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The disposition of trimethoprim and sulfadiazine in neonatal foals after intravenous administration

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Abstract

Background: Septicaemia in the neonatal foal is caused by both Gram positive and Gram negative bacteria. The life-threatening nature of this condition requires treatment to be initiated with broad spectrum antimicrobial drugs pending antimicrobial susceptibility testing. Potentiated sulphonamides, for example, trimethoprim combined with sulfadiazine, could be clinically relevant options but their pharmacokinetics in the neonatal foal are unknown.

Objectives: To describe the plasma disposition of trimethoprim and sulfadiazine in neonatal foals and to relate the results to patterns in the minimum inhibitory concentration (MIC) for *Escherichia coli*, a recognized pathogen in neonatal foal sepsis.

Method: A total of five doses of trimethoprim (2.5 mg/kg) and sulfadiazine (12.5 mg/kg) were administered intravenously every 12 h to eight neonatal foals that were 3 days old at inclusion. A non-linear mixed effects model was fitted to the trimethoprim and sulfadiazine experimental data. The 24 h area under the free plasma trimethoprim and sulfadiazine concentration-time curves (*f*AUC) and the pharmacokinetic/pharmacodynamik (PK/PD)-index *f*AUC/MIC was calculated to evaluate the potential clinical benefits of the administered dose.

Results: For trimethoprim, the typical values were 1.99 L/kg, 0.33 L/h·kg and 4.2 h for the apparent volume of distribution, clearance and terminal half-life, respectively. The 24 h *f*AUC for trimethoprim was 11.3 μg·h/ml (7.2–15.2) and the *f*AUC/MIC ratio for *E. coli* was 23 (16.4–29.2) (population mean (range)). For sulfadiazine, the typical values were 0.61 L/kg, 0.09 L/h·kg and 5.3 h for the apparent volume of distribution, clearance and terminal half-life, respectively. The 24 h *f*AUC for sulfadiazine was 246.8 μg·h/ml (175.6–335.4).

Conclusion: For trimethoprim, the plasma exposure is insufficient in some foals to successfully treat bacterial infections with an MIC-value of 0.5 μg/ml using the studied dosing regimen.

KEYWORDS
antibiotics, horse, pharmacokinetics, potentiated sulphonamides, sepsis

1 | INTRODUCTION

Septicaemia (sepsis) is a life-threatening condition in neonatal foals (Magdesian, 2017). The infection might be caused by Gram positive bacteria, Gram negative bacteria or polymicrobial infections (Gayle et al., 1998; Koterba et al., 1984; Theelen et al., 2014). Empirical treatment with broad-spectrum antimicrobial drugs (AMD), based on most likely pathogens and their current resistance pattern, is therefore indicated pending bacterial identification and results of antimicrobial susceptibility testing. Intravenous (IV) administration is usually recommended because gut and muscle perfusion might be reduced due to sepsis (Magdesian, 2017). Potentiated sulphonamides, for example, the combination of trimethoprim (TMP) with sulfadiazine (SDZ) meet these criteria. To assess the ability of a chosen AMD to kill bacteria with a specified dosage regimen, its pharmacokinetics (PK) is combined with the minimum inhibitory concentration (MIC) of the target pathogen to calculate the value of so-called PK/PD indices (Onufrak et al., 2016). The PK/PD index that is predictive of efficacy for potentiated sulphonamides is fAUC/MIC, that is, the exposure of free plasma concentration over 24 h divided by the MIC value (Asín-Prieto et al., 2015; Cheng et al., 2009; Hagihara et al., 2019; Ronaghinia et al., 2020). The PK of TMP/SDZ has been described in horses (Gustafsson et al., 1999; van Duijkeren et al., 1994). However, the PK in neonatal foals may not necessarily be the same as in adult horses (Caprile & Short, 1987; Vaala, 1985). The PK of TMP has been described in neonatal foals after co-administration with sulphamethoxazole as an IV bolus dose, and limited plasma exposure data were recently published after administration per os in foals (Brown et al., 1990; Swain O'Fallon et al., 2020) However, no published PK data are available for TMP/SDZ after repeated IV administration to neonatal foals. Consequently, there are no quantitative data supporting clinically effective dosage regimens of TMP/SDZ combination in foals with sepsis because the PK of SDZ has, to the best of our knowledge, not been properly described in neonatal foals. The aim of this study was to describe the PK of TMP/SDZ in neonatal foals, and to relate these results to patterns in the MIC distribution of *Escherichia coli*, a recognized pathogen in neonatal foal sepsis.

