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2 **Full Paper**
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4 **Pharmacokinetics/pharmacodynamics cut-off determination for fosfomycin using Monte Carlo**
5 **simulation in healthy horses.**

6 Running title: Fosfomycin PK/PD in horses
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Abstract

Fosfomycin (FOM) is an approved veterinary medicinal product for large animals in Japan, but Clinical breakpoint (CBP) for antimicrobial susceptibility test (AST) is not defined for animals. This study aimed at conducting a pharmacokinetics/pharmacodynamics (PK/PD) analysis to determine the PK/PD cutoff for the CBP in horses. Drug concentrations following single intravenous administration (IV) of 20 mg/kg body weight (BW) FOM in nine horses were measured using liquid chromatography/mass spectrometry. The data were modelled using a nonlinear mixed-effects model, followed by Monte Carlo simulations. A 90% probability of target attainment for a PK/PD target of the ratio of Area Under the free plasma concentration-time curve divided by the minimal inhibitory concentration (MIC) >24 hr was set as PK/PD cut-off. The PK/PD cutoff for FOM 20 mg/kg BW q12 hr IV was estimated with the MIC value of ≤16.0 mg/L, and this regimen was considered effective against *E. coli* (MIC_{90}; 16.0 mg/L) in healthy horses based on the MIC_{90} values of the wild population. Owing to the relevance of FOM to human health, veterinarians should use q 12 hr FOM 20 mg /kg against *E. coli* infections with an MIC < 16 µg/mL, as suggested by our PK/PD cutoff after AST.

KEYWORDS

Fosfomycin, horse, Pharmacokinetics/Pharmacodynamics (PK/PD) cutoff, antimicrobial susceptibility testing, *E. coli*.

Introduction

Fosfomycin (FOM) is a bactericidal antimicrobial agent with broad antibacterial activity against both Gram-positive and Gram-negative pathogens [12]. Specifically, FOM is considered to be active against Gram-positive pathogens, including *Staphylococcus aureus*, and *Staphylococcus* spp. and *Enterococcus* spp. and against Gram-negative pathogens, including *Salmonella* spp., *Escherichia coli*, *Klebsiella*. [20]. In humans, FOM is prescribed mainly for urinary tract infections and for various other infections such as pneumonia, osteomyelitis, and septic arthritis [12].

Pharmacokinetic analysis has been reported in chickens, cattle, dogs, and horses, as well as in humans [6, 12, 14, 26, 29]. In Japan, FOM for intravenous (IV) administration was approved as veterinary medicinal products for cattle [29]. The recommended dosing regimen of FOM for horses and cattle based on pharmacokinetics studies is 20 mg/kg, q 8 to 12 hr [26, 29], and used for horses in Japan. European Committee on Antimicrobial Susceptibility Testing (EUCAST) indicated the Clinical breakpoint (CBP) for a susceptible minimum inhibitory concentrations (MIC) value <32.0 mg/L in *Enterobacterales* and *Staphylococcus* spp. in humans with the dose of 4g/patient q 8 hr IV administration [2]. However, CBP is not established in horses by organizations such as EUCAST or the Clinical & Laboratory Standards Institute (CLSI) to interpret antimicrobial susceptibility test (AST) results. Since CBP has been established in horses, human CBP is currently used for AST in horses. Because CBPs are species-specific and depend on dosage regimens, CBP may be different in the current situation where the dosage is different between horses (20 mg/kg) and humans (60-80 mg/kg) [24]. In this case, the CBP in horses would be expected to be lower than that in humans, and FOM 20 mg/kg IV may be ineffective for horses according to the AST based on the human CBP.

The WHO ranked FOM as a critically important antimicrobial agent for human medicine [10]. Because of its importance for human health, the proper use of FOM in horses requires the implementation of an AST based on horse specific CBP. In this study, a pharmacokinetics/pharmacodynamics (PK/PD) analysis was conducted based on the

