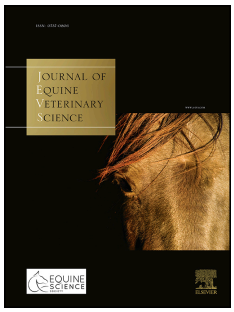
Journal Pre-proof



Plasma And Peritoneal Ceftriaxone Concentrations After Intraperitoneal Administration In Horses With Septic Peritonitis

Juliana de M. Alonso, Evelin S. Martins, Rosangela G. Peccinini, Gustavo S. Rosa, Simony T. Guerra, Márcio G. Ribeiro, Bruna Santos, Henry D.M. García, Marcos J. Watanabe, Regina K. Takahira, Celso A. Rodrigues, Ana Liz G. Alves, Carlos A. Hussni

PII: S0737-0806(20)30401-9

DOI: https://doi.org/10.1016/j.jevs.2020.103310

Reference: YJEVS 103310

To appear in: *Journal of Equine Veterinary Science*

Received Date: 18 May 2020

Revised Date: 28 August 2020

Accepted Date: 16 October 2020

Please cite this article as: Alonso JdM, Martins ES, Peccinini RG, Rosa GS, Guerra ST, Ribeiro MG, Santos B, García HDM, Watanabe MJ, Takahira RK, Rodrigues CA, Alves ALG, Hussni CA, Plasma And Peritoneal Ceftriaxone Concentrations After Intraperitoneal Administration In Horses With Septic Peritonitis, *Journal of Equine Veterinary Science* (2020), doi: https://doi.org/10.1016/j.jevs.2020.103310.

PLASMA AND PERITONEAL CEFTRIAXONE CONCENTRATIONS AFTER INTRAPERITONEAL ADMINISTRATION IN HORSES WITH SEPTIC PERITONITIS

Juliana de M. Alonso[a], Evelin S. Martins[b], Rosangela G. Peccinini[b], Gustavo S. Rosa[a], Simony T. Guerra[c], Márcio G. Ribeiro[c], Bruna Santos[d], Henry D.M. García[a], Marcos J. Watanabe[a], Regina K. Takahira[d], Celso A. Rodrigues[a], Ana Liz G. Alves[a], Carlos A. Hussni[a]*

[a] Department of Veterinary Surgery and Animal Reproduction, School of Veterinary Medicine and Animal Science, Botucatu, Univ. Estadual Paulista (Unesp), Brazil.

[b] Department of Natural Active Principles and Toxicology, School of Pharmaceutical Sciences, Araraquara, Univ. Estadual Paulista (Unesp), Brazil.

[c] Department of Animal Production and Preventive Veterinary Medicine, School of Veterinary Medicine and Animal Science, Botucatu, Univ. Estadual Paulista (Unesp), Brazil.

[d] Department of Veterinary Clinics, School of Veterinary Medicine and Animal Science, Botucatu, Univ. Estadual Paulista (Unesp), Brazil.

* Corresponding author. Tel.: +55 14 38802026.

E-mail address: carlos.hussni@unesp.br (C.A. Hussni).

Juliana M. Alonso: juliana.alonso@unesp.br

Evelin S. Martins: evelin.martins@hotmail.com

Rosangela G. Peccinini: rosangela.peccinini@unesp.br

Gustavo S. Rosa: gustavo.s.rosa@unesp.br

Simony T. Guerra: simony.guerra@unesp.br

Márcio G. Ribeiro: marcio.ribeiro@unesp.br

Bruna Santos: brunasantos.vet@hotmail.com

Henry D.M. García: mogollon.garcia@unesp.br

Marcos J. Watanabe: marcos.jun@unesp.br

Regina K. Takahira: regina.takahira@unesp.br

Celso A. Rodrigues: rodriguesca@fmvz.unesp.br

Ana Liz G. Alves: ana.liz@unesp.br

Carlos A. Hussni: carlos.hussni@unesp.br

35

ABSTRACT

Intraperitoneal ceftriaxone administration in healthy horses results in high and prolonged peritoneal concentrations. Recent findings suggest that intraperitoneal ceftriaxone might increase survival rates in horses affected by peritonitis. The present study aimed to evaluate plasma and peritoneal concentrations of ceftriaxone after intraperitoneal administration in horses with septic peritonitis. Twenty-six horses presenting clinical, laboratorial and sonographic findings compatible with the disease were included. All horses received daily intraperitoneal ceftriaxone (25 mg/kg bwt) in addition or not with other antibiotics and support therapies. High-performance liquid chromatography was used to determine plasma and peritoneal ceftriaxone concentrations before and after 12 and 24 hours of ceftriaxone administration. Mean plasma concentrations 12 and 24 hours after administration were respectively 1.84±0.43 and 0.37±0.07 µg/mL, and mean peritoneal concentrations were 5.7±2.84 and 0.42±0.13 µg/mL. Ceftriaxone concentration was lower in comparison with previous studies in healthy horses, and presented under the MIC for enterobacteria (≤ 1µg / mL) and for *gram* positive isolates (≤ 0.5 µg/mL) at 24 hours. The variation of the results obtained between healthy horses and with septic peritonitis demonstrated that pharmacokinetics/dynamics are different between these patients, and suggests the use of an interval of dose of 12 hours.

