Distribution of orally administered trimethoprim and sulfadiazine into noninfected subcutaneous tissue chambers in adult ponies

E. VAN DUIJKEREN*
J. M. ENSINK† &
L. A. MEIJER‡

**Bacteriology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University; †Department of Equine Sciences, Faculty of Veterinary Medicine, Yalelaan, Utrecht; ‡Dopharma Research, Zalmweg 24, 4941 VX Raamdonksveer, the Netherlands*

van Duijkeren, E., Ensink, J. M., Meijer, L. A. Distribution of orally administered trimethoprim and sulfadiazine into noninfected subcutaneous tissue chambers in adult ponies. *J. vet. Pharmacol. Therap.* **25,** 273–277.

The distribution of trimethoprim (TMP) and sulfadiazine (SDZ) into subcutaneously implanted noninfected tissue chambers was studied in healthy adult ponies. Six ponies were given an oral TMP/SDZ paste formulation at a dose of 5 mg/kg TMP and 25 mg/kg SDZ at 12 h intervals for 2 days in order to reach steady-state concentrations. Plasma concentrations and tissue chamber fluid (TCF) concentrations of both drugs were measured at regular intervals during a period commencing 24 h after the last oral administration. The peak concentration of TMP (mean ± SD) was 2.92 ± 0.86 μg/mL for plasma and 1.09 ± 0.25 μg/mL for TCF. For SDZ, the mean peak concentration was 40.20 ± 14.74 μg/mL for plasma and 23.48 ± 5.84 μg/mL for TCF. TMP peak concentrations in plasma were reached at 3.17 ± 03.48 h and those in TCF at 7.33 ± 03.72 h. SDZ peak concentrations in plasma were reached at 1.83 ± 02.04 h and those in TCF at 8.00 ± 03.10 h. Concentrations of TMP and SDZ in TCF remained above the generally accepted breakpoint for susceptibility (0.5/9.5 for the TMP/SDZ combination) for 12 h. Therefore, in ponies oral administration of TMP/SDZ at a dose rate of 30 mg/kg given twice daily in the form of a paste should be appropriate for effective treatment of infections caused by susceptible bacteria.

(Paper received 8 January 2002; accepted for publication 18 June 2002)

E. van Duijkeren, Bacteriology Division, Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, PO Box 80165, 3508 TD Utrecht, the Netherlands. E-mail: e.duijkeren@vet.uu.nl

INTRODUCTION

Trimethoprim–sulfonamide (TMPS) combinations are used extensively in equine practice because of their broad spectrum of activity against many Gram-positive and Gram-negative bacteria and their clinical efficacy (van Duijkeren *et al.*, 1994a). Moreover, TMPS combinations have a relatively high bioavailability following oral administration (van Duijkeren *et al.*, 1994b; van Duijkeren *et al.*, 1995) with only minor effects on the equine intestinal flora (Ensink *et al.*, 1996b; Gustafsson *et al.*, 1999). Generally, effective antimicrobial therapy should produce and maintain effective concentrations of the drug at the site of infection for a sufficient period of time to kill or inhibit bacteria without toxic effects to the host (Prescott & Baggot, 1993). Thus far, pharmacokinetic studies with TMPS combinations in horses have been limited to plasma, urine, synovial and peritoneal fluid (Brown *et al.*, 1983; Bertone *et al.*, 1988; Brown *et al.*, 1988). Bacterial pathogens, however, often reside in the interstitial fluid and therefore knowledge of the concentrations of antimicrobials achieved in this compartment would assist in predicting their clinical efficacy in soft tissue infections. Tissue chambers implanted subcutaneously provide a convenient method for obtaining samples that are in continuity with interstitial fluid (Clark, 1989; Ensink *et al.*, 1996a).

The objective of the present study was to compare the concentrations of trimethoprim (TMP) and sulfadiazine (SDZ) in plasma and tissue chamber fluid (TCF) in ponies following repeated oral administration of 5 mg/kg TMP and 25 mg/kg SDZ every 12 h.