2 | MATERIALS AND METHODS

2.1 | Animals

Eight standardbred neonatal foals (two females and six males) were included in the study. The foals were deemed healthy after a full clinical examination performed by an experienced equine clinician. The foals were 3 days old and weighed 53–66.5 kg at the time of inclusion. Each foal was held in an individual box together with its dam. They had limited access to paddocks during daytime. The study was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden (C122/7).

2.2 | Experimental design

Before drug administration, the skin over the jugular vein was clipped and pre-treated with a lidocaine + prilocaine cream (EMLA® 25 mg/g + 25 mg/g, Astra Zeneca, Sweden). An IV catheter (MILACATH® 16ga × 7,5 cm, MILA International inc., Kentucky, USA) was placed in the jugular vein and used for TMP/SDZ administration. The labelled dose TMP (2.5 mg/kg) and SDZ (12.5 mg/kg) was administered IV as five consecutive bolus doses every 12 h using a commercially available solution for IV-injection (Hippotrim® vet. TMP 40 mg/ml + SDZ 200 mg/ml, Bayer Animal Health, Copenhagen, Denmark). Since repeated sampling was initiated after the last dose, the time for the last dose was denoted 0 h. Before each bolus dose (time = −48, −36, −24, −12 and 0 h) a pre-dose blood sample was drawn using the same catheter as for TMP/SDZ administration. Additional blood samples were drawn after the last dose at 0.08, 0.25, 0.5, 0.75 1, 2, 4, 7, 12, 16 and 24 h using a new IV catheter placed in the contralateral vein. Before sampling, 5 ml blood was aspirated from the catheter and returned to the foal after sampling. Blood was then collected in 5 ml heparinized tubes before the catheter was flushed with 10 ml saline. Within 5 min after sampling, blood samples were centrifuged (2700 g) for 10 min and the plasma was frozen (−80°C) pending analysis. Full clinical examination was performed daily during the study on all foals by a specialist in equine internal medicine. The foals were then monitored by an equine practitioner at the stud farm for the following 2 months.

2.3 | Protein binding assay

The plasma protein binding of TMP/SDZ was determined by equilibrium dialyses modified from the method described by Gustafsson et al. (1999). Dialyses were performed in triplicates and mean values were used to calculate protein binding. Due to a mistake in handling the samples from one foal, samples for determination of free drug concentration were only available from seven foals. Plasma (1 ml) collected at 0.08 and 12 h after TMP/SDZ administration from those seven foals was pooled to obtain one high concentration and one low concentration sample. The pooled plasma (1 ml) was dialyzed against buffer (1 ml) that consisted of 3.19 g $Na_2HPO_4 \cdot 2H_2O$, 0.78 g $NaH_2PO_4 \cdot H_2O$ and 2.25 g NaCl diluted to 1000 ml using distilled water. Buffer pH was adjusted to 7.4 using 1 M HCl and 2 M NaOH. Plasma pH was adjusted to 7.4 using approximately 6 μl 1 M HCl/ml plasma. During dialysis, plasma and buffer were incubated at 37°C for 5 h, which was the expected time to achieve equilibrium based on preliminary studies (data not shown). The concentration TMP and SDZ in both plasma and buffer was determined using the analytical method described below. The ratios between free concentration in the buffer and total concentration in plasma was calculated and represent the free fraction of the drug(s). The fraction protein bound was 100 minus the free fraction.