Keywords: abdominal infection, HPLC, MIC, peritoneum

INTRODUCTION

Peritonitis presents variable survival rates. It is difficult to compare the therapeutic success rates since there is a wide variation in inclusion criteria between similar studies. In general, studies that include horses without idiopathic peritonitis, as well as cases associated with the gastrointestinal tract and colic surgery remain with high mortality rates in spite of the use of conventional combination of antimicrobial agents and supportive care, demanding the adoption of more effective therapeutic strategies [1-4]. Intraperitoneal administration of antimicrobials is a technique that promotes high peritoneal drug concentrations [5]. Even though this route is routinely used in the treatment of peritonitis in humans [6-9], is not commonly used in horses.

Ceftriaxone is a third-generation cephalosporin with a broad antibacterial spectrum that has a well-established clinical efficacy [10-13]. Its activity is time-dependent and the best therapeutic outcome is obtained using a dosing regimen that provides longer drug concentrations above the minimal inhibitory concentration (MIC) (enterobacteria $\leq 1\mu g$ / mL, and ≤ 0.5 µg/mL for *gram* positive isolates) at the site of infection [14].

Intraperitoneal ceftriaxone administration does not induce peritoneal inflammation [15], results in high and prolonged peritoneal concentrations in healthy horses [16], and is effective in the treatment of peritonitis in horses [17]. Nevertheless, to date, no profile of the plasma and peritoneal concentrations of the ceftriaxone in horses with septic peritonitis is available in the scientific literature. The present study aimed to evaluate plasma and peritoneal concentrations of ceftriaxone after intraperitoneal administration in horses with septic peritonitis.

MATERIALS AND METHODS

The present study was conducted in accordance with the principles of ethics and well-being in animal experimentation and was approved by the Ethics Committee on Animal Use (CEUA) of the School of Veterinary Medicine and Animal Science of UNESP, Brazil (protocol 53/2016).

Animals

The study included horses admitted to the School of Veterinary Medicine and Animal Science of UNESP, Botucatu, São Paulo, Brazil, between 2016 and 2018 with septic peritonitis. A total of 26 horses were included (16 males and 10 females), with mean age of 10.2 ± 6.7 years and mean body weight of 377.4 ± 99.8 kg. The breeds were: 9 Quarter horses, 9 mixed breed, 2 Mangalarga, 1 miniature, 1 Lusitano, 1 Arabian, 1 American Paint,1 Criollo, and 1 Friesian. Animal sampling was convenient, due to the unpredictability of the natural and post-operative cases of infectious peritonitis in horses.

Inclusion criteria

The included horses presented a set of signs that characterized peritonitis: apathy, anorexia, pyrexia, reluctance to move, and presence of an abdominal tension line; peritoneal fluid with nucleated cell count ≥ 10.000/μL [18, 19]; and/or presence of free or phagocytized bacteria; peritoneal fluid protein concentration ≥ 2,5 g/dL and fibrinogen ≥ 100 mg/dL [18-20]; and sonographic findings (thickening of the intestinal wall and increased echogenicity of peritoneal fluid) associated with the diagnosis of septic peritonitis.

In horses included after laparotomy, clinical signs (especially persistent pyrexia) associated with sonographic findings and response to the treatment were used to include the cases, since the surgical procedure increases nucleated cell count and protein concentrations [21].

106

107

108 ***Microbiological culture***

109 Peritoneal fluid was cultured according to Alonso et al. [17], in a defibrinated sheep
110 (5%) and MacConkey agar media. It was maintained under aerobic conditions at 37°C, for 72
111 hours. Simultaneously, an aliquot of the same material was cultured under microaerophilic
112 conditions at 37°C. Further, the peritoneal fluid collected in appropriate media was cultured
113 under anaerobic conditions in defibrinated sheep blood agar (5%) using anaerobic generator
114 (Anaerogen ™, Oxoid, Hampshire, England), maintained for 5-7 days. The microorganisms
115 isolated under aerobic, microaerophilic, and anaerobic conditions were examined based on
116 conventional phenotypic characteristics [22].