MATERIALS AND METHODS

Animals

Eight Shetland ponies, six stallions and two geldings, weighing 135–172 kg and aged 1–12 years were used. The ponies were stabled individually in boxes on wood chips and were fed with 400 g of concentrates and approximately 800 *g* of grass silage twice daily. The concentrate was fed 10 min before and the grass silage 10 min after the drug administration. Water was provided *ad libitum*. One tissue chamber was implanted on each side of the

neck immediately rostral to the scapula as described previously by Beadle *et al.* (1989). The studies were performed 10 weeks after implantation.

Medication

Six ponies were treated and two ponies served as untreated controls. An oral TMP/SDZ paste (Sultrisan® Orale Pasta, Anisane Pet Health Products, Raamdonksveer, the Netherlands) containing 100 mg TMP and 500 mg SDZ per mL paste was administered twice daily for 2 days. The dosage was 5 mg/kg TMP and 25 mg/kg SDZ (1 mL paste per 20 kg bodyweight) every 12 h.

Sampling

At 0, 1, 2, 4, 6, 9, 12 and 24 h after the last dose, blood samples (8 mL each) were collected from a jugular vein using heparinized vacutainer tubes (Vacuette® C.A. Greiner & Söhne, Kremsmunster, Austria). Blood samples were centrifuged immediately at 1000 ***g*** for 10 min and the plasma was stored at −80 °C until assayed. TCF was sampled by aspiration using a 1-mL syringe and a 18G needle from the right tissue chamber. These samples were stored at −80 °C in heparinized tubes until assayed. Sampling times for TCF were 0, 2, 4, 6, 12 and 24 h after the last dose. One blood and one TCF sample was drawn from each of the two untreated ponies to serve as drug-free controls.

Method of analysis

Concentrations of TMP and SDZ were determined by high performance liquid chromatography (HPLC) with detection by mass spectrometry (MS). After addition of an internal standard solution (sulfadoxine) to 500 μL of plasma or TCF, 1 mL of a phosphate buffer pH 7.0 was added to the samples after which the samples were homogenized. Analytes were extracted in 6 mL of a mixture of ethyl acetate and dichloromethane (90/10, v/v). After centrifugation, 5 mL of the organic phase was evaporated to dryness. The residue was reconstituted in 500 μL of 0.1 M NaOH after which 500 μL of 0.1 M ortho-phosphoric acid was added. The HPLC analysis used an analytical stainless steel column (100 × 1.0 mm ID) packed with Aquapore OD 300 C18 packing material (Brownlee Perkin Elmer, Norwalk, USA) (7 μm particle size) and a guard column (Optiguard C18 15 × 1 mm ID) (Alltech, Breda, The Netherlands). The chromatographic separation was achieved at ambient temperature. The mobile phase consisted of a mixture of 0.01 M ammonium acetate and acetonitrile. For the detection, a triple quadrupole MS–MS instrument was used in positive ion-mode. The analytes were fragmented by collisional activated dissociation, using argon. For quantification of SDZ, TMP and sulfadoxine, the transition 251 > 92, 291 > 230 and 311 > 156 were monitored, respectively.

For each analysis, calibration samples and quality control (QC) samples were co-analysed. There were no significant differences in the recovery of either analyte from plasma or TCF following extraction. Because of the limited availability of TCF, this observation justified the preparation of calibration and QC samples in blank equine plasma for the quantitation of both analytes in TCF and plasma. QC samples were co-analysed at three concentrations: at the high end of the calibration curve (24 mg SDZ/L and 2 mg TMP/L), at the mid-range and at the lower limit of quantification (LOQ) (0.10 mg SDZ/L and 0.025 mg TMP/L). The purpose of QC samples was to assure that the complete analytical method, sample preparation, clean-up and instrumental analysis, was performed according to acceptable criteria. Acceptance of series of analyses was based on evaluation of accuracy and the precision of the QC samples according to The Rules Governing Medicinal Products in the European Community (1991): for accuracy, the deviation of the mean measured concentration in the QC samples from the true values were lying within the −30 to +10% limits. The precision for samples with a true concentration of analyte below 0.1 mg/L was better than 20% while above the true concentration of 0.1 mg/L, precision was better than 15%.

