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Comparative pharmacokinetics of sulfamethazine after intravenous administration in bovine (*Bos taurus*) and buffalo (*Bubalis bubalis*) calves

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Sulfamethazine is a sulfonamide that presents a broad spectrum of activity, including gram-positive and gram-negative bacteria, *Chlamydia* spp. and some protozoa. This drug has been reported to be highly efficacious in the treatment of pneumonias, diarrheas and coccidiosis in cattle, as commonly susceptible micro-organisms are *Bacillus* spp., *Brucella* spp., *Listeria monocytogenes*, *Nocardia* spp., *Streptococcus* spp., *Escherichia coli*, *Chlamydia* spp., *Pneumocystis carinii*, *Cryptosporidium* spp., and *Toxoplasma* spp. (Lindsay *et al.*, 1996; Lindsay & Dubey, 1999; Oliveira *et al.*, 2000; Spoo & Riviere, 2001). However, *Leptospira* spp. and *Pseudomonas* spp. are resistant (Prescott, 2000).

The pharmacokinetic (PK) behavior of sulfamethazine in ruminant species is characterized by a relatively high bioavailability after oral administration (58% in sheep), a small volume of distribution (0.24–0.50 L/kg) and an elimination half-life, which oscillated between 2 and 11 h after intravenous administration and approximately 14 h after oral administration. The PK behavior of this drug depends on age, sex and time of day (Mody & Malik, 1997; Spoo & Riviere, 2001; Janus *et al.*, 2004).

In the past, the therapeutic recommendations applied to a single ruminant species were extrapolated to the others because no important differences among cattle, sheep, goats and buffaloes were recognized. However, a different metabolic behavior along the ruminant species (Elsheikh, 1997) and physiological differences between bovines and buffaloes (such as corporal composition, hepatic metabolism or renal excretion) have been described (Mason, 1974; Groves, 1989). The aim of our work was to study the possible inter-species differences in the PK behavior and pharmacokinetic/pharmacodynamic (PK/PD) integration of sulfamethazine after intravenous administration in buffalo (*Bubalis bubalis*) and bovine (*Bos taurus*).

The experiment was performed in five male buffaloes and six male bovine calves (3–4 months old and weighing 120 ± 15 kg). A complete clinical and hematological evaluation was performed throughout the study. The animals were placed in boxes and were given alfalfa hay and had access to water *ad libitum*. The study was approved by Institutional Animal Use Committee. A sodium sulfamethazine formulation was utilized in the PK study as a 30% injectable saline solution (Allignani Hnos. SRL, Santa Fe, Argentina; Batch 05–01).

Both groups received a single 60 mg/kg (0.20 mL/kg) dose of sulfamethazine. The drug was administered intravenously into the right jugular vein. Blood samples (4 mL) were taken in heparinized sterile syringes and centrifuged at 2000 ***g*** for 15 min within 60 min after collection.

Sulfamethazine was extracted using disposable C18 cartridges (Sep-Pak Cartridges; Water Associates Inc., Milford, MA, USA), which were previously conditioned with 5 mL of methanol followed by 3 mL of water (pH 3.0: acetic acid). All samples were applied to the cartridges and then sequentially washed with 5 mL of water and eluted with 3 mL of acetonitrile concentrated to dryness under a stream of nitrogen. Sulfamethazine concentrations were quantified using HPLC/UV according to a modification of a method previously described by Löscher *et al.* (1990). An integrated HPLC system (Konik 500 B; Konik Instruments, Instrumentación Analítica SRL, Buenos Aires, Argentina), with UV detection was used. Separation was accomplished using a reverse-phase column (Water SPHERISORB RP C18 5 μm, 25 × 0.4 cm; Precolumn RP C18, Water Associates Inc.). The liquid phase was acetonitrile: acetic acid solution pH 3.0 (8:92) (Sigma-Aldrich Corporation, St Louis, MO, USA); with a 1.5 mL/min flow, a 270 nm and 35 °C oven temperature.