117 Susceptibility to ceftriaxone was extrapolated from the *Clinical and Laboratory*
118 *Standards Institute* – CLSI [23], using the values of ceftriaxone MIC for enterobacteria of
119 ≤1µg/mL, and for *gram* positive isolates of ≤ 0.5 µg/mL.

120

121 ***Plasma and peritoneal ceftriaxone concentrations***

122 Sample collection

123 Blood and peritoneal fluid samples were collected into tubes containing ethylene
124 diamine tetra-acetic acid (EDTA). Sample collections were performed before, 12 and 24h
125 after intraperitoneal ceftriaxone administration. The peritoneal fluid samples were collected
126 under sterile conditions from the ventral midline of the abdomen through a 21 G hypodermic
127 needle. Plasma samples were obtained through lateral thoracic vein punction. Both samples
128 were centrifuged (1.087 x g for 10 min), then divided into duplicates and frozen at - 80 °C for
129 subsequent determination of ceftriaxone concentrations.

130

Determination of ceftriaxone concentration

Plasma and peritoneal fluid concentrations of ceftriaxone were determined using high-performance liquid chromatography (HPLC; Waters Alliance System, Milford, MA, USA) equipped with an ultraviolet (UV)-Vis detector [16, 24]. Chromatographic separation was performed using a Waters SunFire C18 column (4.6 x 250 mm; 5 µM) protected by a Waters SunFire C18 guard column (4.6 x 20 mm; 5 µM) maintained at 40 °C. The mobile phase consisted of methanol (mobile phase A) and 20 mM ammonium acetate (mobile phase B) in gradient mode. The selected gradient started with 80% of mobile phase B, which was increased to 20% within 10 min and this composition was maintained for 1 min. The initial conditions were re-established within 2 min and held during 2 min more to assure column equilibration. The total run time was 15 min, with a retention time of 6.15 min and 9.15 min for ceftriaxone and cefoperazone (internal standard), respectively. The flow rate was 0.7 mL/min and the injected sample volume was 20 µL. The UV-Vis detector was operated at 260 nm. The ratio of the analyte peak area to the peak area of the I.S. was used for quantification of the drug [24]. The method validation was conducted in accordance with the (US) FDA Guidance for Industry [25] and Brazilian Health Surveillance Agency [26].

Treatment

Supportive therapy based on fluid and electrolyte replacement, analgesia, and anti-inflammatory drugs was provided at admission. Immediately after confirmation of peritonitis, antibiotic therapy was initiated according to the conduct of the veterinarian responsible for the case. All horses received daily intraperitoneal therapy with ceftriaxone (Eurofarma, São Paulo, Brazil) (25 mg/kg bwt). The most common associated drugs were flunixin meglumine

(1.1 mg/Kg IV or IM SID), gentamicin (6.6 mg/Kg IV SID), amikacin (15 mg/Kg IV SID) and metronidazole (15 mg/Kg IV or PO TID).

Ceftriaxone was administered intraperitoneally in accordance to that described by Alonso et al. [17]. Ceftriaxone dosage (25 mg/Kg bwt) was diluted in 250 mL of warmed 0.9% saline solution. The administration was carried out under pressure within 2 minutes.

Plasma and peritoneal protein concentrations

Plasma and peritoneal fluid protein concentrations were determined using refractometry [27] before and after 24 h of ceftriaxone administration.

Statistical analysis

After assessing normality using the Shapiro Wilk the plasma and peritoneal concentrations of the proteins were compared using analysis of repeated measures over time (Proc Mixed) using the time point as a fixed effect; and comparisons were made using pdiff.

Plasma and peritoneal concentrations of ceftriaxone were analyzed using descriptive statistics. When the results of liquid chromatography were undetectable, the considered value was the limit of detection (0.24µg/mL). The results are shown as mean ± standard error of the mean (SEM), and statistically significant difference was considered when $P < 0.05$.

One horse (Animal 2) that presented outlier ceftriaxone plasma and peritoneal concentrations was excluded of the mean calculation.

RESULTS

Microbiological culture

Among the 26 included horses, 19 (73,1%) showed a productive abdominocentesis at admission. Among these, 13 (68,4%) presented isolation of microorganisms. Only one isolate presented mixed culture, whereas the others, isolated a single agent. Totaling 13 isolates.

Among the isolated bacteria, 7 (50%) were *Escherichia coli*, 4 (28,6%) *Streptococcus spp* and 3 (21,4%) *Staphylococcus spp*.

