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Enrofloxacin-based therapeutic strategy for the prevention of endometritis in susceptible mares

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Enrofloxacin (EFX) is often used empirically to prevent uterine infections in mares in order to improve efficiency on Commercial Embryo Transfer Farms. This study investigated the uterine distribution of EFX and its metabolite ciprofloxacin (CFX) in mares and assessed the minimal inhibitory concentrations (MIC) of EFX against various common pathogens as a basis for establishing a rational dosing schedule. Plasma and uterine pharmacokinetic (PK) studies were performed in two groups (n = 5) of healthy mares following intravenous (i.v.) administration of EFX at either 2.5 and at 5 mg/kg bodyweight. Plasma and endometrial tissue samples, taken before for up to 48 h after treatment were analysed by Reverse Phase HPLC. *MIC* values for wild strains of Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive bacteria (β-haemolytic streptococci) ranged from 0.25–2 and 1.5–3.0 μg/mL respectively. In terms of tissue distribution, the sum of the endometrial concentrations of the parent drug (EFX) and its active metabolite (CFX) (in terms of *AUC*), exceeded those in plasma by 249% and 941% following administration of EFX at 2.5 and 5 mg/kg respectively. After i.v. treatment with EFX at 5 mg/kg, endometrial concentrations of EFX and CFX above the *MIC* value were detected for 36–48 and 22–43 h posttreatment for Gram-negative and -positive isolates respectively. Concentrations above *MIC* were maintained for much shorter periods at the lower (2.5 mg/kg) treatment dose. Based on these results, a conventional dose (5 mg/kg) of EFX given prebreeding followed by two further doses at 36–48 h postbreeding are proposed as a rational strategy for using of EFX as a preventative therapy against a variety of common bacterial strains associated with equine endometritis.

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INTRODUCTION

Embryo transfer is a management technique used to facilitate the production of foals from older breeding mares suffering persistent chronic or non-responsive, mating-induced endometritis and/or repeated early embryonic death or abortion (Hurtgen, 2006). On Commercial Embryo Transfer Farms (CETFs) decreasing of fertility in older mares is often a based on their inability to eliminate the uterine fluid accumulation after breeding, and the adherence of bacteria resulting in the development of chronic bacterial endometritis (Watson, 2000). Moreover, practices such as repeated examinations and repeated breeding can lead to uterine contamination such mares, although this problem can be alleviated by using a meticulous preventive management programme. Several therapeutic strategies have been developed to reduce the postbreeding infection in mares susceptible to endometritis. These include prebreeding and postbreeding uterine lavages, the administration of oxytocin and prostaglandin to improve the clearance of accumulated fluid and the local use of antibiotics to prevent infections. However, the administration of treatments, including ecbolic drugs, by the intrauterine route are of limited use during diestrus. One alternative therapeutic strategy to improve breeding efficiency in CETFs and prevent uterine infections in mares is to administer antimicrobial drugs intravenously.

The normal uterus of healthy mares is usually able to eliminate bacterial contamination and detritus introduced during examination, breeding or foaling with a transient innate inflammation (acute phase response) throughout the open cervix or lymphatic drainage. However, older, multiparous mares often require special attention and management because their innate natural defence mechanisms are compromised with uterine contamination in these susceptible mares often leading to infections (Asbury *et al.*, 1982; Blue *et al.*, 1982; Ricketts, 1987; Brinsko *et al.*, 1990; Causey, 2005).

The prophylactic use of antibiotics as a treatment for endometritis, either before breeding or in semen extenders, has long been routine practice on stud farms (Albihn *et al.*, 2003). Recently, the use of fluoroquinolone antibiotics has offered an alternative to established veterinary therapy. Enrofloxacin (EFX) (a second generation fluoroquinolone with broad spectrum activity against Gram-negative and -positive bacteria) has been adopted by clinical practitioners as a common empirical treatment strategy in Argentinian CETFs. Because of its high lipid solubility and low protein binding capacity, EFX has a large volume of distribution, which provides high drug concentrations in many tissues and body fluids (McKellar *et al.*, 2004) with tissue concentrations often being higher that concurrent serum concentrations (Prescott & Baggot, 1994). Interestingly, empirical clinical evidence indicated that pregnancy rates were higher in susceptible healthy mares following i.v. dosing with EFX (5 mg/kg) for 5 consecutive days. This therapeutic regimen is identical to that used for treating severe infections and the irrational use of EFX at such high doses for preventative purposes could, therefore, contribute to the development of drug resistance in the most common pathogenic bacteria in the uterus.