The QC samples were analyzed simultaneously with the test samples evenly dispersed throughout each batch of samples. The concentration of analyte in the QC samples was calculated using the corresponding calibration curve.

Data analysis

The plasma and TCF data for each pony were analyzed using noncompartmental analysis (TopFit; Heinzel *et al.*, 1993). The weighting factor was 1. This analysis produced the following pharmacokinetic parameters: maximal plasma or TCF concentration at steady state (μg/mL) (C_{maxss}), time of maximum plasma or TCF concentration (h) (T_{max}), terminal half life (h) ($t_{½}$), area under the curve (μg h/mL) (AUC_{0-12}) from 0 to 12 h during the last dosing interval, area under the curve from time zero to infinity during the last dosing interval (μg h/mL) ($AUC_{0-\infty}$) and the mean residence time (h) (*MRT*). The *AUC* was calculated using the linear trapezoidal rule. All values were calculated for each individual pony and mean ± SD were determined. For the terminal half-life the harmonic mean was calculated.

RESULTS

Pharmacokinetic parameters (±SD) for TMP and SDZ after the last of four oral dosings of 5 mg/kg TMP and 25 mg/kg SDZ q 12 h are shown in Table 1. Distribution of TMP and SDZ into TCF was slow and resulted in lower peak concentrations than in plasma. Mean peak TCF concentrations were only reached 07.33 h (TMP) and 08.00 h (SDZ) after the last oral administration. However, C_{maxss} and T_{max} for both TMP and SDZ varied considerably between individual ponies in plasma as well as TCF.

The mean (±SD) TMP and SDZ concentration–time curve of six ponies for plasma and TCF are depicted in Figs 1 & 2, respectively. The mean TMP and SDZ plasma concentrations remained above the mean TMP and SDZ TCF concentration for 12 h. The TCF concentrations of both TMP and SDZ were only

Table 1. Pharmacokinetic data (±SD) for trimethoprim (TMP) and sulfadiazine (SDZ) after the last of four oral administrations (dosing interval 12 h) of 5 mg/kg TMP and 25 mg/kg SDZ to six healthy adult ponies

Pharmacokinetic parameter (units)	TMP plasma	TMP TCF	SDZ plasma	SDZ TCF
T_{max} (h) (range)	3.17 ± 3.48 (1–9)	7.33 ± 3.72 (4–12)	1.83 ± 2.04 (1–6)	8.00 ± 3.10 (6–12)
C_{maxss} (μg/mL)	2.92 ± 0.86	1.09 ± 0.25	40.20 ± 14.74	23.48 ± 5.84
Terminal half-life (h)*	4.43	12.38†	12.08	22.52†
AUC_{0-12} (μg.h/mL)	16.0 ± 5.3	11.4 ± 2.7	327.9 ± 84.2	255.8 ± 69.2
$AUC_{0-\infty}$ (μg.h/mL)	23.0 ± 12.5	27.9 ± 11.9†	717.8 ± 118.7	1241.7 ± 152.0†
MRT_{0-12} (h)	6.9 ± 2.0	10.3 ± 1.3	9.6 ± 0.6	11.3 ± 0.6
$MRT_{0-\infty}$ (h)	7.8 ± 2.9	20.6 ± 6.2†	18.5 ± 2.3	43.5 ± 10.1†

*Harmonic mean. †These pharmacokinetic parameters were calculated using the data of five ponies. There were insufficient data points to allow calculation in one animal.

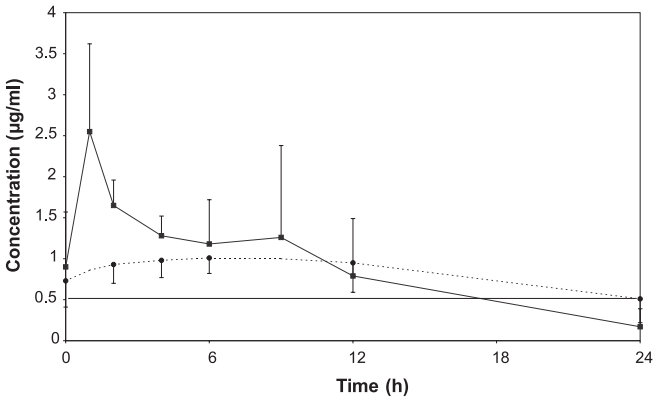

Fig. 1. Time course of plasma (—■—) and tissue chamber fluid (TCF) (–●–) concentrations (SD) of trimethoprim (TMP) after four oral administrations of 5 mg/kg TMP q 12 h. Data are the mean ± SD of six healthy adult ponies. MIC breakpoint for susceptibility (——).

