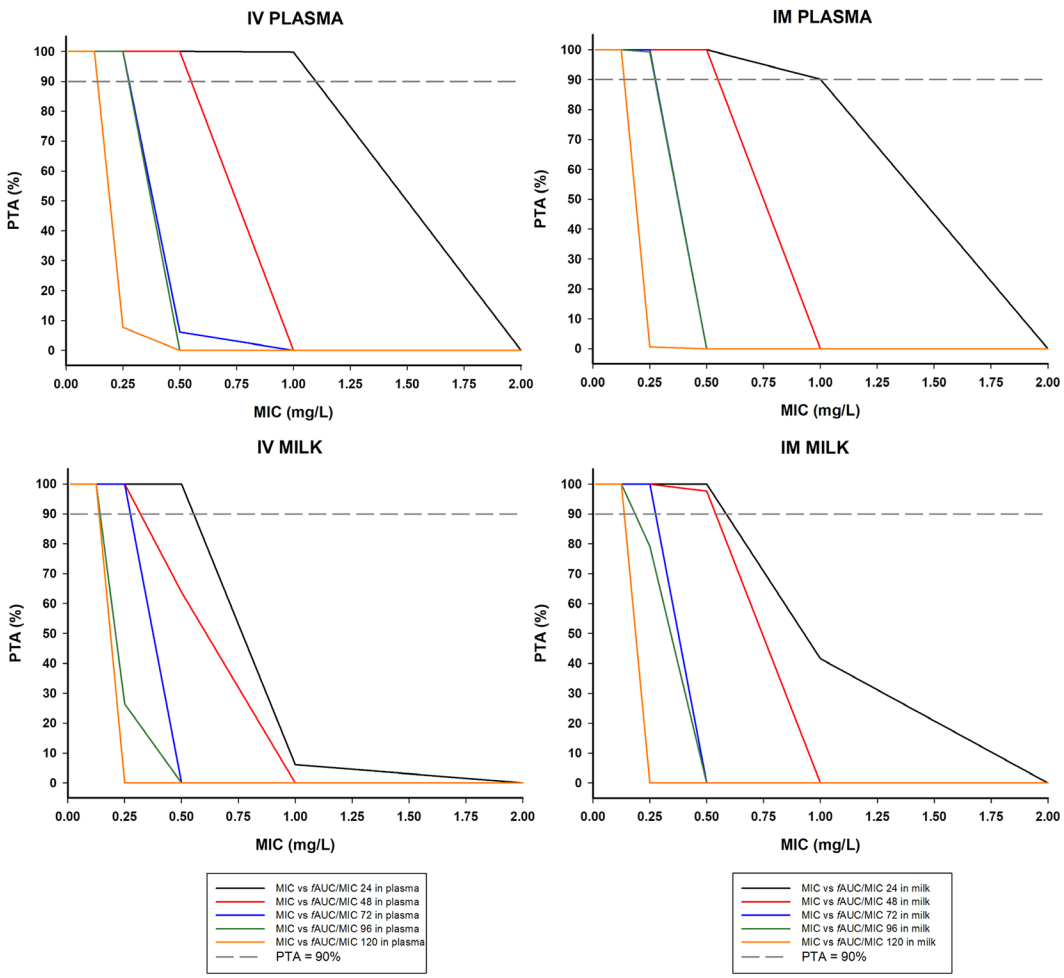

Figure 5. Probability of target attainment values (PTA, %) of marbofloxacin in plasma and milk versus MIC for i.v. and i.m. administrations at 10 mg/kg. The AUC/MIC ratios of 24 h are shown with a black line, AUC/MIC ratios of 48 h with a red line, AUC/MIC ratios of 72 h with a purple line, AUC/MIC ratios of 96 h with a green line, and AUC/MIC ratios of 120 h with an orange line. The PTA value of 90% is plotted in gray dashed line. fAUC = AUC of free drug concentrations over time.

processes mediated by the BCRP proteins (Atkinson and Begg, 1990; McManaman and Neville, 2003; Pulido et al., 2006).