Ceftriaxone concentration

Peritoneal fluid collection was productive at 12 and 24 hours after administration to 57,7% (15/26 horses). At the time point 12 hours of administration all samples had concentrations above the detection limit of the test (0.24 µg/ mL), and the mean peritoneal fluid ceftriaxone concentration obtained was 5.7±2.84 µg/mL. After 24 hours, 53,3% (8/15 horses) had undetectable peritoneal concentrations (<0.24 µg/mL). Among the detectable concentrations, a mean of 0.42±0.13 µg/mL was obtained.

All horses had detectable plasma concentrations at 12 hours, with mean concentration of 1.84±0.43 µg/mL. At 24 hours, 50% (13/26 horses) did not show detectable plasma concentrations. The mean obtained concentration was 0.37±0.07 µg/mL.

Plasma and peritoneal protein concentration

No differences were obtained between admission (plasma: 6,57 ± 0,19; peritoneal: 4,10 ± 0,43 g/dL) and the time point 24 hours after ceftriaxone administration for plasma (6,83 ± 0,20 g/dL) and peritoneal (3,26 ± 0,49 g/dL) protein concentrations.

DISCUSSION

This is the first report that describes both plasma and peritoneal concentrations of ceftriaxone after intraperitoneal administration in horses with septic peritonitis. Recently, using the same included animals that were used in this study, our group [17] described favorable results using the adjuvant intraperitoneal ceftriaxone (25 mg/kg bwt) every 24 hours in the treatment of peritonitis in horses, with an average survival rate of 77%. It is interesting

206 to emphasize that Alonso et al. [17] included peritonitis cases secondary or not to
207 gastrointestinal tract injuries and abdominal surgery, and secondary to intestinal rupture
208 and/or faeces contamination. Currently, when reporting survival rates associated with
209 peritonitis, the variation in inclusion criteria between similar studies makes it difficult to
210 compare therapeutic success rates. In many reports with higher survival rates, peritonitis is not
211 related to the gastrointestinal tract, peritonitis cases associated with gastrointestinal tract, colic
212 surgery and intestinal perforations are excluded [2,28-31]. In Alonso et al study [17], if the
213 group of animals that presented intestinal perforation was excluded, the survival rate would
214 have achieved 86,05%. Despite the slow advance in the treatment of peritonitis, in general,
215 studies with wider inclusion criteria remains with high mortality rates with the use of
216 conventional combination of antimicrobials [1,3,4].

217 As this is a clinical study, it was difficult to standardize the approach and diagnosis of
218 peritonitis, which varied according to the etiology of the disease and the individual
219 manifestation of the cases. Possibly the degree of peritoneal inflammation and the dynamics
220 of diffusion and elimination of the drug varied according to the peritoneal lesion intensity,
221 which is a limitation of this study. Also, animals that presented peritonitis in the postoperative
222 time were not included immediately after surgery, but in the clinical manifestation of the
223 disease, with possible no influence of intraoperative abdominal lavage in ceftriaxone
224 concentrations, since the peritoneum is capable of absorbing large quantities of liquid in one
225 or two days [32]. The peritonitis aetiology, individual plasma and peritoneal ceftriaxone
226 concentrations and outcome are presented in table 1.

227 In order to provide optimal antimicrobial therapy to the diseased horses, three aspects
228 have to be considered: pharmacokinetics-PK (relative solubility, protein binding, clearance
229 mechanism); clinical information (site of infection, severity of illness, body composition,

likely pathogens, local resistance patterns); and pharmacodynamics-PD (time course of bacterial killing, post antibiotic effect-PAE, preclinical infection models) [33-34].

Ceftriaxone is a hydrophilic drug that has high levels of binding to proteins and renal clearance, [10,11,16,35,36]. Regarding the clinical aspects, the site of infection was the abdominal cavity. However, the severity of the illness and body composition varied between patients, and the groups of pathogens were *Enterobacteria*, *Streptococci* and *Staphylococci*. Among PD aspects, the time course of bacterial killing is highlighted, since β-lactams is a class of time-dependent antibiotics, whereby substantially increasing drug concentrations have minimal effects on the overall rate and extent of bacterial killing. Instead, maintaining a free drug concentration above the MIC of the organism for a portion of the dosing interval has been shown to best predict microbiological efficacy. The percentage of time that free drug remains above the MIC over a 24-hour period ($fT \geq _{MIC}$) to provide maximal bactericidal effect is a PD index that varies according to β-lactam subclass, with typical values of ≥ 60% to 70% for cephalosporins acting against *Enterobacteria* and *Streptococci.* whereas $fT \geq _{MIC}$ of 40-50% against *Staphylococci* species [34,37].

Comparing the plasma concentrations between horses with septic peritonitis and healthy horses [16] after daily intraperitoneal ceftriaxone administration (25 mg/kg), both studies showed mean plasma concentrations above the estimated MIC 12 hours after administration, and values below the MIC 24 hours after intraperitoneal administration. It is noteworthy, however, that plasma concentrations of the horses with peritonitis were lower than those obtained in healthy horses [16] 24 hours after intraperitoneal administration.