To date, there is no information on the uterine distribution of EFX in mares, so this study was designed to investigate (i) the relationship between the plasma disposition kinetics and endometrial concentrations of EFX given intravenously at two doses (2.5 and 5 mg/kg), (ii) assess the uterine distribution of EFX its *MIC* against a range of common uterine pathogens, with the overall purpose of proposing a rational dosing strategy.

MATERIAL AND METHODS

Determination of MIC values: microdilution antimicrobial susceptibility tests

International Collection (ATCC, Maryland, MD, USA) and Gram-positive and -negative field bacterial strains were used in this trial. *MIC* was assessed by a microdilution method based on the protocol established by the Clinical and Laboratory Standards Institute (CLSI, 2006). *Escherichia coli*, ATCC 25922, and *Streptococcus pneumoniae*, ATCC 49619 were used as quality control strains. Clinical strains were provided by differential culture of *E. coli*, *Pseudomonas aeruginosa* and β-haemolytic streptococci derived from field isolates from mares suffering endometritis. Isolates of from susceptible, but infection-free, mares were also used to evaluate the comparative drug sensitivity of various common pathogenic bacterial strains. Antimicrobial working solutions were applied to microtiter plates under sterile conditions and dried for 24–48 h at 37 °C. Final drug concentration ranges for EFX and CFX were 0.28–8.00 and 0.03–1.00 μg/mL for testing Gram-positive and -negative bacteria respectively. Bacterial suspensions, equivalent to a 0.5 McFarland standard (1.5×10^8 CFU/mL) were prepared from colonies grown during 24 h incubations on tryptic-soy agar plates (Lab. Britania, Buenos Aires, Argentina) for *E. coli* and *P. aeruginosa*, and 5% sheep blood agar for β-haemolytic streptococci. The broth microdilution method was based on a final volume of 200 μL of Mueller Hinton broth (Lab. Britania) with a 50 μL of inoculum of bacterial suspension diluted to a final concentration of 5×10^4 CFU/mL in Mueller Hinton broth. Lysed horse blood (5%) was also added to the broth for testing Gram-positive strains. Microplates were sealed then incubated at 35 °C before reading after 16–20 h. The *MIC* was defined as the lowest antimicrobial concentration at which, after the designated incubation period, no visible bacterial growth was observed.

Animals, treatments and sampling

Ten cross-breeding mares (418 ± 45 kg) ranging from 12 to 16 years old, were used for this study. All the mares were assessed to be clinically healthy after ultrasound, histological and bacteriological examination revealed no evidence of inflammation and negative bacteriological culture. Samples of plasma and endometrial tissue biopsy were taken before treatment as control blanks. In a parallel experimental design, the mares were allocated into two groups ($n = 5$) which were treated as follows: *Group I:* Mares were treated with an EFX solution (Baytril, 5%, Bayer) by the i.v. route at 2.5 mg/kg. *Group II*: Mares were given with the same EFX formulation by the same administration route but at a higher dose of 5.0 mg/kg. Blood samples were collected into heparinized vacutainers at 0.083, 0.25, 0.5, 1, 2, 4, 6, 12, 24, 36 and 48 h posttreatment by jugular venepuncture (using the vein opposite to that used for EFX administration). Blood samples were centrifuged at 2000 ***g*** for 10 min, and the resultant plasma collected and frozen until HPLC analysis. Endometrial tissue biopsy samples were collected at 1, 3, 6, 9, 12, 24 and 48 h post treatment. Immediately after collection these samples were washed with saline solution water, then wrapped in aluminium foil and kept frozen at −20 °C until analysis. All sampling procedures were approved by The Animal Welfare Committee of The Faculty of Veterinary Medicine, http://www.vet.unicen.edu.ar.

Chromatographic analysis for EFX and CFX

Enrofloxacin and its metabolite CFX were analysed in plasma and endometrial tissues by Reverse Phase High Pressure Liquid Chromatography (RP-HPLC). Chromatography was performed on a Shimadzu (Shimadzu Corporation, Kyoto, Japan) LC system comprising a LC-10AS liquid chromatograph pump with a RF-10A spectrofluorometric detector, a CTO-10A VP column oven (set at 30 °C) and a Communications Bus Module-101. Data

were collected and analysed using the Shimadzu Class LC10 software (SPD-10A; Shimadzu Corporation, Kyoto, Japan) package. Samples were injected manually in a 100 μL injection loop. A Prodigy, 5 μM particle size, 250 mm × 4.6 nm C_{18} column (Phenomenex®, Torrance, CA, USA) was used for separation. Plasma extracts were eluted at a flow rate of 1.2 mL/min, using a mobile phase containing 16% acetonitrile (ACN):methanol (13:1 v/v) and 84% water to which 0.4% triethylamine and 0.4% phosphoric acid (85%) (pH 2.5 adjusted to with phosphoric acid) were also added. For the elution of endometrial tissue extracts, methanol was omitted from the mobile phase and the ACN concentration was modified to 14% the ACN. All analytes were detected by fluorescence at excitation and emission wavelengths of 294 and 500 nm, respectively, according to the methods described by González *et al.*, 2006.