2.4 | Analytical methods

Plasma TMP and SDZ concentrations were determined by means of ultra high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) using an Acquity ultra performance LC (Waters, Manchester, UK) interfaced to a Quattro Ultima Pt (Waters, Manchester, UK) mass spectrometer. The analytical methods were validated in foal plasma using deuterated analogues as internal standards. The standard curves showed linearity and the standard concentrations were 0.25, 0.6, 1.1, 2.5 and 5.3 μg/ml and 1.12, 2.82, 5.58, 11.28, 27.88 and 56.4 μg/ml for TMP and SDZ, respectively. For determination of total plasma concentration, the precision (relative standard deviation [CV%]) was in the range of 3.7%–12.2 % and 2.0%–14.6 % for TMP and SDZ, respectively. The accuracy was 99%–104% and 87%–106% for TMP and SDZ. For determination of free plasma concentration, the CV% was in the range of 2.2%–14.1 % and 0.8%–9.2 % for TMP and SDZ, respectively. The recovery was 97%–100% and 92%–104% for TMP and SDZ. The lower limit of quantification (LOQ) was 0.25 μg/ml for TMP and 1.12 μg/ml for SDZ.

2.5 | Pharmacokinetic model

The PK data were analyzed by non-linear mixed effects modelling using Monolix 2020R1 (Lixsoft SAS, Antony, France) running in the Stochastic Approximation Expectation Maximization (SAEM) mode. One-, two- and three-compartment models were fitted to the observed TMP and SDZ plasma concentration–time data and evaluated by comparing the Akaike information criterion (AIC) and visually inspecting the diagnostic plots (observed data vs. predicted data, weighted residuals vs. time, weighted residuals vs. observed concentration and visual predictive check). Observations below LOQ were treated as censored, that is, any model predicted positive value below LOQ (0.25 μg/ml for TMP and 1.12 μg/ml for SDZ) was considered plausible. The TMP data and the SDZ data were best described with a two-compartment with four parameters, viz., total body clearance (Cl), the volumes of the central (V_c) and peripheral (V_t) compartments and the inter-compartmental distribution clearance (Cl_d). Model parameters were assumed to be log normally distributed and a multiplicative (proportional) residual model was used (Bonate, 2011). A statistical model for between subject variability (BSV) was evaluated based on impact on objective function values and parameter precision. The BSV model was included for the Cl parameter.

During model evaluation, predicted concentrations at times –36, –24 and –12 h were consistently underpredicted compared with observed concentrations (data not shown). This was assumed to be due to sampling and drug administration using the same IV-catheter causing falsely high observed concentrations. Observations from –36 to 0 h were therefore ignored in PK-analyses.

The initial half-life ($t_{1/2\alpha}$), the terminal half-life ($t_{1/2\beta}$) and the half-life from the central compartment ($t_{1/2c}$) for the plasma SDZ concentration-time courses were calculated using standard equations

(Gabrielsson & Weiner, 2006). The $t_{1/2c}$ reflect the mean elimination half-life of a drug in the central compartment.

A more detailed description of the pharmacokinetic model is given in the Supporting Information (S1).

The area under the plasma TMP and SDZ concentration-time curves (AUC$_{0-\infty}$) was estimated from the concentration-time courses predicted by the mixed effects model. From the AUC$_{0-\infty}$, the time between –12 and 12 h were used as 24 h AUC. The 24 h AUC value was then corrected for plasma protein binding to estimate the 24 h AUC for the free fraction (fAUC). The fAUC/MIC for TMP was determined by dividing the 24 h fAUC with the MIC value 0.5 μg/ml. Model parameters were then used in a second step when the fAUC/MIC was simulated for a population of 2000 foals by means of Monte Carlo simulation using Simulx 2020R1 (Lixsoft SAS, Antony, France, 2020).

3 | RESULTS

3.1 | Trimethoprim

After the first drug administration, the population mean for the maximum TMP plasma concentration (C_0) was 1.25 μg/ml. Observed total TMP plasma concentrations were 1.54–2.06 μg/ml 5 min after the last drug administration. Twelve hours after the last TMP/SDZ administration the total TMP plasma concentrations were below LOQ (0.25 μg/ml) in six foals. Twenty-four hours after TMP/SDZ administration, the total TMP plasma concentrations were below LOQ in all foals. The observed data are given in Table S1.

The TMP plasma concentration-time course was best described by a two-compartment model. The model accurately predicted the experimental data. (Figure 1). The PK parameters are presented in Table 1. Shrinkage was estimated for the clearance parameter to –1.7. The fraction TMP bound to plasma proteins was 23%. The 24 h fAUC for TMP was 11.5 μg·h/ml (8.2–14.6) (population mean [range]). The fAUC/MIC ratio was 23 (16.4–29.2). In a simulated population of 2000 foals the fAUC/MIC ratio was 22.9 (13.2–41.6) (median [range]).