Regarding peritoneal concentrations, healthy horses that received intraperitoneal ceftriaxone maintained concentrations above the MIC for 24 hours [14], while in the present study, the mean ceftriaxone peritoneal concentration was above the MIC 12 hours after administration and below the MIC at the time point 24 hours. Peritoneal and plasma

concentrations of the horses with peritonitis were lower than those obtained in healthy horses [16] at both times of evaluation.

It is believed that there was a higher drug elimination rate due to the increase in vascular permeability secondary to inflammation/infection [10]. Additionally, due to the fact that the presence of infection results in significant fluid extravasation and in a high probability of augmented renal clearance, standard therapy that include volume resuscitation can further alter antibiotic PK, with hydrophilic, renally cleared agents as β-lactams being most susceptible [26, 30].

The increased vascular permeability may also have resulted in superior diffusion/distribution of the drug to the abdominal tissues affected by the septic process. In this sense, the favorable outcome obtained in the treatment [17] may have resulted from the more pronounced absorption by the tissues and binding of ceftriaxone to bacteria. However, this study did not evaluate the concentrations of the drug in the tissues nor the PK of the drug to access distribution.

Since ceftriaxone binds to proteins and has a fixed and free form concentration dependent [11,13,36], its binding may have contributed to the minor availability of free drug and consequent lower concentrations in horses with peritonitis, as these horses have higher concentrations of proteins in the peritoneal fluid (4.10 ± 0.43 g/dL) compared to healthy horses (<2.3 g/dL).

The optimal antimicrobial concentration in the tissues and blood is poorly understood, often defined as the concentration above the MIC. However, there are many examples of effective prophylaxis with antibiotic concentrations below the MIC and failures of prophylaxis with concentrations above the MIC [37].

The variation in the results obtained between healthy horses [16] and horses with septic peritonitis demonstrates that PK/PD are different between these conditions. The

undetectable concentrations after 24 hours of administration suggests the necessity to change the dose regimen, with administration taking place every 12 hours; or the conducting of new tests using higher doses with a 24 hours interval of doses. It is also important to point out that a high survival rate was achieved by Alonso et al. [17], despite the lower concentrations achieved with the 25 mg/kg bwt dose regimen every 24 hours in this study.

The estimative of the $fT \geq_{MIC}$ was hampered as a result of the limited sample collection for ceftriaxone concentrations evaluation at two isolated time points (12 and 24 hours). Since there is no time point of evaluation between 12 and 24 hours, it is not possible to know if ceftriaxone presented a $fT \geq_{MIC}$ of 60-70%, necessary for cephalosporins to be effective against *Enterobacteria* and *Streptococci* species [34,37]. Based in the present results it is possible to ensure a $fT \geq_{MIC}$ of 50%, a compatible $fT \geq_{MIC}$ necessary to suppress *Staphylococcus* induced infections. It is suggested, based on the high survival rates that the target was accomplished.

Human antibiotic therapy is guided by the Clinical & Laboratory Standards Institute (CLSI), which provides information and standardization of the MIC for the most common used antimicrobials. It is also important to highlight that there are no available studies about the MIC values and the necessary $fT \geq_{MIC}$ to control peritoneal infection in horses. Thus, veterinary antibiotic therapy is based on human guidelines. It is described that *Escherichia coli*, one of the potential causes of peritonitis in horses, has a MIC of 0.003mg/mL in cats [13] and cattle [39]. However, in the absence of a specific MIC for the main etiological agents of peritonitis in horses, the most widespread and highest value for the group of Enterobacteriaceae (1mg/mL) was selected as the baseline MIC in this study (CLSI), situation that based in the high survival rate, could under estimated the $fT \geq_{MIC.}$

Another possible situation that may have contributed to the outcome of the horses is that

despite the fact that ceftriaxone presents a short PAE (nearly 1 hour for gram negatives and 2 hours for gram positives), it was administered in association with aminoglycosides in most of the presented cases, situation that results in synergism and prolonged PAE (2 to 6 hours) [10,34,40,41].

The main limitations of this study were the lack of time points of evaluation, and the fact that ceftriaxone was used in association with other antibiotics, anti-inflammatory drugs and parenteral solutions, since horses sampled are cases naturally infected. Although no combinations of compounds capable of interaction with ceftriaxone [42] were used, the effect of this associations is unknown, and there are no studies in horses about drug interaction with ceftriaxone. This association also hampered the assessment of the survival rates and the determination of the ceftriaxone effectiveness separately.