Sample preparation

Plasma

For the routine analysis of EFX and CFX, plasma aliquots (1 mL) were placed into 5 mL glass tubes and 10 μL of marbofloxacin (MFX) (100 μg/mL) were added as internal standard (IS). Before HPLC analysis, the analytes and IS were partially prepurified by solid phase extraction on disposable C^{18} cartridges which had been preconditioned with 0.5 mL methanol, followed by 0.5 mL deionized/distilled water. Samples were applied to the cartridges, which were then washed with 3 mL deionized/distilled water and, after allowing as much liquid as possible to drain off, finally eluted with 2 mL of methanol. Eluants were collected in 5 mL glass tubes and evaporated to dryness under vacuum (Speed-Vac®, Savant, Los Angeles, CA, USA) at 40 °C. Dried extracts were reconstituted in mobile phase (2.4 mL) and mixed by vortexing for approximately 30 sec. Aliquots of 100 μL of the reconstituted extracts were injected directly into the chromatographic system for analysis. The Limit of Quantification (LOQ) for both EFX and CFX in plasma was 0.005 μg/mL as determined in the previously published method validation (González *et al.*, 2006).

Endometrial tissue

Endometrial tissue samples (150 mg) were carefully minced and then 37.5 μL of danofloxacin (DFX, 1 μg/mL) was added as IS, followed by 350 μL phosphate buffer (pH 7). Samples were extracted in 1 mL ACN. After stirring for 10 min at room temperature (multi-tube vortexer), samples were centrifuged at 2500 ***g***, for 10 min at 5 °C. The resulting supernatant was transferred into a glass tube and the extraction process was then repeated. The supernatants from the two extractions were combined and evaporated to dryness under vacuum as described above for the plasma extracts. Dried extracts were reconstituted in 1 mL methanol:phosphate buffer (pH 7.0) (20:80) and prepurified by solid phase extraction, also as described above for plasma with the exception that cartidges were washed with 10 volumes of deionized/distilled water before elution. The LOQ for both analytes (again as estimated in the previously reported method validation González *et al.*, 2006) in endometrial tissue was 0.05 μg/g.

Plasma PK analysis

Concentration vs. time curves for EFX and/or its metabolite, CFX, in plasma and endometrial tissue for each individual animal, after the different treatments, were fitted using commercial software (PK Solution 2.0; Summit Research Services, Ashland, OH, USA). Data points generated for the parent drug (EFX) in plasma after i.v. administration were best fitted to a two compartment model: $Cp = Ae^{-\lambda 1t} + Be^{-\lambda 2t}$, where A and B were the primary and secondary disposition intercepts, respectively; $\lambda 1$ and $\lambda 2$ were the primary and secondary disposition rate constants (per h), respectively and Cp was the plasma concentration of EFX at time t (μg/mL). The distribution and elimination half-lives were calculated as $\ln_2$ divided by the rate constants. The estimated plasma concentration of EFX at time zero (Cp_0) after i.v. administration was the sum of the extrapolated zero-time concentrations of the coefficients A and B. Total body clearance (Cl_B) was calculated as Cl_B = Dose/AUC. The volume of distribution (Vd_{area}) was estimated by the following equation: Vd_{area} = Dose/(AUC) (β). The comparative concentration vs. time curves for the major metabolite (CFX) were best-fitted to a biexponential equation as follows: $Cp = Be^{-\lambda 2t} - Be^{-\lambda 1t}$ (see Notari, 1987).

Endometrial tissue PK analysis

The following equation (Notari, 1987) was used to describe bi-exponential concentration–time curves for EFX and CFX in endometrial tissue after the i.v. treatment:

$$Cp = Be^{-\lambda_2 t} - Be^{-\lambda_1 t}$$

where: Cp = endometrial concentration at time t after administration (μg/mL); B = concentration at time zero extrapolated from the elimination phase (μg/g); e = base of the natural logarithm; λ_2 = terminal slope (per h) and λ_1 is the slope obtained by feathering which represents either the first order absorption rate constant (λ_1) or first order metabolite formation rate constant (λ_{for}) (per h). The peak concentration (C_{max}) and time to peak concentration (T_{max}) were displayed from the plotted concentration–time curve of each analyte. The area under the concentration time-curve (AUC) was calculated by the trapezoidal rule (Gibaldi & Perrier, 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (λ_1).