3.2 | Sulfadiazine

After the first drug administration, the population mean for the maximum SDZ plasma concentration (C_0) was 20.5 μg/L. Observed total SDZ plasma concentrations were 25.1–34.1 μg/ml 5 min after the last drug administration. Twelve hours after TMP/SDZ administration the total SDZ plasma concentration ranged between 2.06 and 8.35 μg/ml. Twenty-four hours after TMP/SDZ administration, the total SDZ plasma concentration ranged between 1.40 and 1.98 μg/ml in four foals and were below LOQ (1.12 μg/ml) in four foals. The observed data are given in Table S1.

The SDZ plasma concentration time course was best described by a two-compartment model. The model accurately predicted experimental data without systematic bias (Figure 1). The PK parameters are
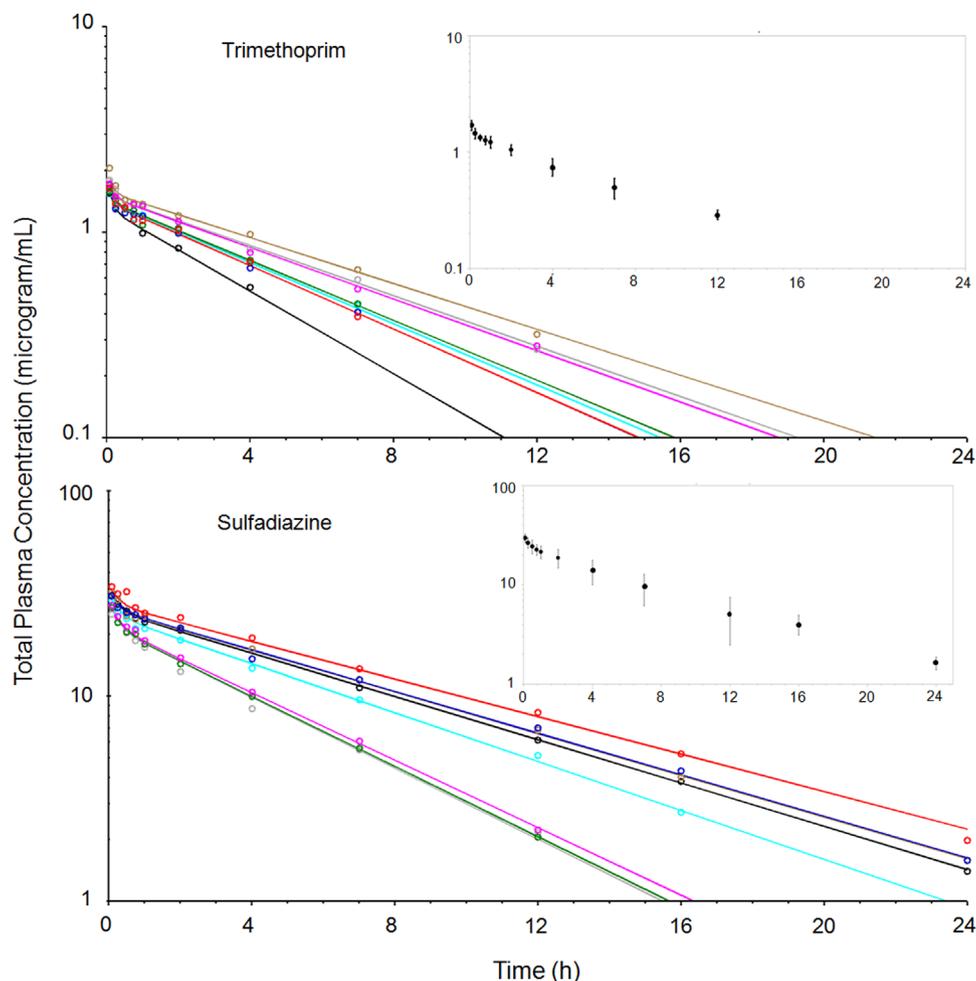
FIGURE 1 Spaghetti plot of observed (circles) and model predicted (lines) trimethoprim total concentration-time courses (upper plot) and sulfadiazine total concentration-time courses (lower plot) following the last of five intravenous 2.5 mg/kg trimethoprim and 12.5 mg/kg sulfadiazine administrations every 12 h to eight neonatal foals. The trimethoprim/sulfadiazine combination was administered at −48, −36, −24, −12 and 0 h. Insets show mean ± SD observed total trimethoprim and sulfadiazine concentrations

presented in Table 1. Shrinkage was estimated for the clearance parameter to −1.4. The fraction SDZ bound to plasma proteins was 14%. The 24 h $fAUC$ was for SDZ 246.8 μg·h/ml (175.6–335.4) (population mean [range]).