Multiple dose regimenx of marbofloxacin in plasma and milk were simulated at 10 mg/kg for 24 h for i.v. and i.m. routes for 5 d, and accumulation ratios were calculated, with values close to one for each route, suggesting a low accumulation ratio with administration every 24 h. However, the AUC_{milk}/AUC_{plasma} ratios calculated for the simulated AUC_{24} and $AUC_{96–120}$ were close to 1.25 for both routes, suggesting the same milk penetration with this simulated dose regimen (Figure 3).

Marbofloxacin is approved the EU in swine and bovine, and consequently, the maximum residue limit (**MRL**) in meat and milk as well as the withdrawal times (**WDT**) have been well established according to the Commission Regulation (EU) No 37/2010 (EMA, 2010). The MRL in milk is 75 µg/kg, close to 75 µg/L (the milk density is around 1,027 L/kg; Stankov et al., 2022). The WDT of marbofloxacin in cattle are 5 d for meat and 48 h for milk (EMA, 2024). Accordingly, this drug can be used extralabel in other food-producing animals such as sheep in compliance with the articles 113, 114, and 115 of Regulation (EU) 2019/6 (EMA, 2019). However, this regulation indicates that the WDT for extralabel use must be calculated after multiplication the time of cattle by a scalar of 1.5 (EMA, 2019). For our milk data in sheep, a value of 72 h can be obtained. However, to harmonize this result with the methods of calculating WDT in other countries, such as the United States, Canada, Japan, Australia, or New Zealand, the EMA also recommends using the guidelines for preclinical veterinary studies with the WTM 1.4 software, validated for the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH, 2015; EMA, 2022). Finally, WDT values of 47.24 h and

69.53 h with a 95% of confidence level were calculated for milk concentrations after i.v. and i.m. administrations at 10 mg/kg dose, respectively.

Our second objective was to determine MIC distributions, MIC_{50}, MIC_{90}, and the TECOFF values for *Staphylococcus aureus* and *Mycoplasma agalactiae* wild-type isolates from sheep. Cumulative counts of MIC distribution data for bacteria were modeled to the cumulative log-normal distribution, and the MIC_{50} and MIC_{90} values of marbofloxacin against *Staphylococcus aureus* and *Mycoplasma agalactiae* obtained were 0.25 and 0.5 µg/mL, and 0.5 and 1.0 µg/mL, respectively. These values are similar to other MIC obtained from field isolates previously reported. (Poumarat et al., 2016; Serrano-Rodríguez et al., 2017). In the same form, TECOFF values of 1.0 µg/mL obtained for *Staphylococcus aureus* were similar to the values observed in caprine strains, but the value of 2.0 µg/mL obtained for *Mycoplasma agalactiae* in this study was higher to the values described from strains of goats (Serrano-Rodríguez et al., 2023). However, comparisons between these values must be made cautiously, because the response of isolates can be different between small ruminants and countries (Poumarat et al., 2016).

The determination of the PTA and the PK/PDco values for the dose of 10 mg/kg of marbofloxacin was the third objective of this research. Consequently, the PK/PD ratios tested in this study of 24, 48, 72, 96, and 120 were related to the typically recommended AUC/MIC ratios of 30 to 55 for gram-positive and 100 to 125 for gram-negative bacteria for plasma concentrations (Papich et al., 2023). However, is important to emphasize that these ratios have been used as a reference in others publications against Mycoplasma spp., as to our knowledge, there are no specific ratios for these bacteria (Serrano-Rodríguez et al., 2023). In this way, and these ratios could tentatively be applied due to the phylogenetic relationship of mycoplasmas with some gram-positive pathogens described by other authors (Mitchell et al., 2012; Gautier-Bouchardon, 2018; Fernández-Varón et al., 2021). Finally, the PK/PD ratios were also used for comparison with concentrations in milk although there are no established reference ratios in this fluid (Schneider et al., 2004; Toutain et al., 2017).