93 pharmacokinetics of FOM and its MICs against bacteria isolated from horses to determine the PK/PD
94 cutoff for CBP.
95
96 **Materials and methods**
97 Nine healthy 2–8 year-old Thoroughbred horses (four stallions and five mares) with body
98 weights (BWs) of 416–557 kg were used. The horses were kept in individual stalls during the
99 experiments and had *ad libitum* access to water and hay. The dose of FOM (20 mg/kg BW) was
100 determined based on previous reports [29]. FOM (FOSMICIN® injection 2 g; Meiji Seika Pharma
101 Co. Ltd, Tokyo, Japan) was dissolved in 50 mL sterile physiological saline and delivered into the right
102 jugular vein by a short bolus infusion (<30 sec). This study was reviewed and approved by the Animal
103 Care and Use Committee of the Equine Research Institute, Japan Racing Association, in accordance
104 with ASPA (1986) legislation Protocol # 21-5.
105 Blood samples were collected at 0 (prior to administration), 5, 10, 20, 30, and 45 min and 1,
106 2, 3, 4, 6, 8, 12, and 21 hr after IV administration. All blood samples were collected from the left
107 jugular vein using a 16G catheter (Becton Dickinson Company, Franklin Lakes, NJ, USA), which was
108 inserted into the skin using local anesthesia and 1 mL lidocaine (Xylocaine Injection Polyamp 0.5%,
109 Sandoz Pharma., Tokyo, Japan). Subsequently, 10 mL blood samples were collected in heparinized
110 vacuum blood collection tubes (Venoject 2; Terumo Corporation, Tokyo, Japan). The samples were
111 immediately centrifuged at 1500 × g for 10 min, and the separated plasma samples were stored at −20
112 °C until analysis.
113 ***Determination of plasma concentrations***
114 Plasma concentrations of FOM were quantified via liquid chromatography/mass spectrometry
115 as previously reported [28]. The FOM calibration curve and quality controls were prepared by spiking
116 blank equine plasma with the reference standard at the concentration from 0.1 to 300 ng/mL. Quality
117 control samples for the calibration of the plasma analysis were prepared by adding standard FOM
118 (Sigma-Aldrich Co., St. Louis, MO, USA) to blank horse plasma. Then, 200 µL of acetonitrile and 20
119 µL of 1 µg/mL rac-fosfomycin-D_5 (Toronto Research Chemicals Inc, Toronto, Canada) in methanol as

120 an internal standard were added to 100 μL of plasma. The samples were incubated for 5 min at 25 °C

121 and centrifuged at 10,000 × g for 5 min. One microliter of each sample was injected into a liquid

122 chromatography system (Nexera X2; Shimadzu Corporation, Kyoto, Japan) connected to a mass

123 spectrometer (QTRAP4500; SCIEX Corporation, Tokyo, Japan). Liquid chromatography was

124 performed on the ZIC-HILIC Guard column (20 mm, 2.1 μm; Merck, Darmstadt, Germany) and ZIC-

125 HILIC column (50 mm × 2.1 mm i.d., 3.5 μm; Merck, Darmstadt, Germany) with a mixture of 25

126 mmol/L ammonium formate (Fujifilm Wako, Osaka, Japan) and acetonitrile (Fujifilm Wako, Osaka,

127 Japan) at a flow rate of 0.2 mL/min. The final calibration curve had a coefficient of correlation (R^2)

128 >0.995 over the concentration range of 0.1–300.0 μg/mL with a $1/y_2$ weighing factor. Accuracy and

129 precision in quality control samples were determined at concentrations of 0.1, 0.3, 5, and 240 μg/mL

130 (five replicates each). Accuracies were between 83.0% and 114.0%, and the precision of coefficient of

131 variation (CV) were < 15 %. The lower limit of quantitation (LOQ) for FOM was 0.1 μg/mL.

132 ***Protein binding***

133 The ultrafiltration method was used to separate free and bound drug for FOM; 200 μl samples

134 were placed in a filter (Centrifree Ultrafiltration Device; Merck KGaA, Darmstadt, Germany) and

135 centrifuged at 15,000 × g for 10 min at 25 °C. The free drug concentration following ultrafiltration

136 and the total drug concentration in samples not subjected to ultrafiltration were quantified using the

137 same assay method, as previously described. Plasma samples for the assay were collected from nine

138 horses 1, 3, and 5 hr after administration. The extent of protein binding and free fraction were

139 calculated by comparing the free and total drug concentrations.

140 ***Pharmacokinetic data analysis***

141 Estimation of the PK/PD cutoff requires the development of a population PK model to

142 quantify typical PK parameters and their between-subject variability (BSV) [27]. Plasma

143 pharmacokinetic analyses were conducted using a Nonlinear Mixed Effect (NLME) model on a

144 commercially available software (Phoenix WinNonlin version 6.4; Certara, Princeton, NJ, USA). The

145 ‘mixed effect” of NLME refers to two types of effects; namely, fixed effects and random effects.

146 Fixed effects correspond to typical pharmacokinetic parameters which characterize the structural

147 model in all subjects in a population. Random effects like the BSV describe the variability around
148 these fixed effects, making it possible to estimate individual values of the PK parameters. The
149 distribution of PK parameters are assumed to be log-normal [19]. In a second step, the NLME model
150 was used to generate a large sample of plasma disposition curves (typically 5000) via Monte Carlo
151 simulations (MCS) based on typical PK parameters and the corresponding BSV to predict exposure
152 for a large virtual population, allowing us to compute the Probability of Target Attainment (PTA).
153 VetCAST recommended the use of this virtual population to estimate the PK/PD cutoffs for different
154 possible MICs [26].

155 A three-compartment structural model was selected based on the likelihood ratio test and the
156 Akaike information criterion. The model was parameterized in terms of clearance and distribution
157 volume. The estimated parameters were the central (V1) and two peripheral (V2 and V3) volumes of
158 distribution, plasma clearance (CL), and inter-compartmental distribution clearances (CL2 and CL3).