Clinically, no adverse effects were observed during the study and the two following months after the study.

4 | DISCUSSION

In this study, SDZ and TMP time-concentration data following IV administration to neonatal foals were fitted to a population-based PK model. The model accurately predicted the experimental data and provided quantitative information about SDZ and TMP disposition with good parameter precision. The population approach was useful in describing and dealing with variability in experimental data between individuals. The relatively sparse experimental data only allowed estimation of BSV for one parameter. Total body clearance (Cl) is the

parameter needed to predict the concentration at steady state for a given dose and was therefore selected to estimate BSV.

The V_{ss} for TMP in this study (1.99 L/kg) was consistent with 1.77 L/kg previously reported in neonatal foals and 1.68–1.96 L/kg reported in adult horses (Brown et al., 1990; Gustafsson et al., 1999; van Duijkeren et al., 1994). The value for Cl (0.33 L/kg·h) was, however, lower compared with 0.7 and 1.0 L/kg·h previously reported in neonatal pony foals and neonatal horse foals, respectively (Brown et al., 1990). The difference in clearance was unexpected. The foals in the current study were 1 day older at inclusion compared with those used by Brown et al. (1990), so similar values were expected. The analytical sensitivity in the present study was lower compared with Brown et al. (1990), which could lead to less precise description of the terminal phase of the concentration-time course and a larger part of the AUC being extrapolated. This could influence the value pharmacokinetic parameters calculated from the AUC, for example, clearance. The reason for the difference in sensitivity between this study and the earlier one is, however, unclear. UHPLC-MS/MS was used in the

TABLE 1 Pharmacokinetic model parameter estimates and secondary parameter estimates after repeated administration of a trimethoprim and sulfadiazine combination to neonatal foals

| Model parameters | Unit | Sulfadiazine | | | | Trimethoprim | | |
		Value	SE	BSV(%)		Value	SE	BSV(%)
V_c	L/kg	0.47	0.02	–		1.38	0.1	–
V_t	L/kg	0.14	0.02	–		0.61	0.097	–
Cl	L/kg·h	0.09	0.01	25.4		0.33	0.02	17.1
Cl_d	L/kg·h	0.41	0.12	–		3.83	1.36	–
Secondary parameters								
α	1/h	3.84	–	–		9.13	–	–
β	1/h	0.13	–	–		0.16	–	–
k_{10}	1/h	0.17	–	–		0.24	–	–
$t_{1/2\alpha}$	H	0.18	–	–		0.08	–	–
$t_{1/2\beta}$	H	5.3	–	–		4.2	–	–
$t_{1/2c}$	H	4.1	–	–		2.9	–	–
V_{ss}	L/kg	0.61	–	–		1.99	–	–
24 h $fAUC$	μg·h/ml	246.8	–	–		11.5	–	–

Pharmacokinetic parameters derived after administration of a total of 5 intravenous doses trimethoprim (2.5 mg/kg) and sulfadiazine (12.5 mg/kg) to eight neonatal foals: V_c, V_t, Cl, and Cl_d are the volumes of the central and peripheral compartments, the total body clearance and the inter-compartmental distribution clearance, respectively. α and β are the initial and terminal rate constants of the two-compartment model. k_{10} is the elimination rate from the central compartment. $t_{1/2\alpha}$, $t_{1/2\beta}$ and $t_{1/2c}$ are the half-lives of the initial phase, the terminal phase and the elimination from the central compartment, respectively. V_{ss} is the apparent volume of distribution at steady state. $fAUC$ is the area under the free plasma TMP and SDZ concentration-time curves. SE is the standard error of the typical value and BSV(%) is the between subject variation.

present study, which is typically more sensitive and precise compared to liquid chromatography used in the older study. The clearance value presented here was also lower than 0.51–0.53 L/kg·h in adult horses. In neonatal foals, both metabolism and excretion are relatively inefficient compared with adults (Vaala, 1985), so a lower clearance value was expected compared with adult horses. The clearance value for TMP presented in the present study is therefore reasonable.