The AUC/MIC ratios related to plasma or tissue concentrations at infection sites have been typically described as surrogate markers of clinical efficacy in human medicine (Papich, 2014). However, the application of these issues in veterinary medicine, and more specifically in the treatment of mammary infections by systemic antimicrobials has not yet been established, but is assumed that drug concentrations in milk can be used as a surrogate marker of efficacy (Toutain et al., 2017). The high exposure and local concentration level that can be reached in milk by systemic or intramammary route due to the drug transfer between milk and tissues (cistern and alveolus) allow to predict the antimicrobial efficacy against mastitis pathogens (Woodward and Whittem, 2019). Different in vivo and ex vivo assays using milk concentrations have proposed effective ratios against *E. coli* or staphylococci in lactating cows and lactating goats (Renard et al., 1996; Lorenzutti el al., 2017; Xiao et al., 2022).

For AUC/MIC ratios of 24 and 48, PK/PDco values of 1.0 and 0.5 µg/mL were observed in plasma and values 0.5 and 0.25 µg/mL were obtained in milk (Table 4 and Figure 5). Lower values from 0.25 to 0.125 were achieved with higher AUC/MIC ratios of 72, 96, and 120, respectively. Based on these observations, i.v. and i.m. dosage regimens of 10 mg/kg per 24 h could be adequate for highly susceptible pathogens, such as gram-negative microorganisms, which are usually described with lower MIC values for fluroquinolones (Scheld, 2003). Nevertheless, it could be also useful for intermediate and lowly susceptible microorganisms such as gram-positive bacteria and mycoplasmas, which are related to higher MIC values (Poumarat et al., 2016). In contrast, simulated dose regimen of marbofloxacin showed a low accumulation ratio in AUC values, with no difference between AUC_{24} and $AUC_{96–120}$ values. These findings show that marbofloxacin at 10 mg/kg over a 5-d treatment could provide an effective treatment option for the MIC range investigated in this study. But, despite this suggestion, comparisons of our data with other MIC values obtained from other isolates would be necessary to determine effectiveness and suggest cutoff points (Toutain et al., 2019; Bousquet-Mélou et al., 2021; Vegas Cómitre et al., 2021).

Results obtained in this study showed that the simulated dosage regimens of 10 mg/kg per 24 h will not achieve the corresponding TECOFF value of 2.0 µg/mL for *Mycoplasma agalactiae* in any of the AUC/MIC ratios tested; only values of 1.0 µg/mL or lower could be achieved. However, this dose regimen could be effective for populations with a target AUC/MIC ratio of 24 for *Staphylococcus aureus* with TECOFF values up to 1.0 µg/mL or low value of 48 for 0.5 µg/mL.

The PK/PDco values obtained indicate that i.v. and i.m. marbofloxacin at 10 mg/kg per 24 h dose could be used successfully in sheep for the treatment of mastitis caused by *Staphylococcus aureus* and *Mycoplasma agalactiae* with MIC values up to 1.0 mg/L for AUC/MIC ratios of 24 and 0.5 µg/mL for AUC/MIC ratios of 48. Closely related results have been reported by Serrano-Rodríguez et al. (2023), using a population PK model of marbofloxacin in goats. These observations suggest that marbofloxacin at 10 mg/kg by i.v. or i.m. administration could be useful in small ruminants as have been previously described and authorized for respiratory diseases and mastitis in cattle in the EU (Paulin et al., 2018; EMA, 2024).