159 In a population model, the statistical model describing the BSV is added to the structural
160 model. The BSV for a given parameter was described using an exponential model of the following
161 form:

162 $$\theta_{parameter_i} = \theta_{tv_parameter} \cdot EXP(\eta_i) \text{ (Eqn. 1)}$$

163 where $\theta_{parameter_i}$ is the value of theta for a given parameter in the ith animal, $\theta_{tv_parameter}$ is the typical
164 population value of parameters, and ηi (eta_i) is the deviation associated with the ith animal from the
165 corresponding theta population value. the distribution of the etas was assumed normal with a mean of
166 0 and a variance ω^2.

167 To report the BSV as a coefficient of variation, Equation 2 was used for conversion of the variance
168 terms (ω^2) into a coefficient of variation (CV%):

169 $$CV(\%) = 100 \times \sqrt{exp(\omega^2) - 1} \qquad \text{(Eqn. 2)}$$

170

171 Shrinkage of the random effects (eta) toward the means was described as:

172 $$shrinkage = 1 - \frac{var(\eta_r)}{\omega^2} \qquad \text{(Eqn. 3)}$$

173 where var(η_r) is the variance of Empirical Bayes ("*post hoc*") estimates (EBEs) of η_s. When the
174 shrinkage of eta was >0.3, the data did not allow for a robust estimation of this random component.
175 Estimates of the random effects for the IV model are given in Table 1, and all the eta shrinkage values
176 were <0.3. A full OMEGA matrix, meaning that both variance and covariance terms were estimated,
177 was used to determine the random components of the model, that is, the BSV associated with the
178 fixed pharmacokinetic parameters.
179 The residual model was an additive plus a multiplicative (proportional) model of the form.
180 $$\mathrm{C(t)} = f(\theta, Time) \times (1 + \varepsilon_1) + \varepsilon_2 \qquad \text{(Eqn. 4)}$$
181 with ε1 is the multiplicative error term having a mean of 0 and a variance noted σ1
182 $$\varepsilon 1 \approx N(0, \sigma 1^2)$$
183 and ε2 is the additive error term having a mean of 0 and a variance noted σ2
184 $$\varepsilon 2 \approx N(0, \sigma 2^2)$$
185 The additive sigma was reported as its standard deviation noted with the same units as plasma
186 concentration (µg/mL) and the multiplicative sigma was reported as coefficient of variation.
187 Moreover, covariates were tested (age, body weight, and sex). The stepwise covariate search mode of
188 Phoenix was used to define the statistically significant covariates for each of the structural parameters.
189 The stepwise forward or backward addition or deletion of covariate effects (by adding one at a time)
190 determines the improvement of the final model based on the Bayesian information criterion (BIC). A
191 BIC value of 6.635 for adding a covariate and a value of 10.823 for deleting a covariate was used [9].
192 The Quasirandom Parametric Expectation Maximization (QRPEM) engine was used to maximize the
193 likelihood.
194 Using the developed model and the free fraction, MCS were used to generate free plasma
195 concentrations in a population of 5000 horses using individual predictions or IPRED (eta was as
196 estimated), corresponding to different dosage regimens. The simulation was performed for 20 mg/kg
197 at four interval patterns for 24 hr from the first administration. We calculated for the 5000 curves the
198 ratio of the Area Under free plasma concentration-time Curve from 0–24 hr after administration
199 divided by the MIC ($f\mathrm{AUC}_{0\text{-}24\,h}$/MIC) >24 [17, 23]. The highest MIC reaching the corresponding

200 Probability of Target Attainment (PTA) of 90% after standard dosage regimen is considered PK/PD
201 cutoff according to the VetCAST approach [27].

202 ***Minimum inhibitory concentrations***

203 The MICs of FOM were obtained from unpublished data on the 138 strains of *S.*
204 *zooepidemicus*, 65 strains of *Staphylococcus aureus* (without methicillin-resistant *S. aureus*), 87
205 strains of *Escherichia coli* and 58 strains of *Pseudomonas aeruginosa* isolated from infected
206 Thoroughbred horses, including those with pneumonia and cellulitis. MICs were determined in
207 accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (M07-A9).
208 Moreover, we compared MIC distributions and Epidemiology cut off (ECOFF) isolated from humans
209 which were reported by EUCAST [4]. For bacteria for which ECOFF was not indicated by EUCAST,
210 ECOFF was calculated from our MIC distributions using EcoFinder [3].
211

212 **Results**

213 Semilogarithmic plots of the disposition curves of the FOM for each horse are shown in Figure
214 1. The plasma concentrations of FOM were above the limit of quantification until 21 hr after
215 administration at last sampling. Logarithmic plots of the observed drug plasma concentrations versus
216 population prediction (PRED) and IPRED are shown in Figure 2. Data were evenly distributed around
217 the line of identity, indicating no major bias in the population components of the model. The plot of
218 the conditional weighted residuals (CWRES) versus time indicated that the residuals were randomly
219 scattered around zero with no systematic trend, supporting the selection of the residual error model
220 (Figure 3). None of the tested covariates (age, sex, or body weight) were significant in this model.
221 Bootstrap estimates of the typical values of the primary structural parameters of the model (theta),
222 secondary parameters, and their associated coefficients of variation as a measure of the precision of
223 their estimation are given in Table 1. A Visual Predictive Check ensured that the simulated and
224 observed data were consistent (Figure 4).