For SDZ, the V_{ss} was 0.61 L/kg and clearance was 0.09 L/kg·h. The volume was consistent with previously reported parameter values for sulphamethoxazole in neonatal foals (0.55 L/kg) and sulphadiazine in adult horses (0.5–0.58 L/kg) (Brown et al., 1990; Gustafsson et al., 1999; van Duijkeren et al., 1994). Also, the Cl value was consistent with previously reported Cl values for SDZ in adult horses (0.09–0.12 L/kg·h), but higher than the Cl for sulphamethoxazole in neonatal horses and pony foals (0.05 and 0.07 L/kg·h).

One consequence of the lower TMP clearance value is longer half-life of elimination $t_{1/2c}$ from the central compartment. Both $t_{1/2c}$ (2.9 h) and the half-life of the terminal phase ($t_{1/2\beta}$) (4.2 h) was longer than the 1.6 h terminal half-life presented previously (Brown et al., 1990). However, it was shorter than the half-life of 6.5 h presented in a recent study (Swain O'Fallon et al., 2020). In this study five 24 mg/kg doses of a 67 mg/ml TMP/333 mg/ml SDZ formulation were administered per os every 12 h to Quarter horse foals. The SDZ elimination half-life after IV administration was approximately 5 h, compared with 10 h in the study by Swain O'Fallon et al. (2020). These foals were 1–1.5 days old at the time of inclusion and were also evaluated at 5 and 10 days of age, respectively. At this age, lower trough concentrations were indicated

in older foals. Lower plasma trough concentrations may be caused by decreased volume of distribution, increased clearance or a combination of both. Vaala (1985) argued that hepatic metabolism develops more rapidly in foals compared with other species. A rapid development of eliminating organs in young foals and increasing clearance could explain the different half-lives between studies. The difference in half-lives could also be attributed to the route of administration. Swain O'Fallon et al. (2020) administered the drug per os, which means that the elimination could have been absorption-rate limited. Future studies are needed to verify this. Other possible explanations might be breed-dependent differences in the PK between Standardbreds and Quarter horses.

There are several reasons why the pharmacokinetics of drugs can differ between neonates and adult horses (Caprile & Short, 1987; Vaala, 1985). Older publications describe the overall body composition of the neonatal foal as having a higher proportion of extracellular water (Baggot & Short, 1984; Kami et al., 1984). This would explain a higher volume of distribution in foals for hydrophilic drugs which distribute throughout the extracellular space. Neither TMP nor SDZ is water soluble which would explain why the volume of distribution of these compounds is not different between neonatal foals and adult horses. The lower elimination rate could be explained by a lower capacity for metabolism and excretion due to immature organs of elimination in the young individual (Baggot, 1994; Baggot & Short, 1984; Vaala, 1985). This has also been shown for TMP and SDZ in several species (Atef et al., 1979; Nielsen & Rasmussen, 1976; Nouws et al., 1983; Shoaf et al., 1989). In the newborn foal however, some metabolic

pathways and renal excretion develop within a few days (Baggot, 1994). This might very well explain the similarity in PK parameter estimates between neonatal foals presented here and adult horses presented elsewhere.

From a clinical perspective, this study provides essential information for designing clinically effective dosing protocols. Successful antimicrobial therapy depends on sufficient exposure to the antimicrobial drug at the site of infection so that the bacteria are either killed or their growth is inhibited. This requires dosing regimens to be adjusted according to the pharmacokinetics of the drug to achieve sufficient exposure given the MIC-value of the infecting bacteria. PK/PD indices that integrate the pharmacokinetics of the drug with the MIC-values of the target pathogen are used to guide dosing regimen design. These indices include the ratio of the maximum concentration over the MIC (C_{max}/MIC), the AUC/MIC or the time that plasma concentrations remain above the MIC (T>MIC) (Onufrak et al., 2016).