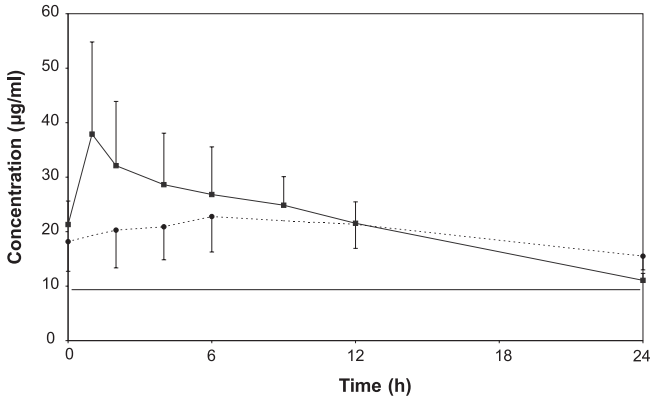

Fig. 2. Time course of plasma (—■—) and tissue chamber fluid (TCF) (–●–) concentrations (SD) of sulfadiazine (SDZ) after four oral administrations of 25 mg/kg SDZ q 12 h. Data are the mean ± SD of six healthy adult ponies. MIC breakpoint for susceptibility (——).

higher than plasma concentrations during the elimination phase (12–24 h). The mean plasma and TCF concentrations of TMP stayed above the minimum inhibitory concentration (*MIC*) breakpoint for 18 h and 24 h, respectively. For SDZ, the mean concentration of both plasma and TCF remained above the *MIC* breakpoint for 24 h. In two ponies absorption was bi-phasic resulting in a second peak for TMP as well as for SDZ at 6–9 h after the last drug administration. TMP:SDZ ratios in plasma ranged from 1:15 at 1 h to 1:65 at 24 h, and in TCF from 1:21 at 4 h to 1:30 at 24 h (see Table 2).

DISCUSSION

This is the first report on TCF concentrations of a TMPS combination in ponies. Concentrations were determined after oral administration of four doses of 5 mg/kg TMP and 25 mg/kg SDZ at 12 h intervals. Gustafsson *et al.* (1999) reported that in horses this regime was sufficient to reach steady-state concentrations of TMP and SDZ in plasma.

The maximal concentration of TMP and SDZ in TCF was lower than in plasma. Also, concentrations of TMP and SDZ in TCF rose more slowly than in plasma and after reaching the peak they decreased slowly because of the longer terminal t½, resulting in a longer *MRT* in TCF than in plasma. This is most probably because of the fact that transport of TMP and SDZ across membranes occurs by passive diffusion. An important factor governing the rate of diffusion in and out of tissue chambers is the ratio of diffusible surface area to tissue chamber volume. Tissue chambers have a relatively small diffusible surface area to volume ratio as compared with the 'true' soft

Table 2. Ratios of TMP:SDZ in plasma and tissue chamber fluid (TCF) after the last of four oral administrations of 5 mg/kg TMP and 25 mg/kg SDZ

Time (h)	TMP: SDZ ratio Plasma	TMP: SDZ ratio TCF
0	1:21	1:25
1	1:15	NT
2	1:19	1:22
4	1:22	1:21
6	1:23	1:23
9	1:20	NT
12	1:27	1:23
24	1:65	1:30

TMP, trimethoprim; SDZ, sulfadiazine; NT, not determined.

tissue interstitial compartment (Clarke, 1989). The differences in pharmacokinetics between plasma and TCF can be explained by considering the TCF to be a third 'deep' compartment, arranged in series with the peripheral and central compartments, with rate constants for transfer of TMP and SDZ between the peripheral and TCF smaller than those describing transfer between the central and peripheral compartments (Clarke *et al.*, 1989; Ensink *et al.*, 1996a). TCF concentrations exceeded plasma concentrations in the elimination phase ($T = 12$–24 h) which can be explained by the slow transfer of TMP and SDZ from TCF to plasma compared with the fairly rapid elimination from plasma. Our findings that C_{max} of TMP and SDZ in TCF were lower and were reached later than in plasma correspond with the results of Piercy (1978) in dogs and sheep.