225 The median plasma protein binding percentage of FOM was 1.5% (-3.9–4.6%), given the
226 median and range, and the free fraction was determined as 1 to make the simulations of free plasma
227 concentration. The PTA for the 5000 free drug concentration profiles obtained by MCS for different

228 possible MICs and FOM regimens are shown in Figures 5. For an IV regimen of 20mg/kg q12 hr IV a
229 PTA of 90% was achieved for a MIC of ≤ 16.0 mg/L, and it was considered as PK/PD cutoff. The
230 MIC_{90} of FOM against *S. zooepidemicus*, *S. aureus*, *E. coli*, and *P. aeruginosa* were 128.0, 2.0, 16.0,
231 and >128.0 mg/L, respectively, isolated from horses. The ECOFF by EUCAST for *S. aureus*, *E. coli*
232 and *P. aeruginosa* determined using EUCAST were 32, 4.0, and 256.0 mg/L, respectively. The
233 ECOFF of *S. zooepidemicus* was calculated to be 256 mg/L using EcoFinder.
234
235 **Discussion**
236 FOM is an old antimicrobial; however, its importance has increased in recent years because of
237 the worldwide emergence of resistant bacteria [12]. In Europe, the Antimicrobial Advice Ad Hoc
238 Expert Group (AMEG) of the European Medicine Agency categorized FOM as a category A
239 antimicrobial; it may be administered to horses under exceptional circumstances [1]. However, FOM
240 has been approved as veterinary product in Japan. This motivated the present study to delimit its use
241 based on an evaluation of the susceptibility of pathogens involved in horse infections. Currently, there
242 is no horse CBP available for interpreting AST results; only human CBP is available. Given that
243 CBPs are species-specific and depend on dosage regimens [27], there is no indication that human
244 CBPs can be used for horses, particularly because of the difference in the recommended dosages in
245 horses (20 mg/kg) and in men (60-80 mg/kg) [24]. The present study aimed to determine the PK/PD
246 cutoff of the FOM, i.e. the highest value of the MIC that can be reached in 90% of horses with the
247 recommended FOM dosage according to VetCAST approach [27]. This PK/PD cutoff, in the absence
248 of CBP, can be used to interpret AST and consider ECOFFs for the involved pathogen, and allows for
249 the prudent use of FOM in horses.
250 PK/PD cutoff of FOM was estimated using NLME model and free fraction according to
251 VetCAST approach [27]. The extent of protein binding was important to establish a PK/PD cutoff
252 because only the free drug concentration is microbiologically active [5]. It was reported that FOM
253 was not to bind to human plasma proteins and good diffusion into tissues and body fluids in humans
254 [15]. In this study the protein binding rate of FOM in horses was also almost 0 at all-time points and
255 considered not bind to equine plasma protein same as humans. The selection of a PK/PD index is

256 necessary to compute a PK/PD cutoff as a surrogate of antimicrobial efficacy based on an in vivo or
257 in vitro infection model [27]. Antimicrobials are classified as time-dependent or concentration-
258 dependent, with the former index being the time for which the free drug concentration exceeds the
259 MIC (*f*T>MIC), and the latter being *f*AUC/MIC [11]. Previous reports on mouse infection models
260 have indicated that FOM has bacteriostatic effects against the Enterobacteriaceae group, with an
261 AUC/MIC (equivalently an *f*AUC/MIC with *f*=1) ratio of 24 [18].

262 In this study, PK/PD cutoff of FOM with 20 mg/kg q12h IV administration were calculated as
263 MIC of ≤ 16.0 mg/L. CLSI indicated the breakpoint for a susceptible MIC value <64.0 mg/L in
264 *Enterobacterales* and *Enterococcus* spp. in humans (M100 Ed32E). The EUCAST indicated a
265 breakpoint for a susceptible MIC value of <32.0 mg/L in *Enterobacterales* and *Staphylococcus* spp. in
266 humans at a dose of 4g/patient q 8 hr IV administration (Version 12.0). Compared with these human
267 breakpoints, the PK/PD cutoff was estimated to be lower in horses. These differences are related to
268 the difference between the dosage in humans (60-80 mg/kg q 8h) and in horses (20 mg/kg q 12h) and
269 the plasma clearance in horses (1.17 mL/kg/min) vs humans (approximately 2 mL/kg/min) [13].
270 Since there is a difference between the human breakpoints and result of the present paper, the dose of
271 20 mg/kg q 12 hr administration may be ineffective for 'susceptible' bacteria by AST based on human
272 breakpoints with the MIC located between human CBP (32-64 mg/L) and PK/PD cutoff in this study
273 (16 mg/L) in horses. We recommend discarding human CBP and considering the PK/PD cut-off
274 estimated in this study for horses.

