

## Dispositions of enrofloxacin and its major metabolite ciprofloxacin in Thai swamp buffaloes

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**ABSTRACT.** Given the limited information available in this species, the aim of this study was to investigate the pharmacokinetic characteristics of enrofloxacin (ER) and its major metabolite ciprofloxacin (CP) in buffaloes, *Bubalus bubalis*. ER was administered intravenously (i.v.) or subcutaneously (s.c.) to buffaloes at doses of 5.0 and 7.5 mg/kg BW, and plasma, urine and fecal samples were collected until 48 hr post-administration. The concentrations of ER and CP in the plasma, urine and feces were analyzed using high-performance liquid chromatography equipped with a fluorescence detector. The plasma concentrations of ER and CP could be determined up to 24 hr and 32 hr after i.v. and s.c. administrations at doses of 5.0 and 7.5 mg/kg BW, respectively. CP concentrations were always lower than those of parental drug. The s.c. bioavailability of ER was  $52.36 \pm 4.24\%$  and  $72.12 \pm 5.39\%$  at doses of 5.0 and 7.5 mg/kg BW, respectively. Both ER and CP were detectable in urine and feces up to 24 hr. ER and CP were mainly excreted via the urine. Based on the pharmacokinetic data and PK-PD indices, s.c. administration of ER at doses of 5.0 and 7.5 mg/kg BW might be appropriate for the treatment of susceptible bacterial diseases in Thai swamp buffaloes.

**KEY WORDS:** ciprofloxacin, enrofloxacin, pharmacokinetic, Thai swamp buffalo

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Enrofloxacin (ER) is a synthetic bactericidal agent of the quinolones group, which acts by inhibiting the DNA gyrase enzyme, and it is predominantly used in veterinary practice for the treatment of respiratory and gastrointestinal infections. In both mammalian and non-mammalian species, it is partly de-ethylated to the main active metabolite ciprofloxacin (CP). Indeed, CP has been found as the metabolites that account almost 25–35% of the exposure to circulating parent drug [30]. It possesses antibacterial actions against a wide range of Gram-negative aerobes, a number of Gram-positive bacteria, mycoplasmas and some rickettsial micro-organisms [21]. ER has received growing attention, because of its potential efficacy and safety for the treatment of diseases in animals [28]. The dispositions of ER have been investigated in cattle [17, 18], sheep [25], horses [11, 13], pigs [3, 4, 24], rabbits [5], dogs [21], poultry [2] and exotic animals [12], and both intra- and inter-species differences in pharmacokinetics have been reported. For this reason, adequate information on the pharmacokinetics of ER in each species is pivotal

for a successful therapeutic plan. Thus, the main aim of this study was to elucidate the disposition and s.c. bioavailability of ER in Thai swamp buffaloes, *Bubalus bubalis*, following i.v. and s.c. administration at two different doses.

### MATERIALS AND METHODS

**Standards and chemicals:** Enrofloxacin (ER) (Baytril® 100, Bayer AG, Leverkusen, Germany) for injection was used. Enrofloxacin and ciprofloxacin standards were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other reagents and chemicals of analytical grade were purchased from Sigma Chemical Co. Purified water was produced using the Milli-Q water purification system from Millipore, Inc. (Bedford, MA, U.S.A.).

**Animals:** Ten female adult Thai swamp buffaloes between 5 and 10 years of age with an average body weight of 500 kg were selected. All ten buffaloes were housed together at the Animal Unit, Faculty of Natural Resources and Agro-industry, Chalermphrakiat Sakon Nakhon province campus, Kasetsart University, Sakon Nakhon province, Thailand. All experimental procedures were performed according to the Guidelines for Animal Experiments and approved by the Animal Ethics Research Committee of the Faculty of Veterinary Medicine, Kasetsart University. Prior to the study, each buffalo was assessed to be healthy based on a review of health history, physical examination, complete blood count and serum chemistry analysis.