CONCLUSIONS

Intraperitoneal ceftriaxone administration (25 mg/Kg bwt) in horses with septic peritonitis resulted in lower ceftriaxone plasma and peritoneal concentrations compared to previous studies in healthy horses. At the time point of 12 and 24 h, ceftriaxone concentrations were respectively above and under the MIC for the most prevalent bacteria related to peritonitis. The variation of the results obtained between healthy and diseased horses demonstrated that pharmacokinetics/dynamics are different between these conditions. Even though previous studies demonstrated that administration of ceftriaxone (25 mg/Kg bwt) using the interval of doses of 24 hours is effective for the treatment of peritonitis in horses, our data suggest that the dose interval may be switched to twice a day.

CONFLICT OF INTEREST

None of the authors has any financial or personal relationships that could

inappropriately influence or bias the content of the paper.

FUNDING SOURCES

This study was supported by São Paulo Research Foundation (Grant Number # 2016/08713-8).

REFERENCES

[1] Browning A. Diagnosis and management of peritonitis in horses. In Practice 2005; 27: 70-75.

[2] Nógrádi N, Tóth B, Macgillivray KC. Peritonitis in horses: 55 cases (2004–2007). Acta Vet Hung 2011;59(2):181-93.

[3] Dyson S. Review of 30 cases of peritonitis in the horse. Eq Vet J 1983; 15:25-30.

[4] Mair TS, Hillyer MH, Taylor FG. Peritonitis in adult horses: a review of 21 cases. Vet Rec 1990;126: 567-570.

[5] Varghese JM, Roberts JA, Wallis SC, Boots RJ, Healy H, Fassett RG, Lipman J, Ranganathan D. Pharmacokinetics of intraperitoneal gentamicin in peritoneal dialysis patients with peritonitis (GIPD study). Clin J Am Soc Nephrol 2012; 7: 1249-1256.

[6] Wiggins KJ, Johnson DW, Craig JC, Strippoli GF. Treatment of peritoneal dialysis-associated peritonitis: a systematic review of randomized controlled trials. Am J Kidney Dis 2007; 50: 967-988.

[7] De Vin F, Rutherford P, Faict D. Intraperitoneal administration of drugs in peritoneal dialysis patients: a review of compatibility and guidance for clinical use. Perit Dial Int 2009; 29: 5-15.

[8] Taegtmeyer AB, Konowa N, Fasel D, Haschke M, Burkehalter F. Successful treatment of a pacemaker infection with intraperitoneal daptomycin. Perit Dial Int 2016; 36: 114-117.

[9] Hesame MA, Alipour H, Daylami HN, Alipour B, Bazargan-Hejazi S, Ahmadi A. Irrigation of abdomen with imipenem solution decreases surgical site infections in patients with perforated appendicitis: a randomized clinical trial. Iran Red Crescent Med J 2014; 16: e12732.

360[10] Ringger NC, Pearson EG, Gronwall R. Pharmacokinetics of ceftriaxone in healthy horses.
361 Eq Vet J 1996; 28: 476-479.
362[11] Lamb HM, Ormrod D, Scott LJ, Figgitt DP. Ceftriaxone: an update of its use in the
363 management of community-acquired and nosocomial infections. Drugs 2002; 62: 1041–
364 1089
365[12] Bijie H, Kulpradist S, Manalaysay M, Soebandrio A. In vitro activity, pharmacokinetics,
366 clinical efficacy, safety and pharmacoeconomics of ceftriaxone, compared with third and
367 fourth generation cephalosporins: review. J Chemoth 2005; 17(1): 3-24.
368[13] Albarellos GA, Kreil VE, Landoni MF. Pharmacokinetics of ceftriaxone after intravenous,
369 intramuscular and subcutaneous administration to domestic cats. J Vet Pharmacol Ther
370 2007; 30:345–352.
371[14] Levison ME, Levison JH. Pharmacokinetics and Pharmacodynamics of Antibacterial
372 Agents. Clin Infect Dis 2009; 23:791–815.
373[15] Alonso JM, Nitta TY, Akutagawa TYM, Crescencio AP, Takahira RK, Santos B, Alves
374 ALG, Watanabe MJ, Rodrigues CA, Peccinini RG, Campos ML, Hussni CA. Evaluation
375 of *Tenckhoff* catheter use and ceftriaxone intraperitoneal administration in horses. J Eq
376 Vet Sci 2017; 53: 55-63.
377[16] Alonso JM, Peccinini RG, Campos ML, Nitta TY, Akutagawa TYM, Crescencio AP, Alves
378 ALG, Watanabe MJ, Rodrigues CA, Hussni CA. Plasma and peritoneal fluid
379 concentrations of ceftriaxone after intravenous and intraperitoneal administration in
380 horses. The Vet J 2018; 234: 72-76.
381[17] Alonso JM, Rosa GS, Santos B, Guerra S, Ribeiro MG, Watanabe MJ, Alves ALG,
382 Rodrigues CA, Takahira RK, Hussni CA. Adjuvant intraperitoneal ceftriaxone in the
383 treatment of septic peritonitis in horses. Vet Rec, 2020; vr.105570.
384[18] Murray P. Peritonite. In: Medicina interna equina, Reed SM, Bayly WM, Guanabara
385 Koogan, Rio de Janeiro, 2000; 941-949.
386[19] Davis JL. Treatment of peritonitis. Vet Clin N Am Eq Pract 2003;19: 765-78.
387[20] Van Hoogmoed L, Rodger LD, Spier SJ, Gardner IA, Yarbrough TB, Snyder JR. Evaluation
388 of peritoneal fluid pH, glucose concentration and lactate dehydrogenase activity for
389 detection of septic peritonitis in horses. J Am Vet Med Ass 1999; 241: 1032-1036.
390[21] Sapper C, Gerhards H. Examination of peritoneal fluid after diagnostic and therapeutic
391 laparotomies in horses. Pferdeheilkunde Eq Med 2005;21(1):20-8.
392[22] Quinn PJ. Microbiologia Veterinária e Doenças Infecciosas. Lippincott Williams & Wilkins,
393 Philadelphia, PA, USA. 2001.