275 The determination of a PK/PD cut-off is first required to develop a population model to
276 subsequently simulate a large meta-population using MCS. In the present study, we used only nine
277 horses, which is a limited number. That said, for the IV route, the only pharmacokinetic determinant
278 of the *f*AUC/MIC index for FOM was plasma clearance (0.070 L/kg/h) and its BSV (15.9%). If the
279 BSV of plasma clearance is increased for a given typical PK value, the PK/PD cut off to reach 90%
280 PTA is expected to be lower than our results. Since this study only included a limited number of
281 healthy young thoroughbred horses and none of the tested covariates (age, sex, and body weight) were
282 significant, it is difficult to predict what the PK/PD cutoff would be in other populations. In quarter
283 horses aged 5 to 15 years, the clearance was shown to be lower and AUC to be higher than in the

284 present study. It is therefore likely that the threshold value that we proposed is also valid in that
285 population, since the PK/PD cutoff is inversely proportional to plasma clearance. [29]. To explore the
286 robustness of PTA for AUC/MIC, meta-analysis including various horses and conditions using
287 multiple previously published data based on NLME model with covariate model may be efficient and
288 have recently reported on marbofloxacin and penicillin in horses [8, 16].

289 The pharmacokinetics of FOM have also been reported in cattle, chicken, pig, dog, and
290 humans [6, 13, 14, 21, 25, 26]. The plasma clearance was higher in horses than in chickens,
291 cattle, and dogs, lower than in pigs, and similar to humans' clearance [13, 21]. Since AUC is
292 controlled by the administered dose and the plasma clearance, the PK/PD cutoff is expected
293 to decrease with increased clearance and/or lower doses. Dosing regimens are also different,
294 so different PK/PD cutoffs are expected in different animal species. FOM breakpoints have
295 not been indicated in other animals from any organizations such as CLSI and EUCAST.
296 Since FOM is considered a critically important antimicrobial agent for human medicine [10],
297 its use in animal medicine is limited. It's use has been reported in chickens and pigs in
298 Central and South America, and in cattle in Japan [21]. Whatever the treated species, an
299 appropriate use of FOM after AST based on scientific PK/PD cutoffs is recommended to
300 prevent antimicrobial resistance.

301 Furthermore, ECOFF and MIC distributions are important for CBP along with PK/PD cutoff
302 and clinical cutoff [27]. The clinical cutoff is an MIC cutoff related to clinical outcomes, but the data
303 required for this value are limited in veterinary medicine. ECOFF is defined as the upper end of the
304 wild-type MIC distribution and is a biological parameter that is not affected by the source (human or
305 animal). If the PK/PD cut-off is below the ECOFF, the current dosage regimen is too low to treat the
306 wild-type population. In this case, VetCAST does not establish a CBP dividing the wild-type MIC
307 distributions to prevent the wild-type strain from becoming resistant [7, 27]. Compared to PK/PD
308 cutoff (16.0 mg/L), ECOFF (4.0 mg/L) and MIC distribution in horses (MIC_{90}; 16.0 mg/L) for *E. coli*,
309 16 mg/L may be set as possible CBP for this pathogen. *E. coli* is sometimes isolated from the lower
310 respiratory tract in horses and is associated with death and severe pleuropneumonia [22], and FOM 20

311 mg/kg q 12 hr administration was expected to be controlled from this study. Because the PK/PD
312 cutoff (16 mg/L) in this study was below the ECOFF of *S. zooepidemicus* (256 mg/L), *P. aeruginosa*
313 (256 mg/L), and *S. aureus* (32 mg/L), the PK/PD cutoff cannot be set as the CBP for these pathogens.
314 In particular, the ECOFF and MIC distributions in horses of *S. zooepidemicus* and *P. aeruginosa* were
315 extremely high; FOM is ineffective and should not be used for these infections. For *S. aureus*, the
316 PK/PD cutoff was higher than the MIC_{90} in horses (2.0 mg/L), but lower than the ECOFF (32.0
317 mg/L). Currently, 20 mg/kg q 12 hr administration is expected to be effective against the MIC_{90} value
318 of *S. aureus* isolated from horses, however, q 6h administration is required to cover all wild-type
319 strains. The results of this study will help clarify which pathogens can be targeted in horses by FOM
320 and should promote more responsible prescription trends by reducing empirical use, thus preventing
321 the development of resistant pathogens.

322 Our study indicated that the PK/PD cutoff in 20 mg/kg BW q12 hr IV were MIC value ≤16.0
323 mg/L and attained therapeutic concentrations to control *E. coli* in healthy horses up to the MIC_{90}
324 values of the wild population. Owing to its importance in human health, veterinarians should use
325 FOM 20 mg /kg q 12 hr mainly against *E. coli* infections with a MIC < 16 µg/mL, as suggested by our
326 PK/PD cutoff after AST.

