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Pharmacokinetics of Enrofloxacin and its Metabolite Ciprofloxacin in Badri Cows

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ABSTRACT

Background: Breeds of animals differ with respect to anatomical, physiological and behavioural traits hence the study of inter-breed variation in pharmacokinetics becomes necessary. The aim of the current investigation was to determine the pharmacokinetics of enrofloxacin following intravenous bolus administration to assess the influence of breed on drug pharmacokinetics and availability.

Methods: In the present study the pharmacokinetics of a common antimicrobial drug, enrofloxacin and its metabolite, ciprofloxacin was studied in adult, non-pregnant and dry Badri cows, wherein a commercial preparation of enrofloxacin (10% v/v) was administered at a dose rate of 7.5 mg/kg intravenously as a single dose. The plasma samples were collected at regular intervals and subjected to analysis by HPLC after appropriate processing. The results obtained were analysed using a software tool, "PK Solver" to obtain the pharmacokinetic parameters.

Result: From the study, it was revealed that the Badri cattle showed a distribution half-life of 0.11 h, a volume of distribution of 7.63 $L.kg^{-1}$, an elimination half-life of 4.27 h and an MRT of 5.93 h for enrofloxacin. Whereas, a maximum plasma concentration level of 0.12 $\mu g.ml^{-1}$ was observed for ciprofloxacin in the current study. According to the results obtained in the pharmacokinetic study, an individualized dosage regimen containing a priming dose of 5 mg/kg and a maintenance dose of 4.5 mg/kg with an interval of 24 hours was suggested for the enrofloxacin in Badri cattle. The results of our current study when compared with the previously available literature on other cattle breeds suggested that the Badri cattle differed with respect to pharmacokinetic properties and further studies will be required to determine the conclusive reason for the differences.

Key words: Badri cow, Ciprofloxacin, Enrofloxacin, High-performance liquid chromatography, Pharmacokinetics.

INTRODUCTION

A breed is defined as a group of animals with a common ancestor and certain distinguishable traits, developed through artificial selection and maintained through controlled propagation (Fleisher *et al*., 2008). Different breeds within a species differ in anatomical, physiological and behavioural traits hence, it would be inappropriate to generalize the pharmacokinetic (PK) and pharmacodynamic (PD) properties of a "species" as a whole (Toutain *et al*., 2010). So, studies investigating the differences in pharmacokinetic profiles within the different breeds of a species become essential to facilitate the proper usage of drugs in those breeds.

In the present study the Badri cattle, also known as Red Hill cattle or Pahadi cattle is studied to understand the pharmacokinetics of a commonly used antimicrobial drug, Enrofloxacin (ENR), IUPAC name: 1-cyclopropyl-7-(4-ethylpiperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. The breed is native to the Indian state of Uttarakhand and it also happens to be the first registered indigenous breed in the state. Since its recognition as a distinct breed, researchers have been curious about the various morphological, physiological and biochemical characteristics of the cattle and the economic and ecological niche of this breed in the hilly regions of the state which make these cattle unique asset of the state. The main aim of the study is to gain a better understanding of breed-dependent variation in pharmacokinetics if any, present in these cattle.

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MATERIALS AND METHODS

Location and ethical compliance

The animals were procured from the Livestock Instructional Dairy Farm, Nagla, Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Udham Singh Nagar district, Pantnagar and were housed at Livestock farm, Beni, Uttarakhand, India. The geographical coordinates of the area are 28.97°N latitude 79.41°E longitude with an elevation of 243.84 meters above mean sea level. The

study protocols of pharmacokinetics in experimental animals were approved by Institutional Animal Ethics Committee (IAEC).