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**Experimental design:** To obtain the pharmacokinetic data, the ten Thai swamp buffaloes were randomly divided into two groups (n=5 per group). Each group was administered ER intravenously (i.v.) or subcutaneously (s.c.) (Baytril, 100 mg/ml) at a dose of 5.0 or 7.5 mg/kg BW. The experiments were performed in a two-phase, two-period, cross-over design with an interval of 3 weeks between the periods and the phases. Ten ml of blood was collected from the jugular vein of each animal with heparinized syringes at 0, 15 and 30 min and at 1, 2, 4, 6, 8, 12, 24, 32 and 48 hr after administration. The plasma was separated by centrifugation ( $1,986 \times g$ ) for 15 min. The urine and fecal samples were collected from 0 to 48 hr by natural collection and rectal palpation. All of the plasma, urine and fecal samples were frozen at  $-20^{\circ}\text{C}$  until analysis.

**Sample extraction:** The extraction method of ER and CP in plasma, urine and feces was performed as described previously [19, 26, 36], after revalidation. Briefly, plasma samples were extracted using liquid-liquid extraction. 300  $\mu\text{l}$  of acetonitrile (ACN) was added to 200  $\mu\text{l}$  plasma in a tube and then shaken for 15 sec. The mixture was centrifuged at  $4,500 \times g$  and  $4^{\circ}\text{C}$  for 10 min. A 150  $\mu\text{l}$  sample of the supernatant was transferred to a new tube and mixed with 300  $\mu\text{l}$  of water. After passing it through a Ministart<sup>®</sup> RC filter (pore size 0.45  $\mu\text{m}$ , Sartorius AG, Göttingen, Germany), an aliquot of 200  $\mu\text{l}$  of this mixture was injected for high performance liquid chromatography (HPLC).

One hundred  $\mu\text{l}$  of urine was diluted with 900  $\mu\text{l}$  of distilled water in a microcentrifuge tube. The solution was vortex-mixed and filtered using a Ministart<sup>®</sup> RC filter (pore size 0.45  $\mu\text{m}$ , Sartorius AG); then, the sample (50  $\mu\text{l}$ ) was injected onto HPLC.

Fecal samples (100 mg) were homogenized with 400  $\mu\text{l}$  of distilled water in a motor driven glass homogenizer for 2–3 min. To each sample, 2 ml of extraction solution was added, and the samples were mixed. Samples were stored at room temperature, in the dark, for 45 min before 1.5 ml of 0.07 M *ortho*-phosphoric acid were added. Samples were then vortexed and allowed to stand for a further 10 min before being centrifuged at  $5,800 \times g$  at  $4^{\circ}\text{C}$  for 10 min. The solution was filtered with a Ministart<sup>®</sup> RC filter (pore size 0.45  $\mu\text{m}$ , Sartorius AG); then, the sample (50  $\mu\text{l}$ ) was injected onto HPLC.

**HPLC conditions:** The analytical method was performed in accordance with Otero *et al.* [27]. The HPLC analysis was performed using an Agilent 1260 series system consisting of a binary pump, an automatic sample injector, a column thermostat and a fluorescence detector (Agilent Technologies, Waldbronn, Germany) with excitation and emission wavelength of 294 and 500 nm, respectively. Separation was achieved using a PLRP-S column (5  $\mu\text{m}$ ,  $4.6 \times 150$  mm) with an RP18-E guard column (5  $\mu\text{m}$ ,  $4 \times 40$  mm) (Polymer Laboratories Inc., Church Stretton, U.K.). The column was maintained at a temperature of  $35^{\circ}\text{C}$  using a column oven. The mobile phase consisted of 14% ACN and 86% 0.07 M *ortho*-phosphoric acid. The flow rate was 1.2 ml/min. The retention times of ER and CP were 7.93 and 5.45 min, respectively. The limit of quantification (LOQ) of ER and CP

in plasma was 0.1 and 0.05  $\mu\text{g/ml}$ , respectively.