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328

329 **Conflict of Interest Statement**

330 The authors disclose no conflict of interest.

331

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448 horses. *J Vet Pharmacol Ther* **31**: 321-327.
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451 **Figure 1**: Semilogarithmic spaghetti plots of the disposition curves of fosfomycin after a single dose

452 administration of 20 mg/kg BW fosfomycin in nine horses.

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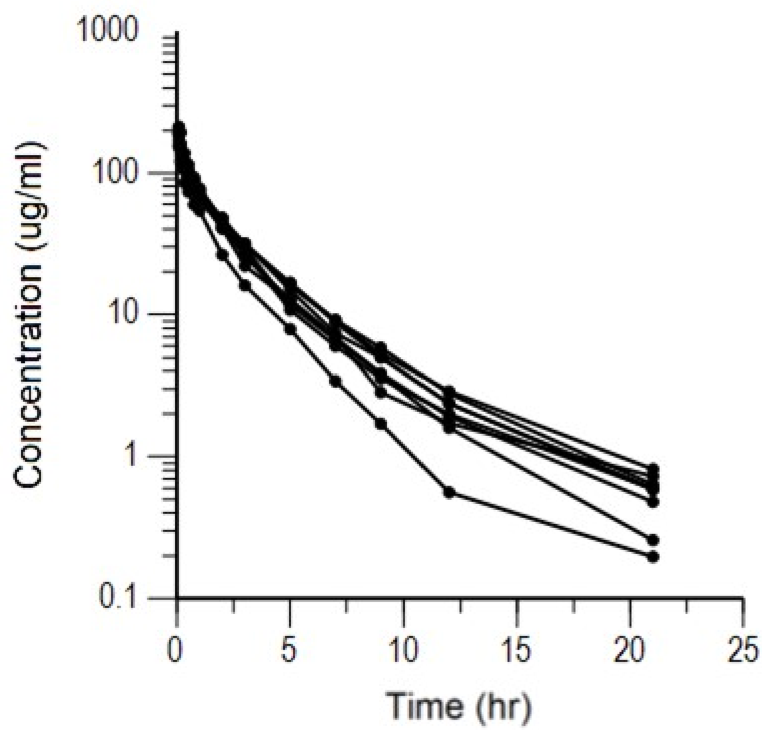

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456 **Figure 2**: Arithmetic scale (left) and logarithmic scale (right) of observed fosfomycin plasma

457 concentrations vs. population predictions (PRED) (top plots) and individual predictions (IPRED)

458 (bottom plots).

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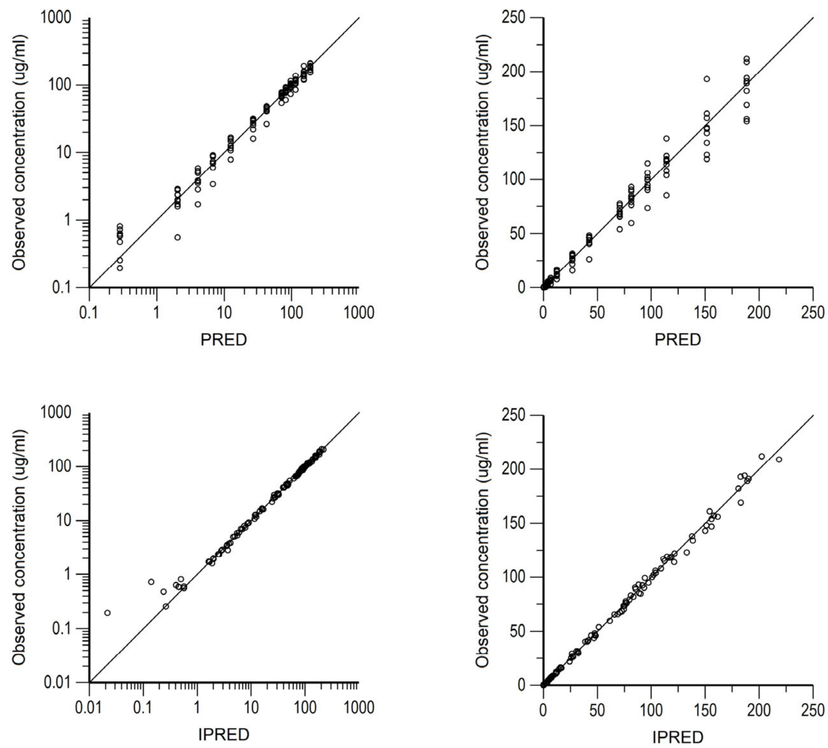