394[23] CLSI - Clinical and Laboratory Standards Institute (NCCLS), 2014. Performance Standards
395 for Antimicrobial Susceptibility Testing: Twenty-Second Informational Supplement
396 M100-S24. CLSI, Wayne, PA, USA.
397[24] Campos ML, Alonso JM, Martins ES, Oliveira JA, Hussni CA, Peccinini RG. Ceftriaxone
398 pharmacokinetics by new simple and sensitive ultra-high-performance liquid
399 chromatography method. Diagn Microbiol Infect Dis 2017
400[25] Food and Drug Administration (FDA), 2001. Bioanalytical method validation. In Rockville:
401 U.S.: Food and Drug Administration (FDA) Center for Drug Evaluation and Research
402 (CDER) Center for Veterinary Medicine (CVM).
403[26] National Sanitary Inspection Agency (ANVISA); Guide for validation of bioanalytical
404 method: Resolution n◦ 27, São Paulo, Brazil, (May 17, 2012), Accessed April 2016.
405[27] Allison WA. Avaliação laboratorial das proteínas do plasma e do soro sanguíneo. In: Thrall,
406 M.A. (Ed.), Hematologia e Bioquímica Clínica Veterinária, 2ª edição. Guanabara Koogan,
407 Rio de Janeiro, 2015; pp. 979-1010.
408[28] Matthews S, Dart A, Dowling B, Hodgson J, Hodgson D. Peritonitis associated with
409 *Actinobacillus equuli* in horses: 51 cases. Aust Vet J 2001;79: 536-539.
410[29] Henderson ISF, Mair TS, Keen JA, Shaw DJ, McGorum BC. Study of the short- and long-
411 term outcomes of 65 horses with peritonitis. Vet Rec. 2008;163:293–7.
412[30] Odelros E, Kendall A, Hedberg-Alm Y, Pringle J. Idiopathic peritonitis in horses: a
413 retrospective study of 130 cases in Sweden (2002–2017). Acta Vet Scand 2019;61:18.
414[31] Javsicas LH, Giguère S, Freeman DE, Rodgerson DH, Slovis NM. Comparison of Surgical
415 and Medical Treatment of 49 Postpartum Mares with Presumptive or Confirmed Uterine
416 Tears. Vet Surg 2010;39:254-260.
417[32] Alonso JM, Alves AL, Watanabe MJ, Rodrigues CA, Hussni CA. Peritoneal response to
418 abdominal surgery: the role of equine abdominal adhesions and current prophylactic
419 strategies. Vet Med Int. 2014;2014:279730.
420[33] Giguère S, Prescott JF, Baggot JD. Terapia Antimicrobiana em medicina veterinária. Roca,
421 São Paulo, 2010;683.
422[33] Onufrak NJ, Forrest A, Gonzalez D. Pharmacokinetic and Pharmacodynamic Principles of
423 Anti-infective Dosing. Clin Therapeut 2016;38(9): 1930–1947.
424[34] Ringger NC, Brown MP, Kohlepp SJ, Growall RR, Merrit K. Pharmacokinetics of
425 ceftriaxone in neonatal foals. Eq Vet J 1998; 30: 163-165.
426[35] Sun H, Chow MS, Maderazo EG. Characteristics of ceftriaxone binding to immunoglobulin
427 G and potential clinical significance. Antimicrob Agents Chemother 1991; 35, 2232-2237.