**Fortification and method validation:** The calibration curves were obtained by spiking the working standard solution into buffalo blank plasma, urine and feces to yield final concentrations of 0.1, 0.5, 1, 5 and 10  $\mu\text{g/ml}$  of ER and CP. Buffalo blank plasma, urine and feces (for quality control samples) were obtained some days before the treatment protocol commenced. Linearity of the regression curve in the range 0.1–10  $\mu\text{g/ml}$  was assessed on the basis of the residual plot, the lack of fit test and the back calculation (within 20% of known amount). Five duplicates of the quality control samples at concentrations of 0.5, 5 and 10  $\mu\text{g/ml}$  were prepared and used to determine the recoveries, intra- and inter-day precision, and accuracy of the method. The procedure was repeated five times within the same day to gain intra-day run precision and accuracy and five times for each concentration over five consecutive days to obtain inter-day run precision and accuracy. The extraction recoveries of ER from plasma were  $85.19 \pm 2.52$ ,  $87.12 \pm 1.95$  and  $86.24 \pm 3.11\%$  for 0.5, 5 and 10  $\mu\text{g/ml}$ , respectively. The extraction recoveries were  $84.22 \pm 4.52$ ,  $83.26 \pm 2.9$  and  $83.24 \pm 3.33\%$  for urine, and  $82.63 \pm 3.32$ ,  $81.64 \pm 3.68$  and  $82.45 \pm 4.31\%$  for feces, respectively. The intra- and inter-day precision values in plasma, urine and feces were lower than 7% and accuracy was more than 95%, respectively. The variation coefficient ( $r^2$ ) value of the ER and CP calibration curves was  $>0.9997$ .

**Pharmacokinetic calculation and statistical analysis:** The concentrations of ER and CP in Thai swamp buffaloes versus time were pharmacokinetically analyzed by applying a two-compartment and non-compartmental model, respectively, using the PK Solution 2.0<sup>™</sup> Program (Summit Research Services, Montrose, CO, U.S.A.). The absolute subcutaneous bioavailability ( $F$ ) was calculated using the following equation:

$$(\%) F_{(S.C.)} = (AUC_{S.C.}) / (AUC_{I.V.}) \times 100$$

The statistical analyses were evaluated using the student-*t* test. The results were presented as mean  $\pm$  standard deviation (SD). All the analyses were conducted using GraphPad InStat (GraphPad Software, Inc., La Jolla, CA, U.S.A.). In all the experiments, differences were considered significant, if the associated probability level ( $P$ ) was lower than 0.05.

## RESULTS

The determination of the ER and CP concentrations showed that ER and CP were detectable in the plasma of buffaloes following single i.v. and s.c. administrations. The semi-logarithmic plots of the mean plasma concentration-time curves of ER and CP at doses of 5.0 and 7.5 mg/kg BW are shown in Figs. 1 and 2. ER was measurable from 5 min, while CP was detectable from 30 min after s.c. administration. However, the drug ER and its metabolite CP were below the limit of detection of the method at 32 hr after i.v. and s.c. administrations, respectively, at doses of 5.0 and 7.5 mg/kg BW. Data for ER best fit a two-compartment model. The main pharmacokinetic parameters are reported in Table 1.

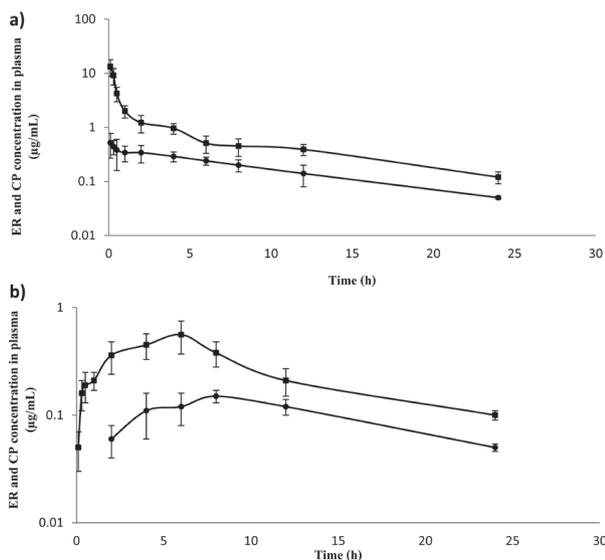


Fig. 1. Mean values ( $\pm$  SD) of enrofloxacin (ER) (■) and ciprofloxacin (CP) (●) concentrations in plasma of Thai swamp buffaloes after intravenous (a) and subcutaneous (b) administrations at a dose of 5.0 mg/kg BW (n=5).