Statistical analysis

Pharmacokinetic parameters and concentration data are reported as mean ± SD. The Mann–Whitney Test was used to compare selected PK parameters. A value of $P < 0.05$ was considered statistically significant.

RESULTS

The *MIC* values for EFX (effectively representing the sum of the activities of the parent drug, EFX, and its biologically active metabolite, CFX) against Gram-negative strains (*E. coli*,

P. aeruginosa) and Gram-positive (β-haemolytic) strains ranged from 0.25–2.0 and 1.5–3.0 μg/mL respectively. No adverse effects were observed in any of the mares after the i.v. administration of EFX at either 2.5 and 5.0 mg/kg bodyweight.

Plasma PK parameters obtained for both analytes after both treatments are reported in Table 1. Both EFX and CFX were detected in plasma for up to 48 h posttreatment following i.v. administration of the parent drug (EFX) at either 2.5 and 5.0 mg/kg (Fig. 1). However, no statistical differences were observed when the Cp0 values obtained after both treatments were compared (Table 1). EFX and CFX plasma concentrations correlated with the distribution of the parent drug and its metabolite to the target tissue (endometrium) (Fig. 2). The main parameters resulting from the endometrial PK analysis performed on data for each experimental animal are summarized in Table 2. The PK parameters obtained from the endometrial comparison indicated that the C_{max} and *AUC* values for both EFX and CFX were significantly greater in the group treated with 5 mg/kg *t* EFX (Table 2). Relationships between the concentrations of the parent drug (EFX) and its active metabolite (CFX) detected after both treatments and the *in vitro MIC*'s obtained for various bacterial isolates are illustrated in Fig. 3 and Table 3. After i.v. treatment at 5 mg/kg, endometrial concentrations of EFX and CFX exceeding the *MIC*'s for Gram-negative isolates and the Gram-positive strains were detected in individual mares for 36–48 and 22–43 h respectively. Concentrations above *MIC* were generally maintained for much shorter periods in mares treated at 2.5 mg/kg (Table 3).

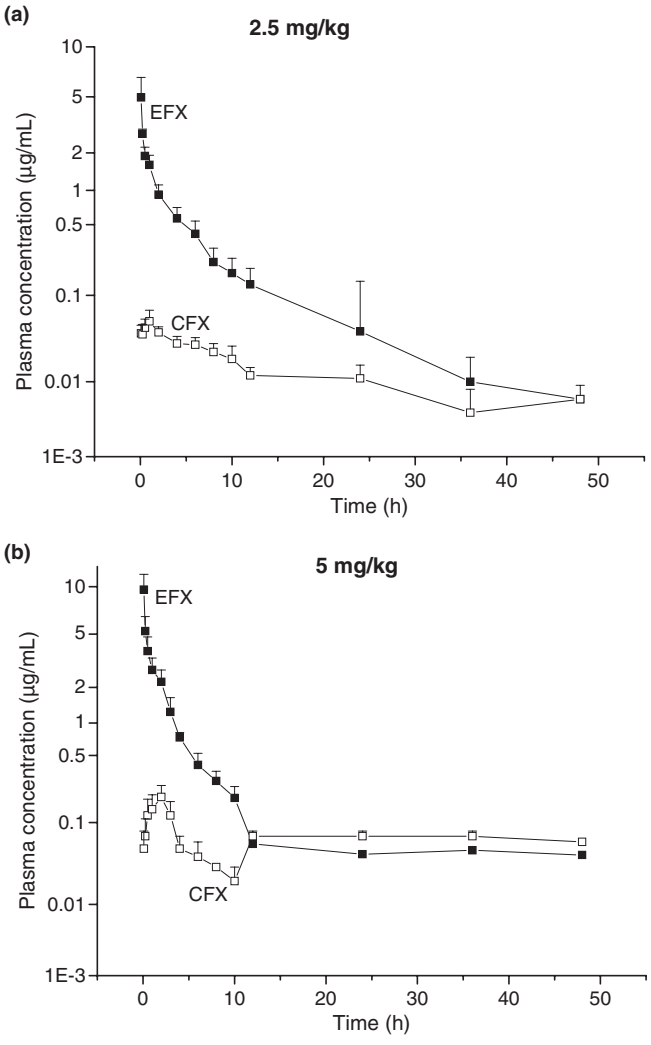

Fig. 1. Mean (±SD) plasma concentrations (μg/mL) obtained for enrofloxacin (EFX) and its metabolite ciprofloxacin (CFX) after the intravenous administration of EFX at (a) 2.5 and (b) 5.0 mg/kg in healthy mares.