The mean peak plasma concentrations of TMP (2.92 μg/mL) and SDZ (40.20 μg/mL) after 48 h of drug administration in our study in ponies were higher than those reported by Gustafsson *et al.* (1999) (1.06 μg/mL and 22.4 μg/mL for TMP and SDZ, respectively). This difference is probably because of the different TMP/SDZ formulations employed. The time points at which peak plasma concentrations of TMP and SDZ were reached corresponded with those found by Gustafsson *et al.* (1999). The great variation in T_{max} between individual ponies found in the present study (range 1–9 h) has also been found by others (van Duijkeren *et al.*, 1995; Gustafsson *et al.*, 1999). The bi-phasic absorption seen in two ponies has been described earlier for a TMP/sulfachlorpyridazine combination in horses fed concentrates (van Duijkeren *et al.*, 1995) and for phenylbutazone in ponies fed hay (Maitho *et al.*, 1986). When horses were fasted 12 h before TMPS administration no double peaks were obtained (van Duijkeren *et al.*, 1994b). In the present study the ponies were fed just before and after the drug administrations. Possible explanations for the bi-phasic absorption are individual differences in gastric emptying in fed horses or adsorption of the drugs to food ingredients. Binding of TMP and sulfachlorpyridazine to food has been reported and this binding is probably reversible resulting in a second absorption phase after digestion in the large intestine. This second absorption phase is thought to be responsible for the double peaks (van Duijkeren *et al.*, 1996).

The data indicate that the terminal $t_{½}$ of TMP (4.4 h) and SDZ (12.1 h) after oral administration were longer than following i.v. administration (2.7 h for TMP; 4.7 h for SDZ) (van Duijkeren *et al.*, 1994b; Gustafsson *et al.*, 1999). This can be explained by the 'flip-flop' effect which occurs when absorption is relatively slow in relation to elimination.

The TMP:SDZ ratio for plasma as well as TCF ranged between 1:15 and 1:27 during the dosing interval of 12 h. Although the optimum TMP:sulfonamide ratio is approximately 1:20, *in vitro* synergistic activity can occur at a wide range of ratios between 1:1 and 1:160 (van Duijkeren *et al.*, 1994c). Hence, synergy will be present in plasma as well as TCF almost continuously in the interdosing period after 48 h.

According to Adamson *et al.* (1985) the *MIC* breakpoint for TMPS combinations is 0.5/9.5 μg/mL. The mean TCF concentrations of TMP and SDZ exceeded this *MIC* breakpoint for 24 h. Ensink *et al.* (1993) used a higher *MIC* breakpoint for the TMP/SDZ combination, namely 1/20 μg/mL. If this breakpoint had been used in this study, the mean TCF concentrations of TMP and SDZ would only exceed the breakpoint for 12 h. In addition, in view of the great variation in C_{max} and T_{max} between individual ponies, TCF concentrations of TMP only exceeded the *MIC* breakpoint of 0.5/9.5 μg/mL for 10–12 h in three of six ponies.

In this study we measured TMPS concentrations in sterile tissue chambers, but infection of the chamber results in an increase of protein concentration, which may influence the amount of protein-bound drugs, and a decrease of the pH in TCF, altering the pH gradient between blood and TCF (Clarke *et al.*, 1989). TMP (pka 7.6) is a weak organic base, whereas SDZ (pka 6.4) is a weak organic acid. Therefore a decrease of the pH of infected TCF will favor the diffusion of TMP into TCF. Futhermore, there will be an increased permeability of the diffusional barrier between blood and TCF. Thus, concentrations of TMPS achieved in infected tissue chambers will differ from those in uninfected ones. Inoculation of tissue chambers with *Mannheimia haemolytica* resulted in increased concentrations of protein and albumin and a decrease in the pH (7.02 vs. 7.22) (Clarke *et al.*, 1989). The concentrations of both TMP and SDZ increased in the infected tissue chambers compared with noninfected chambers in cattle, and this might also apply to ponies (Clarke *et al.*, 1989).