428[36] Craig WA. Interrelationship between pharmacokinetics and pharmacodynamics in
429 determining dosage regimens for broad-spectrum cephalosporins. Diagn Microbiol Infect
430 Dis 1995; 22: 89–96.
431[37] Martin C, Ragni J, Lokiec F, Guillen JC, Auge A, Pecking M, Gouin F. Pharmacokinetics
432 and tissue penetration of a single dose of ceftriaxone (1,000 milligrams intravenously) for
433 antibiotic prophylaxis in thoracic surgery. Antimicrob Agents Chemother 1992;36(12):
434 2804-2807.
435[38] Soback S, Ziv G. Pharmacokinetics and bioavailability of ceftriaxone administered
436 intravenously and intramuscularly to calves. Am J Vet Res 1988;49;535–538.
437[39] Craig WA. The postantibiotic effect. Clin Microbiol Newsl 1991; 13, 121–124.
438[40] Craig WA. Post-antibiotic effects inexperimental infection models: relationship to in-vitro
439 phenomena and to treatment of infections in man. J Antimicrob Chemother 1993;31(Suppl
440 D):149–158.
441[41] Hardman JG, Limbird LE (editors). Goodman & Gilman: the pharmacological basis of
442 therapeutics. 10th ed. New York: McGraw-Hill; 2001
443

Table 1

Peritonitis aetiology, plasma and peritoneal ceftriaxone concentrations 12 and 24 hours after ceftriaxone administration and outcome of the horses with septic peritonitis and treated with intraperitoneal ceftriaxone (n=26). Botucatu, SP, Brazil, 2016-2018.

Animal	Peritonitis aetiology	Plasma ceftriaxone concentrations (µg/mL)		Peritoneal ceftriaxone concentrations (µg/mL)		Outcome
		12h	24h	12h	24h	
1	Colitis	0,408	U	UC	U	Discharged
2**	Sand impaction	1,457	15,720	47,578	179,635	Discharged
3	Sand impaction	0,339	U	0,508	U	Discharged
4	Sand impaction and gastric ulceration	1,667	0,249	2,031	0,280	Discharged
5	Fecalith removal*	0,343	NC	0,187	NC	Died
6	Correction of colon displacement*	4,010	0,564	UC	IV	Discharged
7	Correction of colon displacement*	2,182	0,269	UC	UC	Discharged
8	Correction of colon displacement*	2,084	0,487	UC	UC	Discharged
9	Jejunal granulomas excision*	1,724	U	UC	UC	Discharged
10	Surgical cecal impaction*	0,242	U	UC	UC	Discharged
11	Surgical cecal impaction*	2,186	0,643	UC	UC	Discharged
12	Surgical sand impaction*	0,393	U	UC	UC	Discharged
13	Surgical sand impaction*	1,241	U	1,495	U	Discharged
14	Abdominal wall abscess	1,518	0,519	1,391	0,209	Died
15	Funiculitis	0,337	0,276	0,391	U	Discharged
16	Vaginal rupture	2,321	U	3,761	0,198	Discharged
17	Abdominal perforation	1,160	U	26,110	0,508	Discharged
18	Umbilical herniorrhaphy*	1,260	U	1,035	U	Discharged
19	Umbilical herniorrhaphy*	1,738	U	2,200	U	Discharged
20	Incisional herniorrhaphy*	1,112	U	UC	UC	Discharged
21	Removal of a vesical urolithiasis*	2,804	U	UC	IV	Discharged
22	Grade IV rectal rupture	0,295	U	1,063	0,780	Discharged
23	Small intestine perforation secondary to a foreign body*	2,602	0,391	0,730	U	Died
24	Jejuno-jejunal anastomoses failure*	NC	NC	NC	NC	Euthanised
25	Tiflostomy dehiscence*	10,664	1,926	34,660	2,091	*Euthanised*
26	Jejunal perforation*	1,469	0,283	4,839	U	Discharged

*Horses subjected to surgery; **outlier (excluded of the ceftriaxone concentrations analysis); UC=unproductive collection; NC=Not collected (animal died); U=Undetectable (<0,24µg/mL); IV=Insuficient volume for analysis.

Highlights

- Peritonitis remains with high mortality rates in horses
- Intraperitoneal ceftriaxone administration in healthy horses results in high and prolonged peritoneal concentrations
- Intraperitoneal ceftriaxone might increase survival rates in horses
- No profile of the plasmatic and peritoneal concentrations of the ceftriaxone in horses with septic peritonitis is available