DISCUSSION

Maintenance of uterine hygiene is pivotal in avoiding endometritis in susceptible mares and to ensure that breeding mares are free of uterine inflammation when mating. However, current therapy of susceptible mares has focused primarily on the postbreeding period and the removal of free fluid and inflammatory products from the uterus. Infections with highly pathogenic bacteria, such as those that adhere or

Table 1. Mean ± SD plasma pharmacokinetics parameters, obtained for enrofloxacin (EFX) and its active metabolite ciprofloxacin (CFX) after the intravenous (i.v.) administration of EFX at single dose of 2.5 and 5.0 mg/kg in healthy mares

	Dose			
	2.5 mg/kg		5 mg/kg	
Plasma PK parameters	EFX	CFX	EFX	CFX
$C_{P0\ 5'}$(μg/mL)	6.70 ± 3.30	NA	9.60 ± 2.12	NA
C_{max} (μg/mL)	NA	0.10 ± 0.00	NA	0.20 ± 0.10
T_{max} (h)	NA	1.00 ± 0.60	NA	1.60 ± 0.50
$AUC_{(0-t)}$ (μg h/mL)	9.10 ± 2.00	0.60 ± 0.20	13.2 ± 1.90**	3.40 ± 0.40**
MRT (h)	5.90 ± 1.10	13.9 ± 6.90	23.3 ± 2.25***	99.7 ± 26.7**
$T½_{\lambda 2}$ (h)	5.40 ± 1.30	12.8 ± 3.20	17.9 ± 6.80**	66.3 ± 21.6**
Cl_β (mL/h/kg)	287 ± 65.1	NA	356 ± 40.9	NA
V_d (L/kg)	1.02 ± 0.23	NA	3.81 ± 0.92**	NA

NA, not applicable. **$P < 0.01$, ***$P < 0.001$.

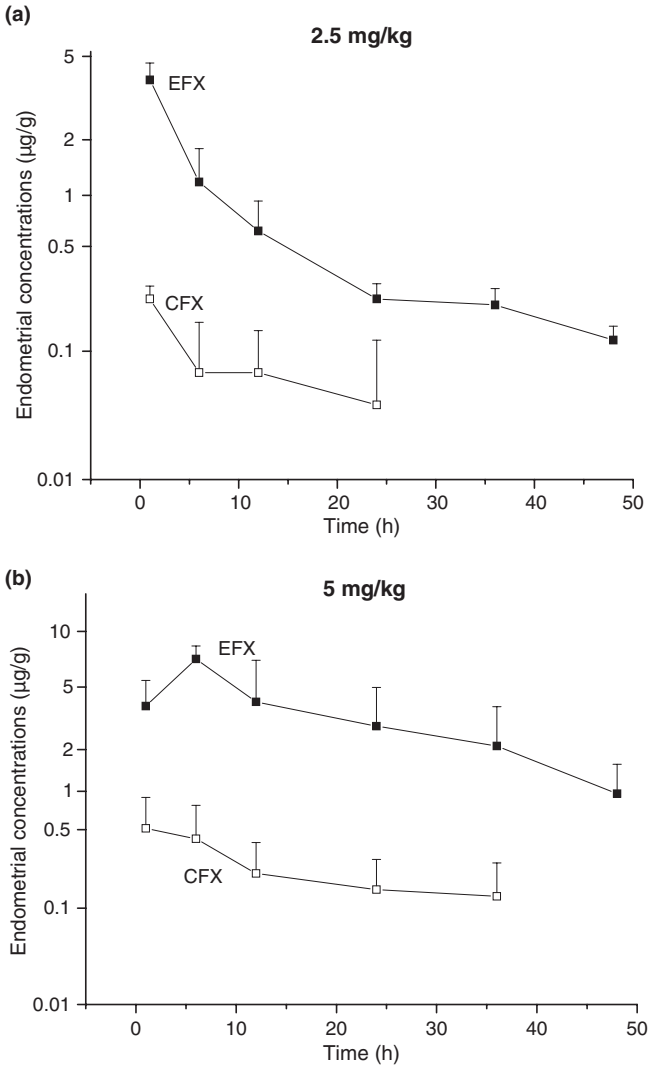

Fig. 2. Mean (±SD) endometrial concentrations (μg/g), obtained for enrofloxacin (EFX) and its metabolite ciprofloxacin (CFX) after the intravenous administration of EFX at (a) 2.5 and (b) 5.0 mg/kg in healthy mares.