Linear calibration curves were obtained between 0.30 and 300 μg/mL concentration range (bovine: $R^2 > 0.997$; buffalo: $R^2 > 0.994$). Limit of quantification (LOQ) were 0.36 and 0.50 μg/mL for bovine and buffalo, respectively. Precision was

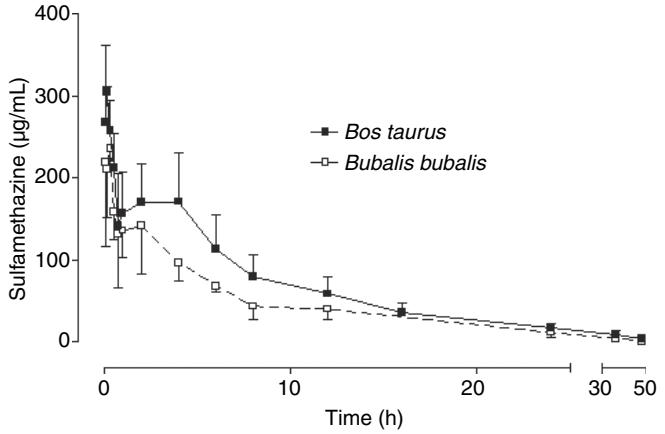

Fig. 1. Sulfamethazine plasma concentration [mean (SD)] vs. time curves after intravenous administration of a 60 mg/kg dose in (■) bovine ($n = 6$) and (□) buffalo ($n = 5$) calves.

calculated as the coefficient of variation of the average value found for each added concentration was <20% and accuracy ranged between 80% and 120%. The precision and accuracy of the LOQ were 9.08% and 95.48 ± 8.6% and 9.75% and 89.23 ± 9.1%, to bovine and buffalo, respectively. Mean analytic recovery for sulfamethazine in plasma was 97.6 ± 0.7% and 98.8 ± 0.2%. For both species, the inter-assay and intra-assay coefficients were <10% and <7.5%, respectively. The stability of the drug in spiked samples stored at −18 °C for up to 2 months was assessed.

Plasma concentrations of sulfamethazine after intravenous administration were subjected to a noncompartmental analysis using PCNONLIN V4.0 software package (Statistical Consultants Inc., Lexington, MA, USA).

The statistical analysis was performed using the SPSS® 12.0 software package (SPSS Inc., Chicago, IL, USA). Comparisons between groups were performed using a Mann–Whitney *U*-test or an ANOVA test, depending on the results obtained in normality study.

Mean (SD) sulfamethazine plasma concentration vs. time curves after intravenous administration to bovines and buffalo calves are illustrated in Fig. 1. Plasma concentration vs. time curves showed higher plasma concentrations in bovine than in buffaloes (Fig. 1). Sulfamethazine $V_{d(a)}$ in buffaloes (0.399 L/kg) did not differ significantly from the values found in bovines (0.317 L/kg). These values are in agreement with other studies in buffaloes (Mody & Malik, 1997), bovines (Witkamp *et al.*, 1992), and other ruminant species (Bulgin *et al.*, 1991; Witkamp *et al.*, 1992).

Differences between bovine and buffalo calves were found in λ and $t_{1/2\lambda}$. The permanence of sulfamethazine in buffaloes ($t_{1/2\lambda} = 6.17 \pm 0.58$ h) is shorter than in bovine cattle ($t_{1/2\lambda} = 7.46 \pm 1.05$; Table 1). These values are lower than terminal half-life (9.37 h) reported by Atef *et al.* (1981) in cows, but similar to $t_{1/2\beta}$ described by Witkamp *et al.* (1992) for bovines and goats. The *Cl* differed between buffaloes (45.31 mL/h·kg) and bovines (30.34 mL/h·kg). As a consequence of the lower clearance in bovines, the *AUC* and $t_{1/2\lambda}$ values were higher in this species. An explanation for clearance differences could be found in the metabolic characteristics of these species, due to the elimination of sulfamethazine in ruminants depended mainly on the extent of the metabolism (Nouws *et al.*, 1988). Jain *et al.* (2000) described a comparatively low extent of acetylation of sulfamethazine and they suggested its safe use in buffalo calves without much risk of toxicity.

Table 1. Pharmacokinetic parameters after intravenous administration of sulfamethazine (60 mg/kg) in cattle ($n = 6$) and buffaloes ($n = 5$)

	Bovine		Buffalo	
Parameters	Mean	SD	Mean	SD
Cl (mL/h/kg)**	30.34	6.39	45.31	10.63
$V_{d\ (ss)}$ (L/kg)	0.311	0.041	0.383	0.120
$V_{d\ (a)}$ (L/kg)	0.317	0.035	0.399	0.121
λ (h^{-1})*	0.090	0.013	0.112	0.009
$t_{1/2\lambda}$ (h)***	7.46	1.05	6.17	0.58
MRT (h)	10.48	1.77	8.44	1.21
AUC (μg·h/mL)*	2009	387	1365	310

*$P = 0.014$–0.015, **$P = 0.017$–0.018, ***$P = 0.037$.
AUC, area under the plasma concentration–time curve from time zero to infinity; *Cl*, total plasma clearance ($Cl = \text{Dose}/AUC$); $V_{d(a)}$, volume of distribution area ($V_{d(a)} = D/\beta \cdot AUC$); $V_{d(ss)}$, volume of distribution at steady state ($V_{d(ss)} = Cl \cdot MRT$); λ, rate constant; $t_{1/2\lambda}$, terminal half-life ($t_{1/2\lambda} = 0.693/\lambda$); *MRT*, mean residence time from time zero to infinity ($MRT = AUMC/AUC$).