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462 **Figure 3:** CWRES (conditional weighted residuals) vs time plot for fosfomycin. Values of CWRES
463 should be approximately N (0, 1) and hence concentrated between y = −3 and y = +3. Inspection of the
464 figure indicates that data were evenly distributed about zero and that the trends (as given by the blue
465 line and the red line, its negative reflection) did not show any fanning, indicating no bias in the structural
466 model.
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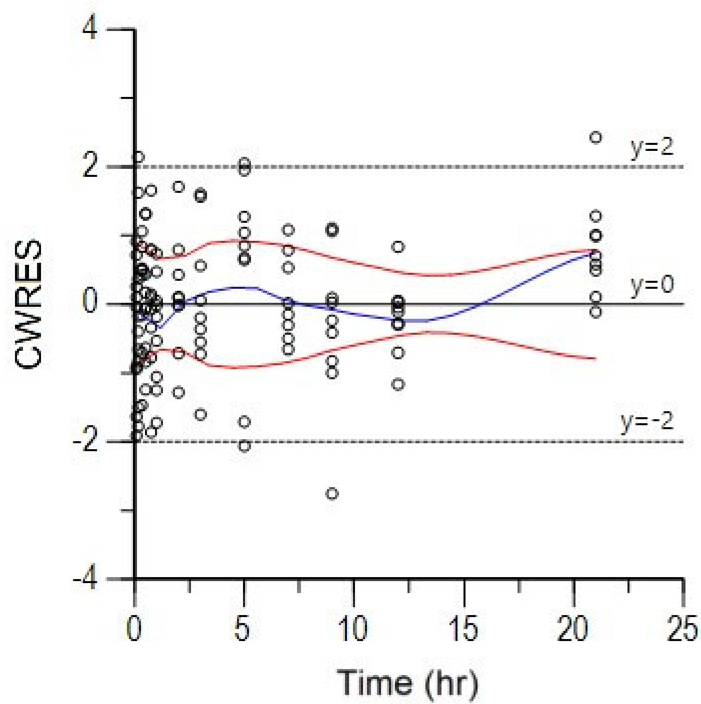

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470 **Figure 4:** Visual Predictive Check of a single dose of 20 mg/kg BW fosfomycin. The observed and
471 predicted 10th and 90th percentiles are shown in solid black and red lines, respectively. The observed
472 and predicted 50th percentiles (median) are shown in black and red broken lines, respectively. Black
473 dots are individual raw data.
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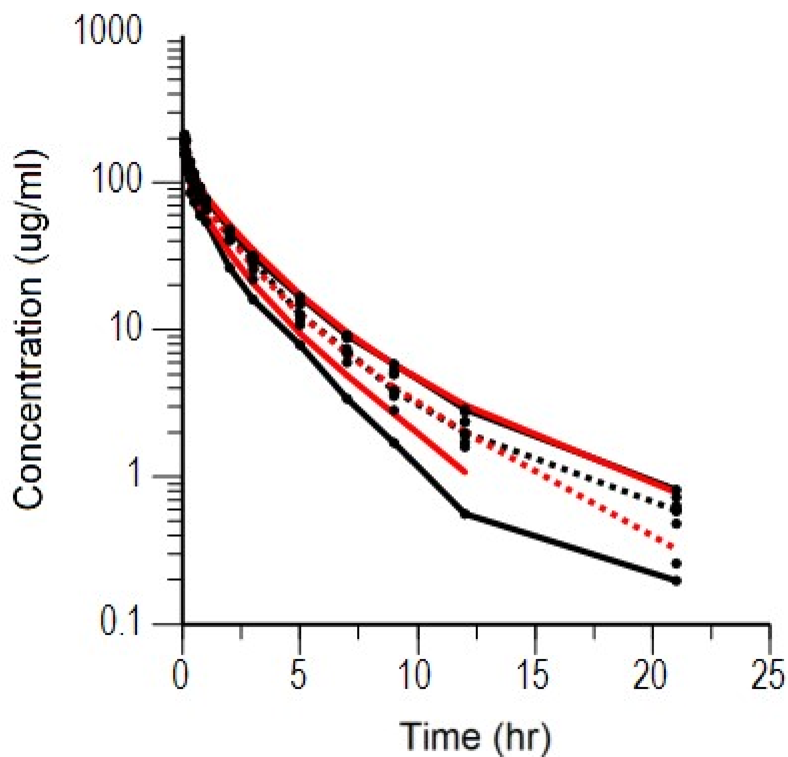