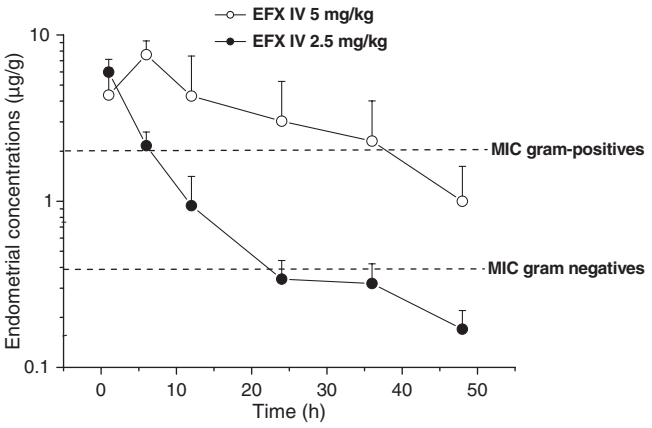

Fig. 3. Comparative endometrial total concentrations for enrofloxacin (EFX) + ciprofloxacin (CFX) after the intravenous administration of EFX at dose of 2.5 and 5 mg/kg in healthy mares.

Table 3. Relationship of the minimum inhibitory concentrations (*MIC*) obtained for Gram-negative and -positive bacteria with the endometrial concentrations of EFX + CFX (sum of metabolites) at the endpoint above the *MIC*, after the intravenous administration of EFX at 2.5 and 5.0 mg/kg

	Endometrio		
MIC (μg/mL)	Dose (i.v.) (mg/kg)	Concentrations of EFX + CFX (μg/g)	Time (h), range
Gram-negative	5.0	2.3–0.96	36/>48
0.5–2.0	2.5	1.3–0.5	6/18
Gram-positive	5.0	3.0–1.7	22/43
1.5–3.0	2.5	2.6–1.5	4/8

produce biofilms, may require antibiotic therapy (Causey, 2005). The empirical use of antimicrobials such as cephalosporins, ampicillin, gentamycin and EFX by either intrauterine or i.v. route, in endometritis-susceptible mares, is a common practice in most intensive reproductive units. However, irrational use of these drugs could accelerate the development of drug resistance, and further understanding of the distribution pattern of antibiotics into the uterus is needed to develop more rational therapeutic strategies. This study correlated the susceptibility of bacteria most commonly isolated from mares suffering endometritis, with the plasma PK and endometrial distribution of the second-generation fluoroquinolone, EFX

Table 2. Endometrial pharmacokinetics parameters (mean ± SD), obtained for enrofloxacin (EFX) and its active metabolite ciprofloxacin (CFX) after the intravenous (i.v.) administration of EFX at single dose of 2.5 y 5.0 mg/kg in healthy mares

	Dose			
	2.5 mg/kg		5 mg/kg	
Endometrio PK parameters	EFX	CFX	EFX	CFX
C_{max} (μg/g)	3.90 ± 0.80	0.20 ± 0.00	7.38 ± 1.08*	0.60 ± 0.30*
T_{max} (h)	1.00 ± 0.00	1.00 ± 0.00	5.00 ± 2.00	1.00 ± 0.00
$AUC_{(0-t)}$ (μg h/g)	29.9 ± 7.90	2.90 ± 1.80	164 ± 67*	8.90 ± 3.40*
Q. period (h)	1 to 48	1 to 24	1 to 48	1 to 36
Concentration at 48 h	0.12 ± 0.03	0.00 ± 0.00	0.96 ± 0.60**	0.00 ± 0.00

Values are statistically different to the 2.5 mg/kg group at *$P < 0.05$ and **$P < 0.01$. Q. period = period where samples were quantified above the LOQ.

and its active metabolite, CFX, in order to establish a rational therapeutic approach for treating healthy mares to prevent the disease and minimize selection pressure for bacterial resistance.

To minimize the dangers of potentiating bacterial drug resistance an essential prerequisite for this study was to determine the susceptibility of target bacteria to the treatment drugs. For this purpose quantitative data (*MIC* values) were generated using Microdilution Antimicrobial Susceptibility Tests: the broth microdilution method being considered the method of choice for *in-vitro* susceptibility testing of bacterial pathogens (Schwars *et al.*, 2003; Rocksin *et al.*, 2007). Quantitative susceptibility varies between bacterial genera and species, as well as between strains of a particular species. Moreover, individual members of drug families differ quantitatively in their antimicrobial activity (Prescott, 2000). The *MIC* values obtained in this study for *E.coli*, *P. aeruginosa* and *β*-haemolytic strains ranged 0.5–2.0, 0.25–2.0 and 1.5–3.0 μg/mL, respectively. Interestingly, and perhaps importantly these values were comparable to those reported in other studies on isolates of the same species from clinical cases of equine endometritis in other countries (Jenkins & Friedlander, 1992; Ensink *et al.*, 1993; Prescott & Baggot, 1994; Bermingham *et al.*, 2000; Haines *et al.*, 2000; Albihn *et al.*, 2003).