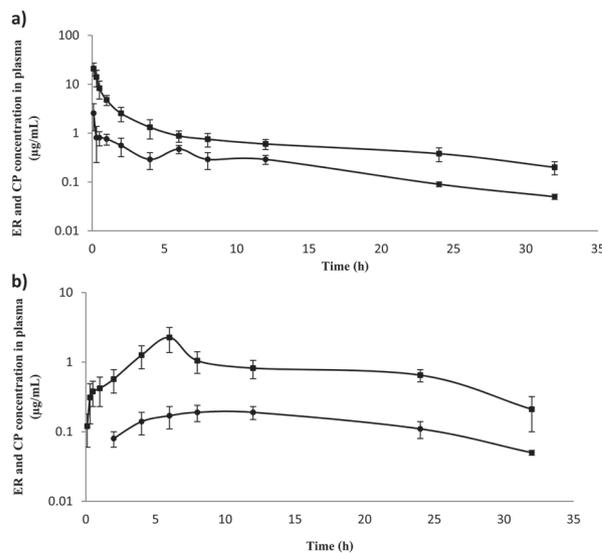


Fig. 2. Mean values ( $\pm$  SD) of enrofloxacin (ER) (■) and ciprofloxacin (CP) (●) concentrations in plasma of Thai swamp buffaloes after intravenous (a) and subcutaneous (b) administrations at a dose of 7.5 mg/kg BW (n=5).

Table 1. Mean  $\pm$  SD values of the pharmacokinetic parameters of enrofloxacin following intravenous and subcutaneous administrations at doses of 5.0 and 7.5 mg/kg BW in Thai swamp buffaloes (n=5 per group)

Pharmacokinetic parameter (unit)	Intravenous		Subcutaneous	
	5.0 mg/kg	7.5 mg/kg	5.0 mg/kg	7.5 mg/kg
$Cp^0$ ( $\mu\text{g/ml}$ )	16.32 $\pm$ 3.16	23.56 $\pm$ 5.23	-	-
$K_{el}$ ( $\text{hr}^{-1}$ )	0.1 $\pm$ 0.008	0.08 $\pm$ 0.005	0.20 $\pm$ 0.02*	0.14 $\pm$ 0.01*
$T_{1/2\alpha}$ (hr)	3.35 $\pm$ 0.66	6.12 $\pm$ 0.86	2.21 $\pm$ 0.19*	4.31 $\pm$ 0.61*
$t_{1/2\beta}$ (hr)	7.05 $\pm$ 1.32	8.64 $\pm$ 1.15	3.43 $\pm$ 1.12*	4.91 $\pm$ 0.91*
$Cl_s$ (l/hr/kg)	0.28 $\pm$ 0.04	0.21 $\pm$ 0.13	0.63 $\pm$ 0.11*	0.28 $\pm$ 0.04*
$Vd_s$ (l/kg)	2.82 $\pm$ 0.48	2.67 $\pm$ 0.33	3.12 $\pm$ 1.16	1.97 $\pm$ 0.32*
$MRT$ (hr)	15.0 $\pm$ 2.63	21.3 $\pm$ 3.12	8.70 $\pm$ 1.19*	13.3 $\pm$ 2.65*
$AUC_{0-24}$ ( $\mu\text{g}\cdot\text{hr/ml}$ )	16.93 $\pm$ 3.66	33.27 $\pm$ 4.63	8.12 $\pm$ 1.16*	24.37 $\pm$ 3.28*
$AUC_{0-\infty}$ ( $\mu\text{g}\cdot\text{hr/ml}$ )	18.1 $\pm$ 3.29	35.0 $\pm$ 5.33	9.9 $\pm$ 2.23*	27.0 $\pm$ 4.23*
$C_{max}$ ( $\mu\text{g/ml}$ )	-	-	0.66 $\pm$ 0.15	2.26 $\pm$ 0.61
$T_{max}$ (hr)	-	-	6.0 $\pm$ 0.00	6.0 $\pm$ 0.00
$F$ (%)	-	-	52.36 $\pm$ 4.24	72.26 $\pm$ 5.39