Sulfonamides are classified as short-, intermediate- and long-acting according to plasma concentration–time profile. These drugs are considered to be short-acting if blood concentration after one therapeutic dose remains above 50 μg/mL for <12 h. Intermediate-acting sulfonamides are considered to maintain this plasma concentration between 12 and 24 h after administration and long-acting sulfonamides show concentrations of 50 μg/mL or more for at least 24 h after dosing (Spoo & Riviere, 2001). Sulfamethazine plasma concentrations oscillated from 304.42 to 58.12 μg/mL at 0 and 12 h (14.45 ± 3.23 h above 50 μg/mL) in cattle. This result is similar to those reported by Srivastava *et al.* (1989) (76.2 μg/mL after 18 h in cross-breed bovines) and Pulido *et al.* (1998) (58–65 μg/mL after 12 h in sheep). Therefore, in cattle, this drug could be classified as an intermediate-acting sulfonamide. On the other hand, in our study, buffaloes showed plasma concentrations from 235 to 67.87 μg/mL at 0 and 6 h, that remained above 50 μg/mL only for 10 h (9.98 ± 2.22 h); thus, it behaves as a short-acting sulfonamide. In contrast, Mody and Malik (1997) classified sulfamethazine as an intermediate-acting drug in buffaloes.

PK/PD modeling is a good alternative for selecting a rational dosage regimen. Sulfonamides could be considered as time-dependent drugs. There is evidence from disease model studies to indicate that the time for which concentration exceeds *MIC* ($t > MIC$) is an important determinant of the outcome of therapy. In periods when concentrations decrease below *MIC* regrowth of organisms occurs. Therefore, it is recommended that $t > MIC$ should be achieved for a whole and not only for some proportion of the inter-dose interval (Frimodt-Møller, 2002). We have included the calculation of weighted *AUC*

Table 2. Pharmacokinetic/pharmacodynamic parameters after intravenous administration of sulfamethazine (60 mg/kg) in bovine ($n = 6$) and buffalo ($n = 5$) calves

	$t > MIC$ (h)		*WAUC* (h)	
	Cattle	Buffalo	Cattle	Buffalo
4 μg/mL[†]	42.68 ± 6.26*	34.72 ± 5.20	838 ± 277*	437 ± 136
8 μg/mL[‡]	34.94 ± 5.29**	28.04 ± 4.22	342 ± 116*	177 ± 58
32 μg/mL[‡]	19.44 ± 3.47**	14.68 ± 2.51	47.89 ± 18.06*	24 ± 9.3

*$P = 0.017$–0.018, **$P = 0.030$.
[†]Oliveira *et al.*, 2000.
[‡]Prescott, 2000.
$t > MIC$, time the drug concentration remains over the *MIC*; *WAUC*, weighted *AUC*.

$[(WAUC = (AUC/MIC)(t > MIC/(t > MIC)_{max})]$, a new empirical PD index for which *AUC*/*MIC* is weighted by $t > MIC$, to take into account the concentration-dependent part of the antibiotic efficacy and the concentration-independent part. This index considers the total dose administered and the clearance of the drug through the *AUC*, the sensitivity of the bacteria to the *MIC* and the percentage of time for which serum drug level is above the *MIC* through the ratio $t > MIC$. It shows a more direct relationship between its values and bacterial killing both for the concentration-dependent drug and for the time-dependent drug (McKellar *et al.*, 2004).

The values obtained for calculated PK/PD ratios $t > MIC$ and *WAUC* are present in Table 2. *MIC* values used in this work were 4 μg/mL which is the MIC_{90} value for *Staphyloccocus aureus* strains isolated from bovine mastitis (Oliveira *et al.*, 2000), and 8, 32 and 128 μg/mL, which has been described by Prescott (2000). This author indicate that *MIC* of 8–32 μg/mL is a reasonable definition of susceptibility and *MIC* of ≥64–128 μg/mL can be interpreted as evidence of resistance to sulfonamides. According to the data shown in Table 2, important differences between bovine and buffalo exist for micro-organisms that have a *MIC* value <32 μg/mL. Hence, a different dosage regimen should be used in these species; however, further studies are necessary to establish an optimal dosage regime.

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