At present no fluoroquinolones have been approved for use in horses although interest in their use in this species is increasing (Papich & Riviere, 2009). EFX is a second generation fluoroquinolone which undergoes phase I biotransformation by a de-ethylation reaction to form the primary metabolite, CFX. Both EFX and CFX are biologically active and, as a consequence, both are beneficial to the recipient. However, EFX is not recommended for use in horses younger than 3 years old due to secondary effects, such as degenerative damage of articular cartilage in weight-bearing joints, of dosing growing animals. More recently, PK studies in adult horses using both the intragastric and i.v. routes have demonstrated that EFX has a suitable PK profile, with high distribution to the target tissues in which most the important infections are located (Giguère *et al.*, 1996; Langston *et al.*, 1996; Kaartinen *et al.*, 1997; Haines *et al.*, 2000; Papich *et al.*, 2002; Peyrou *et al.*, 2006). As a consequence, clinical practitioners have been using EFX in adult horses to control of infections in various tissues.

Postbreeding infections occur following either natural mating or artificial insemination. Such infections are usually transient, lasting <48 h, but in susceptible mares they can be sufficiently prolonged to prevent conception (Causey, 2005). One study demonstrated that the phagocytic activity of uterine neutrophils was significantly lower in susceptible mares, although serum chemotactic activity was significantly higher than in resistant mares (Watson *et al.*, 1987). Other studies have concluded that uterine neutrophils from susceptible mares have significantly lower phagocytic activity and reduced responses to chemotactic stimuli than those from resistant mares (Cheung *et al.*, 1985; Liu *et al.*, 1985). Another recent report demonstrated that, in humans, CFX and most other fluorquinolone derivatives at clinically achievable concentrations of >5 μg/mL significantly inhibited several pro-inflammatory cytokines (IL6, IL 1β and TNFα) induced by lipopolisaccharides (LPS) (Riesbeck, 2006). Similar immunomodulatory effects of CFX have also been reported in other animal models (Riesbeck, 2002; Dalhoff, 2005). The combination of their anti-inflammatory effects and their bactericidal activity make EFX and CPX useful candidates for preventing endometritis in susceptible mares.

Using other analytical methods (microbiological assay and HPLC by ultraviolet detection, respectively), Giguère *et al.* (1996) and Papich *et al.* (2002), reported on preliminary studies to assess the distribution of EFX and its active metabolite (CFX) in the endometrium. However, the published data were insufficient to provide a full understanding of the distribution and disappearance of this molecule from endometrial tissue. In the present study, EFX and CFX were analysed using a much more sensitive and specific HPLC method, with fluorescence detection. This allowed concentrations of EFX and CFX to be measured separately, in both plasma and endometrial tissue samples, over a period of 48 h posttreatment. The high sensitivity of method, also allowed a detailed profile the drug depletion from the target tissue (endometrium) to be generated. In turn, this facilitated accurate correlations to be made between *in vivo* drug concentration endpoints and *in vitro MIC* values obtained for target bacteria. Overall, this approach has yielded data which form an appropriate basis for the proposal of a rational dose schedule for the prevention of infection during embryo transfer in healthy mares.

Persistent endometritis and oviductitis lead to the loss of the conceptus. Mares susceptible to endometritis have impaired uterine innate defences that allow the retention of inflammatory exudate and adherence of commensal bacteria and/or reproductive pathogens to the endometrium. Treatment strategies generally involve the use of drugs promoting contractility (e.g. oxytocin) or anti-inflammatory steroids such as dexamethasone although, once bacteria have adhered to the endometrium, antibiotics should be the drugs of choice.