$Cp^0$ : plasma concentration at initial time;  $K_{el}$ : elimination rate constant of the beta phase;  $T_{1/2\alpha}$ : distribution half-life;  $T_{1/2\beta}$ : elimination half-life;  $Cl$ : clearance;  $AUC_{0-\infty}$ : Area under the curve from time 0 to infinity;  $AUC_{0-24}$ : Area under the curve from time 0 to 24 hr;  $V_d$ : Volume of distribution;  $MRT$ : Mean residence time;  $C_{max}$ : Maximum concentration;  $T_{max}$ : Time at maximum concentration. <sup>§</sup>This datum is normalized for bioavailability when calculated for the s.c. administration. \* $P < 0.05$  (statistically significant value if compared to the corresponding i.v. group).

Following i.v. administration, the  $AUC$  values increased with the dose, but not in a dose proportional fashion. In the s.c. treatment groups,  $C_{max}$  and  $AUC$  values also increased, but not in a dose proportional fashion. Interestingly,  $K_{el}$  and  $T_{1/2\beta}$  showed more rapid elimination of ER in the s.c. injection than in the i.v. injection. The absolute bioavailabilities of ER were 52.36  $\pm$  4.24% and 72.26  $\pm$  5.39% after s.c. administration at doses of 5.0 and 7.5 mg/kg BW, respectively (Table 1).

The plasma concentrations of CP were lower than the respective concentrations of parental drug (Fig. 1). The curves were analyzed according to a non-compartmental method (Table 2). The average CP/ER  $AUC$ s ratio was 24.0  $\pm$  2.1% and 18.3  $\pm$  3.1% for the i.v. treatments (5.0 and 7.5 mg/kg, respectively) and 28.3  $\pm$  3.97% and 26.6  $\pm$  4.41% for s.c. treatments (5.0 and 7.5 mg/kg, respectively).

The concentrations of ER and CP following i.v. and s.c.

Table 2. Mean  $\pm$  SD values of the pharmacokinetic parameters of ciprofloxacin following intravenous and subcutaneous administrations of enrofloxacin at doses of 5.0 and 7.5 mg/kg BW in Thai swamp buffaloes (n=5 per group)

Pharmacokinetic parameters (unit)	Intravenous		Subcutaneous	
	5.0 mg/kg	7.5 mg/kg	5.0 mg/kg	7.5 mg/kg
$\lambda z$ (hr <sup>-1</sup> )	0.11 $\pm$ 0.025	0.11 $\pm$ 0.020	0.075 $\pm$ 0.013*	0.11 $\pm$ 0.01
$t_{1/2}\lambda z$ (hr)	6.44 $\pm$ 1.88	6.66 $\pm$ 1.35	9.43 $\pm$ 1.69*	6.72 $\pm$ 1.28
MRT (hr)	9.63 $\pm$ 2.23	8.88 $\pm$ 1.15	16.44 $\pm$ 1.88*	9.24 $\pm$ 1.04
$AUC_{0-24}$ ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	4.13 $\pm$ 0.40	5.97 $\pm$ 1.68	2.25 $\pm$ 0.28*	6.59 $\pm$ 1.29
$AUC_{0-\infty}$ ( $\mu\text{g}\cdot\text{hr}/\text{ml}$ )	4.50 $\pm$ 0.39	6.42 $\pm$ 1.76	2.85 $\pm$ 0.34*	7.11 $\pm$ 1.22
$C_{max}$ ( $\mu\text{g}/\text{ml}$ )	-	-	0.15 $\pm$ 0.022	2.29 $\pm$ 0.57
$T_{max}$ (hr)	0.14 $\pm$ 0.089	0.10 $\pm$ 0.00	6.0 $\pm$ 2.0*	0.11 $\pm$ 0.00