Dosage regimens are usually based on serum- or plasma concentrations of healthy individuals. Transport of drugs by circulation into normal healthy tissue is often rapid, and plasma-free drug concentration may be used to predict drug concentrations in a superficial peripheral compartment. However, as bacterial infections often lead to encapsulation and fluid accumulation, tissue chambers can be considered to be useful models for drug penetration into a deep peripheral compartment. This study demonstrates that relying on plasma concentrations alone would result in overestimation of the efficacy of this TMPS paste formulation, especially in infections at sites with slow drug penetration like abcesses or arthritis. TCF concentrations may be more useful in predicting clinical efficacy than plasma concentrations in these infections. Moreover, dosage regimens are often based on the average of several animals, but differences in TCF concentrations between individuals in our study were considerable. It would therefore be better to establish dosing regimens taking into account individuals with the lowest TCF concentrations. Most TMP/SDZ products are labeled for administration once daily to horses, but our study indicates that the interdosing interval should not be longer than 12 h. As TCF concentrations increase much slower than plasma concentrations, an intravenous loading dose (30 mg/kg) given simultaneously with the first oral dose is recommended at the start of the treatment. A pilot study has shown that this regimen results in TCF concentrations above the *MIC* breakpoint within 2 h of administration (Ensink, unpublished data).

This study provides the first data on the distribution of TMP and SDZ into tissue chambers in ponies. The combination of TMP and SDZ given orally at a dose of 5 mg/kg TMP and 25 mg/kg SDZ with a 12-h dosing interval resulted in plasma as well as

TCF concentrations of both drugs that exceeded the *MIC* necessary to inhibit growth of susceptible bacteria. Our results indicate that TCF concentrations are better suited to designing dosage regimens for antimicrobial drugs in infected tissues than plasma concentrations.

ACKNOWLEDGEMENTS

We thank Mr A.J. de Wit and his staff, Mr R. Frieling and H. ter Keurs for taking care of the ponies and for technical assistance.

REFERENCES

Adamson, P.J., Wilson, W.D., Hirsh, D.C., Baggot, J.D. & Martin, L.D. (1985) Susceptibility of equine bacterial isolates to antimicrobial agents. *American Journal of Veterinary Research*, **46**, 447–450.

Beadle, R.E., Short, C.R., Corstvet, R.E., Pawlusiow, J., Nobles, D.D., McClure, J.R., Guthrie, A.J. & Clarke, C.R. (1989) Characterization of a soft-tissue infection model in the horse and its response to intravenous cephapirin administration. *Journal of Veterinary Pharmacology and Therapeutics*, **12**, 73–86.

Bertone, A.L., Jones, R.L. & McIlwraith, C.W. (1988) Serum and synovial fluid steady-state concentrations of trimethoprim and sulfadiazine in horses with experimentally induced infectious arthritis. *American Journal of Veterinary Research*, **49**, 1681–1687.

Brown, M.P., Gronwall, R. & Castro, L. (1988) Pharmacokinetics and body fluid and endometrial concentrations of trimethoprim-sulfamethoxazole in mares. *American Journal of Veterinary Research*, **49**, 918–922.

Brown, M.P., Kelly, R.H., Stover, S.M. & Gronwall, R. (1983) Trimethoprim-sulfadiazine in the horse: serum, synovial, peritoneal, and urine concentrations after single-dose intravenous administration. *American Journal of Veterinary Research*, **44**, 540–543.

Clarke, C.R. (1989) Tissue-chamber modeling systems – applications in veterinary medicine. *Journal of Veterinary Pharmacology and Therapeutics*, **12**, 349–368.