The antimicrobial activity of TMP/SDZ is purported to be time-dependent (Magdesian, 2017), that is, the free plasma concentration must exceed the MIC of the target the bacteria (T>MIC) for at least 40%–50% of the dosing interval (Levison & Levison, 2009). More recently, fAUC/MIC has been proposed as the PK/PD index to use for potentiated sulphonamides as it is a combination of both the duration and the extent of exposure (Asín-Prieto et al., 2015; Cheng et al., 2009; Hagihara et al., 2019; Ronaghinia et al., 2020).

TMP has a shorter half-life than SDZ. Dosing regimens should therefore be calculated based on the MIC for TMP to ensure adequate exposure and potentiation of SDZ over the entire dosing interval (Ronaghinia et al., 2020). In 2019, the MIC for TMP against *E. coli* was 0.5 μg/L for 85% of clinical samples collected from the genital tract of 244 mares (SVA, 2020). Based on the results of this study, the population mean (range) of the fAUC$_{24h}$/MIC ratio for TMP was 23 h (16.4–29.2 h). In five of the eight foals included in this study, the value of this ratio was lower than the target values of 24 and 25 h suggested by some authors (Cheng et al., 2009 and Ronaghinia et al., 2020) This should be interpreted as the mean plasma TMP concentration being lower than the *E. coli* MIC in more than 50% of the studied population, which might lead to treatment failure if the dosing regimen is not adjusted. This was further confirmed by simulating TMP exposure in a larger population.

The vast majority of MIC values for clinical isolates of *Streptococcus equi* (73 %) and *Staphylococcus aureus* (83 %) are 0.25 μg/ml (SVA, 2020). For these target pathogens, the fAUC/MIC ratio is twice as high as the value for *E. coli* (16.4–29.2 vs. 32.8–58.4, respectively). Hence, clinical recovery should be expected in animals with these infections.

The therapeutic outcome of the registered dosing regimen of 2.5 + 12.5 mg/kg TMP + SDZ twice daily IV to foals is dependent on both the sensitivity of the infecting bacteria and the variability in PK between individual animals. Higher doses are needed to successfully treat less sensitive isolates with an MIC of 0.5 μg/ml or higher. A recent study showed that a total dose of 24 mg/kg administered per os using a formulation with 67 mg/ml TMP and 333 mg/mL SDZ formulation was effective for treating bacterial infections with TMP MIC of 0.5 μg/ml (Swain O'Fallon et al., 2020). No adverse effects were observed in the healthy foals used in this study. Higher doses could therefore be use-

ful in the therapy against sepsis in neonatal foals and warrant further attention.

This experimental study was performed in healthy foals. Foals suffering from sepsis might have altered PK (Magdesian, 2017). Septic foals are also treated with other drugs and given fluid therapy, both of which might influence the PK either alone or in combination (van der Harst et al., 2005). Consequently, it is difficult to predict clinical efficacy in foals with systemic disease. This is a limitation of the present study, and a clinical study exploring the plasma exposure and PK in septic foals is warranted.

5 | CONCLUSION

The plasma disposition of TMP and SDZ was characterized in healthy neonatal foals and quantitative PK information was provided. Despite that the PK in foals with systemic disease may not necessarily be identical to the PK in healthy foals, the results in this study provide evidence that the dosage regimen of 2.5 mg/kg TMP and 12.5 mg/kg SDZ may not be clinically effective in all foals. A modified dosing regimen (higher dose or more frequent dosing) could, however, constitute an initial broad spectrum therapy pending results of bacterial isolation and sensitivity testing. The safety of such modified dosing regimens has not been fully investigated.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

Authors confirm that both the national rules regarding use of animals in research and the ethical policies of the journal have been adhered to and the appropriate ethical review committee approval has been received.

AUTHOR CONTRIBUTIONS

Formal analysis, methodology, visualization, writing—original draft, review and editing: Carl Evert Ekstrand. Conceptualization, investigation, methodology, writing—review and editing: Katarina Nostell. Formal analysis, methodology, writing—original draft, review and editing: Ronette Gehring. Investigation, methodology, validation, writing—review and editing: Ulf Bondesson. Conceptualization, funding acquisition, investigation, methodology, project administration, supervision, writing—review and editing: Johan Bröjer.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

PEER REVIEW

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