$\lambda z$ : elimination constant;  $t_{1/2}\lambda z$ : Elimination half life;  $Cl$ : clearance;  $AUC_{0-\infty}$ : Area under the curve from time 0 to infinity;  $AUC_{0-24}$ : Area under the curve from time 0 to 24 hr; MRT: Mean residence time;  $C_{max}$ : Maximum concentration;  $T_{max}$ : Time at maximum concentration. <sup>§</sup>This datum is normalized for bioavailability when calculated for the s.c. administration. \* $P < 0.05$  (statistically significant value if compared to the corresponding i.v. group).

Table 3. Mean  $\pm$  SD values of enrofloxacin (ER) and ciprofloxacin (CP) in urine following intravenous and subcutaneous administrations at doses of 5.0 and 7.5 mg/kg BW in Thai swamp buffaloes (n=5 per group)

Time (hr)	5.0 mg/kg				7.5 mg/kg			
	Intravenous		Subcutaneous		Intravenous		Subcutaneous	
	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )
0-4	2.34 $\pm$ 1.18	16.92 $\pm$ 4.93	0.50 $\pm$ 0.31*	4.92 $\pm$ 3.83*	5.14 $\pm$ 2.94	21.89 $\pm$ 7.49	0.35 $\pm$ 0.18*	5.36 $\pm$ 2.13*
4-12	0.42 $\pm$ 0.31	18.09 $\pm$ 3.57	0.70 $\pm$ 0.21	15.99 $\pm$ 4.70	0.54 $\pm$ 0.31	19.19 $\pm$ 1.48	0.56 $\pm$ 0.29	15.61 $\pm$ 4.91*
12-24	0.12 $\pm$ 0.02	5.33 $\pm$ 3.86	0.36 $\pm$ 0.23	3.90 $\pm$ 1.51	0.16 $\pm$ 0.02	3.04 $\pm$ 1.32	0.31 $\pm$ 0.15	7.28 $\pm$ 2.66*

\* $P < 0.05$  (statistically significant value if compared to the corresponding i.v. group).

Table 4. Mean  $\pm$  SD values of enrofloxacin (ER) and ciprofloxacin (CP) in feces following intravenous and subcutaneous administrations at doses of 5.0 and 7.5 mg/kg BW in Thai swamp buffaloes (n=5 per group)

Time (hr)	5.0 mg/kg				7.5 mg/kg			
	Intravenous		Subcutaneous		Intravenous		Subcutaneous	
	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )	ER ( $\mu\text{g}/\text{ml}$ )	CP ( $\mu\text{g}/\text{ml}$ )
0-4	NF	NF	0.03 $\pm$ 0.003*	0.05 $\pm$ 0.04*	0.24 $\pm$ 0.05	ND	NF	NF
4-12	3.63 $\pm$ 1.12	3.46 $\pm$ 0.72	0.57 $\pm$ 0.33*	0.20 $\pm$ 0.17*	2.06 $\pm$ 0.13	0.51 $\pm$ 0.45	0.33 $\pm$ 0.06*	0.14 $\pm$ 0.06
12-24	0.10 $\pm$ 0.33	0.32 $\pm$ 0.03	0.62 $\pm$ 0.14*	0.28 $\pm$ 0.08	0.24 $\pm$ 0.10	0.20 $\pm$ 0.08	0.86 $\pm$ 0.53*	0.53 $\pm$ 0.21*

NF: no fecal collection; ND: not detected. \* $P < 0.05$  (statistically significant value if compared to the corresponding i.v. group).

administrations at doses of 5.0 and 7.5 mg/kg BW in urine and feces, respectively, are shown in Tables 3 and 4. Both ER and CP were detectable in urine and feces up to 24 hr. Generally, ER and CP had higher concentrations in urine than in feces. The concentration of CP was higher than that of ER in urine.