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477 **Figure 5:** Probability of Target Attainment (PTA%) vs. minimal inhibitory concentration (MIC)
478 (µg/mL) of fosfomycin for repeated administration of fosfomycin 20 mg/kg BW different dosing
479 intervals ranging from 8 to 24 hr. Values were obtained from 5000 simulated fosfomycin concentrations
480 profiles generated from the population model by Monte Carlo simulations. PTA 90% is indicated by
481 the solid blue line, which is considered as the target to achieve, and MIC that corresponds to PTA 90%
482 is indicated by the dotted blue line.
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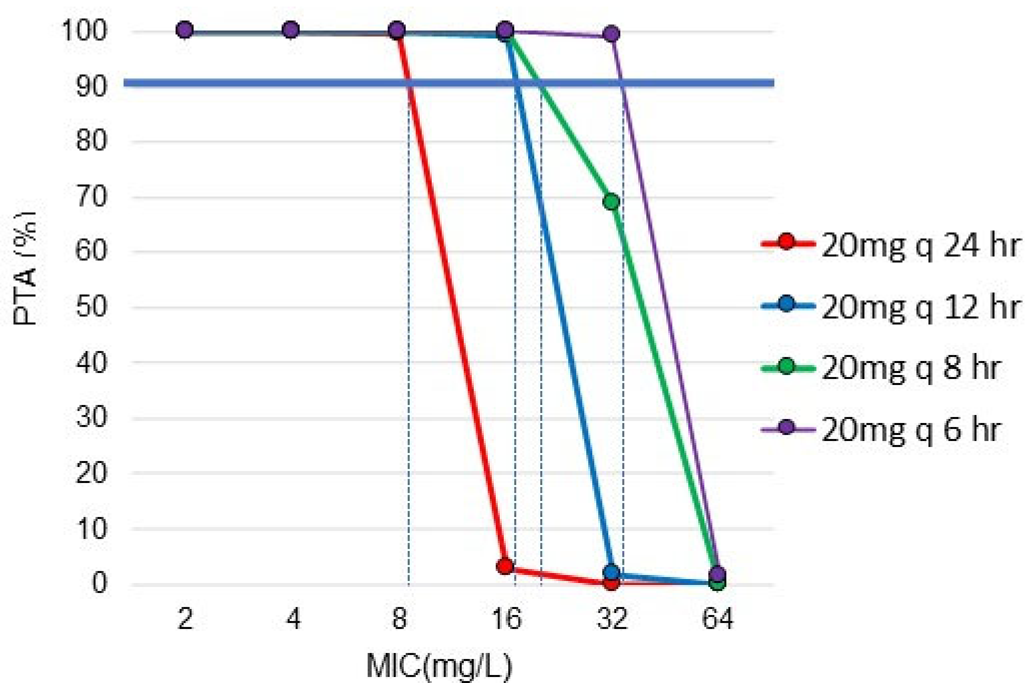

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Table 1: Estimates of the random effects (full variance/covariance matrix) and shrinkage with a 3 compartment model.

Label	nV	nV2	nV3	nCl	nCl2	nCl3
Omega (variance/covariance)						
nV	0.0292					
nV2	0.0049	0.0249				
nV3	-0.0139	0.0592	0.2708			
nCl	-0.0515	0.0222	0.1256	0.2206		
nCl2	-0.0099	0.0089	0.042	0.0228	0.0103	
nCl3	-0.0159	-0.0006	-0.0257	0.0727	-0.0035	0.052
Correlation						
nV	1					
nV2	0.1816	1				
nV3	-0.156	0.7201	1			
nCl	-0.6417	0.2999	0.5138	1		
nCl2	-0.5678	0.5546	0.7973	0.4789	1	
nCl3	-0.4081	-0.0174	-0.2167	0.679	-0.1521	1

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Table 2: Population primary parameters of Fosfomycin in horses with a 3-compartment model (Legend: between-subject variability (BSV)%, CV%, and 2.5% and 97.5% percentiles give the precision of typical value estimates).

Primary structural Parameters	Units	BSV%	Typical values (Median)	CV%	2.50%	97.50%
tvV	L/kg	17.2	0.08	9	0.066	0.094
tvV2	L/kg	10.2	0.055	9.8	0.044	0.066
tvV3	L/kg	23.1	0.059	13.2	0.044	0.074
tvCL	L/kg/hr	15.9	0.07	7.4	0.06	0.08
tvCL2	L/kg/hr	55.8	0.017	28.1	0.008	0.027
tvCL3	L/kg/hr	49.7	0.22	29.6	0.09	0.35
tvCMultStdev (residual, proportional)	Scalar		0.048	18.5	0.03	0.066
stdev0 (residual, additive)	µg/L		0.249	19.5	0.153	0.346
Secondary parameters						
Half_life_alpha	hr		0.099	26.3	0.047	0.151
Half_life_Beta	hr		1.029	17.2	0.677	1.381
Half_life_Gamma	hr		3.191	20.2	1.91	4.472
Vss (steady-state volume of distribution)	L/kg		0.194	4.8	0.176	0.213
MRT (Mean residence time (IV))	hr		2.768	8.3	2.313	3.223
AUC	µg*hr/mL		288.7	5.8	247.8	311.8
AUMC	µg*hr^2/mL		798.9	11.4	626	980.3

The primary estimated parameters were the volume of distribution of the central compartment (V1), the volume of distribution of the peripheral compartments (V2, V3), the plasma clearance (CL) and the distribution clearances (CL2, CL3). CMultStdev corresponds to the proportional component of the residual error and stdev0 is the additive component of the residual. The estimated fixed parameters were reported as their typical values (tv) with their CV% and their confidence interval that is a measure of the precision of their estimation. Secondary parameters are the half-life of the different phases, the steady-state volume of distribution (Vss), the mean residence time (MRT), area under the curve (AUC) and area under the moment curve (AUMC).

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