Consistent with other reports, mean plasma concentrations of EFX (expressed as *AUC*) were significantly greater ($P < 0.05$) than those obtained for CFX. Further evidence for the rapid biotransformation of the parent drug was provided by other PK studies, in which it was found that CFX represented approximately 20.5% of the total (i.e. EFX + CFX) plasma fluoroquinolone concentrations (Giguère *et al.*, 1996; Papich *et al.*, 2002) (Fig. 1 and Table 1). Moreover, it should be noted that these plasma CFX concentrations were sufficient to be effective therapeutically. Interestingly, when the concentrations of the same metabolite were assessed in the endometrium (target tissue), the proportion of CFX was substantially less (2–8% total EFX + CFX concentration) after mares had been treated i.v. with either 2.5 or 5 mg/kg EFX (Fig. 2). In terms of drug distribution, total EFX + CFX concentrations (in terms of *AUC*s) in endometrial tissue were 249% and 941% higher than the equivalent plasma concentrations following treatment with 2.5 and 5 mg/kg EFX respectively (Tables 1 and 2). These results confirm that the lipophilic parent drug, EFX, and its active metabolite, CFX, are readily distributed from plasma to the

endometrium of healthy mares, after the i.v. administration of EFX. The latter was confirmed by comparison of the endometrium/plasma EFX + CFX *AUC* ratios which were 3.6 and 10.3 after treatment with 2.5 and 5 mg/kg EFX respectively. The relatively higher tissue/plasma ratio after the higher dose reported here is in contrast to values reported earlier by Papich *et al.*, 2002. The reasons for these differences are unclear but may be because of factors related to breeding, reproduction cycle (e.g. changes in uterine physiological activity) and/or the time of season (onset or ending) when sampling was undertaken. The extensive distribution of EFX and CFX from plasma to the target tissue (endometrium), could explain the clinical success of using this molecule in preventing and/or treating bacterial endometritis.

Basic predictive PK and pharmacodynamic (PK/PD) values, such as C_{max}:*MIC* and AUC_{0-24}:*MIC*, which can be used to predict efficacy and the impact on bacterial resistance, were also calculated from plasma concentration data obtained. However, the parameters cited should be interpreted with caution since the experimental design was specifically aimed at evaluating the potential use of EFX as a preventative in the healthy uterus to improve conception rates and avoid possible infection and consequent inflammatory effects in susceptible mares. The results obtained here, after dosing mares intravenously with 2.5 or 5 mg/kg EFX, showed that, for plasma, the PK/PD surrogate markers mentioned above, did not achieve the accepted values required to indicate efficacy against either the Gram negatives (*AUC*:*MIC* < 125) or Gram-positive (*AUC*:*MIC* 50–60) bacteria tested respectively. The relationship between the cited PK/PD markers was markedly lower for Gram-negative bacteria than those reported by Papich *et al.*, 2002. This discrepancy appeared to be largely because of the higher (>10- to 15-fold) *MIC* values obtained in this study for the wild isolates stated rather than differences in the plasma drug concentrations reported in both studies. However, if *AUC*:*MIC* integration was applied to the target tissue (endometrium), using the minimum *in vitro MIC* obtained, values of 340 and 680 for Gram-negative and -positive bacteria were obtained following treatment with 5 mg/kg EFX in mares with a healthy uterus. This tissue data is likely to be of far greater importance than the corresponding plasma data in designing a rational therapeutic strategy. Interestingly, fluoroquinolones appear to exhibit a 'concentration-dependent' activity against Gram-negative bacteria, but a 'time-dependent' activity against Gram-positives (Martinez *et al.*, 2006). The most common forms of bacterial endometritis involve mixed infections with *E. coli* and β-haemolytic streptococci, of which *Streptococcus zooepidemicus* has been the mostly widely isolated species (Rogan *et al.*, 2007). Based on these observations, the time above the *MIC* calculated for target tissue (endometrium) is likely to be the best parameter on which to base the development of a rational EFX-based therapeutic regimen for use in embryo transfer mares.

Endometrial EFX and CFX concentrations above the *MIC* for the Gram-negative and -positive isolates tested were detected over 36–48 and 22–43 h, respectively, following i.v. treatment at 5 mg/kg. Concentrations above *MIC* were maintained for much shorter periods following treatment at 2.5 mg/kg although there were quantitative differences between the bacterial species tested (Table 3). Based on the results obtained in this study, a recommended rational regime for the use of EFX as a preventive therapeutic tool to prevent infection with either Gram-positive and -negative bacterial strains in embryo-transfer mares would be: 1 × dose of 5 mg/kg bodyweight intravenously (i.v.) prebreeding and 2 × i.v. doses at 5 mg/kg 36–48 h postbreeding.

In conclusion, the strategic use EFX by clinical practitioners, to prevent bacterial adherence and provide effective bactericidal concentrations *in utero*, in susceptible embryo-transfer mares should facilitate improved the pregnancy rates, avoid toxicity due to drug overdose and avoid low drug concentrations in the uterus, which could increase the risk of development of bacterial resistance.

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