The results described in this research were conducted in healthy animals and the effects on the drug disposition in unhealthy sheep due to mastitis are unknown. Nevertheless, there is evidence that inflammation and infection can alter distribution processes by decreasing the protein binding, and modifying the elimination and excretion by downregulations of CYP-450 enzymes or overexpression of transporters (Don and Kaysen, 2004; Martinez et al., 2020). However, the plasma and milk pharmacokinetics of marbofloxacin in goats at 10 mg/kg per 24 h i.m. for 5 d, using a mastitis disease model was studied with infected and noninfected udders, describing higher milk concentrations in the diseased udder and lower plasma concentrations at the start of treatment, but reporting microbiological and clinical cure of all animals on the fifth day of treatment without differences between udders (Lorenzutti et al., 2021). Other authors have reported for marbofloxacin no changes to protein bindings in nonlactating calves infected with *Mannheimia haemolytica* but with a reduction of clearance, in the same way that nonlactating sheep infected with the same pathogen or LPS-induced endotoxemia (Ismail and El Kattan, 2007; Altan et al., 2019; Coskun et al., 2020). These findings suggest that the pharmacokinetics and milk excretion of marbofloxacin in sheep with mastitis could be altered and further studies in this way should be considered (Martinez et al., 2020).

Finally, is necessary to note that several limitations in this research. First, this research is a preclinical pharmacokinetics assay with healthy lactating animals, but as previously indicated, the effect of diseases on drug disposition in sheep should be considered in further studies (Martinez et al., 2020). Second, due to nonavailability of data associated with gram-negative, only AUC/MIC ratios were calculated from gram-positive and mycoplasmas. Third, MIC values used were only available from a small MIC distribution (Toutain et al., 2017), and could be a limiting factor in relation to the TECOFF obtained, as well as its relationship with the PK/PD ratios and the PK/PDco determined, consequently should be taken in account (Chua and Tam, 2022). Fourth, only a small number of animals was used (n = 12) because this is a preclinical assay, but higher animal populations should be studied. Nevertheless, the results derived from this study offer important insights to the prudent use of marbofloxacin in lactating sheep, and also could represent a potential foundation for future trials involving healthy and diseased animals. This information, combined with the obtained TECOFF values, could be taken into consideration by committees responsible for establishing CBP determination for mastitis pathogens in lactating sheep, as have been adopted for other antimicrobials in dogs (Vegas Cómitre et al., 2021).

CONCLUSIONS

Plasma and milk concentrations of marbofloxacin were well described by the NLME PK model at 10 mg/kg by i.v. and i.m. administrations. The PK/PDco values predicted by the Monte Carlo simulations ranging from 0.50 to 1.0 µg/mL for *Staphylococcus aureus* and could be adequate from sheep with mastitis. However, the values presented in this research showed that *Mycoplasma agalactiae* achieved higher TECOFF, which could not be achieved with the usual AUC/MIC ratios and doses defined from fluoroquinolones. Finally, this information could be useful for further studies of these mastitis pathogens with marbofloxacin in lactating and nonlactating sheep in a clinical context.

NOTES

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Nonstandard abbreviations used: AST = antibiotic susceptibility tests; AUC = area under the curve; AUC_{24} = AUC from 0 to 24 h; $AUC_{96–120}$ = AUC from 96 to 120 h; AUC_{milk} = AUC for milk concentrations; AUC_{plasma} = AUC for plasma concentrations; CBP = clinical breakpoints; C_{max} = maximum plasma and milk concentrations observed; ECOFF = epidemiological cutoff values; EMA = European Medicines Agency; EU = European Union; FDA = Food and Drug Administration; F_{IM} = bioavailability of the i.m. route; K_a = absorption rate constant; MRL = maximum residue limit; NLMEM = nonlinear mixed effect models; PD = pharmacodynamic; PK = pharmacokinetic; PK/PD = pharmacokinetic and pharmacodynamic analysis; PK/PDco = PK/PD cutoff values; PTA = probability of target attainment; TECOFF = tentative ECOFF; T_{max} = time to reach C_{max}; VETCAST = Veterinary Antimicrobial Susceptibility Testing; VPC = visual predictive check; WDT = withdrawal times.

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