## DISCUSSION

Our study was designed to characterize the disposition of ER following i.v. and s.c. administrations at doses of 5.0 and 7.5 mg/kg BW in Thai swamp buffaloes. This animal is important for the rural economy of Thailand, but is often affected by bacterial infections that can reduce its food-production ability (<http://www.dld.go.th/th/index.php/2010-02-16-08-16>) [8]. Several pharmacokinetic differ-

ences have been previously reported for ER within various animal species. For this reason, it is important to have a clear pharmacokinetic profile of this drug in each target species to formulate a sound therapeutic treatment plan.

The elimination half-life range of ER after i.v. administration was 7.05–8.64. This is slightly lower than the value reported for donkeys (9.54 hr) [35], but higher than those reported in buffalo calves (2.92 hr) [20], sheep (3.25 hr) [10], goats (2.73 hr) [10], lactating dairy cows (3.69 hr) [16] and beef steers (5.15 hr) [16]. Compared to other ruminant species, the plasma clearance in Thai swamp buffaloes was lower than those reported in buffalo calves (1.94 l/hr/kg) [20], sheep (0.53 l/hr/kg) [10], goats (0.70 l/hr/kg) [10], lactating dairy cows (1.45 l/hr/kg) [16] and beef steers (0.7 l/hr/kg) [16]. Data from the present study indicated a slower rate of elimination of ER in Thai swamp buffaloes compared to

the aforementioned ruminant species. Parameters related to elimination (such as  $K_{el}$  and  $T_{1/2\beta}$ ) showed rapid elimination of s.c. injection than i.v. injection. It might be due to the saturated elimination of ER at highest concentration of drug. In fish species, ER has been reported to inhibit cytochrome P450 [37]. On the contrary,  $Cl$  values (5.0 vs. 7.5 mg/kg i.v. treatments) appeared to be unchanged, despite a large variability has been found in the 7.5 mg/kg group. However, further studies (with a larger animal sample size) are warranted to support this hypothesis. On the other hand, the  $V_d$  of ER found in the present study was large after both i.v. and s.c. administrations. These data are in agreement with the results from previous investigations in other ruminant species [14, 18, 20, 25, 32], which indicated that ER was able to penetrate well into various tissues.

Surprisingly, the  $AUC$  values increased irrespective of the dose (2 and 3 times after i.v. and s.c. administrations, respectively). This is in disagreement with other animal species where ER showed dose-dependent pharmacokinetic parameters [7]. The reason for this phenomenon is not clear, and a study with larger animal sample size should be used to verify this datum.

Following s.c. administration, ER given at a dose of 7.5 mg/kg was absorbed more efficiently than when given at a dose of 5.0 mg/kg as indicated by its higher bioavailability (72.26%). Some studies involving ER injected via extravascular injective routes, reported that local irritation and soft tissue necrosis can occur in the injection area [28]. Although this possibility was not checked at the time of experiment, the higher dose of the drug might have evoked a stronger inflammation response, increasing the portion of drug absorbed by dilated blood vessels. It might be a concern in clinical practice, however, some recent studies reported in turtles have shown that an intramuscular injection of a diluted concentration of ER (10 mg/ml) [10, 34] did not produce irritative effects as the more concentrate drug marketed solution (25 mg/ml) for pets [6, 31]. However, the nonlinear  $AUC$  values increase on increasing dose might suggest elimination saturation and consequently a non-linear pharmacokinetic. Under this condition, the  $F\%$  data should have a negligible value and should be used with caution. Further studies are necessary to clarify this issue.

CP is the main active metabolite of ER, possessing a similar spectrum of antibacterial activity. Its concentration has been reported as always being lower than that of parental molecule in mammals, birds and reptiles. CP is known to account for about 25–35% of the amount of the parental drug in mammals [33]. These earlier data are in line with the results of the CP/ER  $AUC$  ratio reported in the present study (range 18.3–28.3%).