Clarke, C.R., Short, C.R., Corstvet, R.E. & Nobles, D. (1989) Effect of *Pasteurella haemolytica* infection on the distribution of sulfadiazine and trimethoprim into tissue chambers implanted subcutaneaously in cattle. *Journal of Veterinary Research*, **50**, 1551–1556.

van Duijkeren, E., Vulto, A.G. & van Miert, A.S.J.P.A.M. (1994a) Trimethoprim/sulfonamide combinations in the horse: a review. *Journal of Veterinary Pharmacology and Therapeutics*, **17**, 64–73.

van Duijkeren, E., Vulto, A.G., Sloet van Oldruitenborgh-Oosterbaan, M.M., Mevius, D.J., Kessels, B.G.F., Breukink, H.J. & van Miert, A.S.J.P.A.M. (1994b) A comparative study of the pharmacokinetics of intravenous and oral trimethoprim/sulfadiazine formulations in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, **17**, 440–446.

van Duijkeren, E., van Klingeren, B., Vulto, A.G., Sloet van Oldruitenborgh-Oosterbaan, M.M., Breukink, H.J. & van Miert, A.S. (1994c) In vitro susceptibility of equine Salmonella strains to trimethoprim and sulfonamide alone or in combination. *American Journal of Veterinary Research*, **55**, 1386–1390.

van Duijkeren, E., Vulto, A.G., Sloet van Oldruitenborgh-Oosterbaan, M.M., Kessels, B.G.F., van Miert, A.S.J.P.A.M. & Breukink, H.J. (1995) Pharmacokinetics of trimethoprim/sulfachlorpyridazine in horses after oral, nasogastric and intravenous administration. *Journal of Veterinary Pharmacology and Therapeutics*, **18**, 47–53.

van Duijkeren, E., Kessels, B.G., Sloet van Oldruitenborgh-Oosterbaan, M.M., Breukink, H.J., Vulto, A.G. & van Miert, A.S. (1996) In vitro and in vivo binding of trimethoprim and sulphachlorpyridazine to equine food and digesta and their stability in caecal contents. *Journal of Veterinary Pharmacology and Therapeutics*, **19**, 281–287.

Ensink, J.M., Klein, W.R., Barneveld, A., Vulto, A.G., van Miert, A.S. & Tukker, J.J. (1996a) Distribution of penicillins into subcutaneous tissue chambers in ponies. *Journal of Veterinary Pharmacology and Therapeutics*, **19**, 439–444.

Ensink, J.M., Klein, W.R., Barneveld, A., van Miert, A.S. & Vulto. A.G. (1996b) Side effects of oral antimicrobial agents in the horse: a comparison of pivampicillin and trimethoprim/sulphadiazine. *Veterinary Record*, **138**, 253–256.

Ensink, J.M., van Klingeren, B., Houwers, D.J., Klein, W.R. & Vulto, A.G. (1993) In-vitro susceptibility to antimicrobial drugs of bacterial isolates from horses in The Netherlands. *Equine Veterinary Journal*, **25**, 309–313.

Gustafsson, A., Båverud, V., Franklin, A., Gunnarsson, A., Ögren, G. & Ingvast-Larsson, C. (1999) Repeated administration of trimethoprim/sulfadiazine in the horse-pharmacokinetics, plasma protein binding and influence on the intestinal microflora. *Journal of Veterinary Pharmacology and Therapeutics*, **22**, 20–26.

Heinzel, G., Woloszczak, R. & Thomann, P. (1993) Topfit, Version 2.0, Pharmacokinetic and pharmacodynamic data analysis system for the PC. G. Fischer, New York.

Maitho, T.E., Lees, P. & Taylor. J.B. (1986) Absorption and pharmacokinetics of phenylbutazone in Welsh Mountain ponies. *Journal of Veterinary Pharmacology and Therapeutics*, **9**, 26–39.

Piercy, D.W. (1978) Distribution of trimethoprim/sulphadiazine in plasma, tissue and synovial fluids. *Veterinary Record*, **102**, 523–524.

Prescott, L.F. & Baggot, J.D. (1993) Principles of antimicrobial drug disposition. In *Antimicrobial Therapy In Veterinary Medicine.* Eds. Prescott, J.F & Baggot, J.D. pp. 37–73. Blackwell Scientific Publications, Boston.

The Rules Governing Medicinal Products in the European Community, Vol. VI (1991). pp. 108–109. Commission of the European Communities.