ER and CP were detectable in urine and feces up to 24 hr after i.v. and s.c. administrations, in the present study. However, the concentration of CP in urine was significantly higher than that detected in feces. This indicated that the urine was a major route of excretion of ER metabolite. This is in line with a previous study on the ER excretion in pigs [40].

It has been reported that ER can kill bacteria in a concentration-dependent manner [1, 9]. The minimum inhibitory

concentration ( $MIC$ ) values of ER for most veterinary pathogens range from 0.001 to 0.1  $\mu\text{g/ml}$  [29]; however, bovine pathogens are usually susceptible to ER at  $MIC \leq 0.06 \mu\text{g/ml}$  (Baytril-100<sup>®</sup> package insert). In addition, ER was proven to be one of the most active antimicrobial agents against *Pasteurella multocida* with an  $MIC$  of 90% of the isolates ( $MIC_{90}$ ) of 0.05  $\mu\text{g/ml}$  [39]. This organism causes a disease called hemorrhagic septicemia (HS) or bovine respiratory disease (BRD), which is considered to be a livestock disease of major economic importance in Southeast Asia [30]. The maximum serum concentration/minimum inhibitory concentration ( $C_{max}/MIC$ ) ratio and the area under the serum concentration-time curve ( $AUC_{0-24}/MIC$ ) ratio are considered as the major pharmacokinetic-pharmacodynamic (PK-PD) indices for antimicrobial activity and optimal dose determination. The criteria of  $C_{max}/MIC$  ratios of 8–10 or  $AUC/MIC$  ratios >100–125 have been suggested for maximum therapeutic efficacy and clinical effectiveness [15, 22, 23, 38]. Using an  $MIC$  breakpoint of 0.06  $\mu\text{g/ml}$ , the values of the  $C_{max}/MIC$  ratio and the  $AUC_{0-24}/MIC$  ratio obtained from s.c. administration were 11.0 and 135.3 hr, respectively, whereas they were 37.67 and 406.2 hr at a dose of 5.0 and 7.5 mg/kg BW, respectively. All of these ratio values were above the suggested values. Hence, s.c. administration of ER at a dose of 5.0 and 7.5 mg/kg BW might provide effective plasma concentration for the treatment of susceptible bacterial diseases ( $MIC \leq 0.06 \mu\text{g/ml}$ ) in Thai swamp buffaloes. However, the  $C_{max}/MIC$  (6.6) and  $AUC_{0-24}/MIC$  (81.2 hr) values were below the recommended ratios for an  $MIC$  value of 0.1  $\mu\text{g/ml}$  after s.c. administration of ER at 5.0 mg/kg BW. It is noteworthy that in this theoretical calculation, the efficacy due to CP is not included. CP is reported to have similar efficacy to its parental drug, and an increase of around 20% (equivalent to the fraction of metabolite formed) should be expected in the *in vivo* activity of the drug. Regarding our study, the drug plasma level was maintained at a higher level than the  $MIC$  (0.06  $\mu\text{g/ml}$ ) for 12 and 24 hr after ER s.c. administration at doses of 5.0 and 7.5 mg/kg BW. The side effects of ER, such as vomiting, anorexia, diarrhea and nervous signs, were not observed after s.c. drug administration with either dose.

In conclusion, the plasma concentrations of ER and its metabolite CP were measured in Thai swamp buffaloes, *B. bubalis*, after s.c. administration at doses of 5.0 and 7.5 mg/kg BW. The metabolism of ER to CP was identified also in this species, as occurs in other ruminants. ER and CP were excreted via the urine. Based on the pharmacokinetic data derived from our study and PK-PD indices, s.c. administration of ER at a dose of 5.0 and 7.5 mg/kg BW would be appropriate for the treatment of susceptible bacterial diseases in Thai swamp buffaloes. However, the dose rate of 7.5 mg/kg is recommended to treat infectious diseases caused by other veterinary pathogens having  $MIC \leq 0.1 \mu\text{g/ml}$ .

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