Experimental design

Six healthy adults, non-pregnant and non-lactating Badri cows of 3 to 4 years of age and 130 to 140 kgs. body weight were selected for pharmacokinetic studies. The animals were acclimatized to standard experimental conditions over a pre-experimental period of one month. None of the animals had any prior exposure to antimicrobial agents for at least 3 months from the start of the experiment. The animals were maintained on an *ad-libitum* ration of stall-fed green fodder supplemented with concentrate ration and partial grazing throughout the experimental period. Access to fresh and clean drinking water was provided to these animals. Proper physical and clinical examination was done before the commencement of the experiment and the animals were found to be clinically normal. All the experimental animals were constantly monitored during the entire period of study for the appearance of any drug-related adverse effects.

Injection of drug and collection of blood samples

The commercial preparation of ENR (10% v/v) was injected intravenously as a single intravenous bolus at a dose rate of 7.5 $mg.kg^{-1}$, into the jugular vein of the Badri cattle and the blood samples were collected from the cannulated contralateral jugular vein of the animals at 5, 10, 15, 30, 60, 120, 240, 480, 720 and 1440 min intervals into heparinized blood collection vials.

Sample clean-up and elution of analyte

The plasma from each blood sample was separated and processed as per the method proposed by Nielsen and Gyrd-Hansen (1997) with few modifications as suggested by Rao *et al*. (2002) in order to elute the drug from the plasma sample before subjecting to an analysis by High-Performance Liquid Chromatography (HPLC). The method is described as follows:

Blood samples were centrifuged at 5000-6000 rpm for 15 minutes to separate plasma. For 1 mL of plasma pipetted into a microcentrifuge tube. 1.5 mL of acetonitrile was added to precipitate out proteins. The mixture was again vortexed for 10 seconds followed by centrifugation at 5000 rpm for 10 minutes at 4°C. The supernatant thus obtained was separated and double the amount of HPLC grade water was added to it. This mixture was again filtered through a 0.22 µm filter and an aliquot of 20 µL of the filtrate was injected into the HPLC system for analysis.

Method validation

The method of analysis used in the study was validated for specificity, linearity, precision (intra-day and inter-day variability), recovery, the limit of detection (LOD) and limit of quantification (LOQ) (Guideline, I.H.T., 2005). The current method utilized for this investigation was found to be specific, meaning the blank sample of plasma matrix did not contain any potential interfering substances that could interfere with the retention of the analyte. The retention time of ciprofloxacin (CIP), IUPAC name: 1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid, was found to range between 5.576 to 5.861 minutes with an average retention time of 5.72±0.012 minutes. Whereas, the retention time of ENR was observed to be in the range of 7.16 to 7.59 minutes, wherein the average retention time was observed as 7.41±0.013 minutes. The standards for ENR and CIP were found to be linear and reproducible at ranges of 0.05-10 µg. ml^{-1} and 0.1-10 µg. ml^{-1} for ENR and CIP, respectively. The inter- and intra-day coefficient of variation for ENR and CIP were calculated to be less than 15%. The recovery percentage of enrofloxacin was found to be 90.52% whereas it was estimated as 88.6% for ciprofloxacin from the plasma sample using this method.

HPLC analysis

Plasma samples were analysed simultaneously for enrofloxacin and ciprofloxacin concentrations by reversed-phase high performance liquid chromatography. The HPLC system (Shimadzu, Japan) consisted of a Parallel double micro plunger type, model number LC-20 AD solvent delivery unit, a Rheodyne manual loop injector with 20 µL loop, CTO-10 ASVP column oven and an SPD-M10AVP model diode array detector. The column used for analysis was LiChroCART ® (Merck KGaA, Germany) 125-4 RP-18 end-capped (5 µm) chromatography column fitted with a LiChroCART ® (Merck KGaA, Germany) 4-4, 5 µm, RP-18 guard column. The data acquisition and chromatogram analysis were carried out by “LC Solution software”.

An isocratic mobile phase containing acetonitrile, methanol and HPLC grade water in a 17:3:80 ratio with pH 2.5-3.0 adjusted using 0.4% orthophosphoric acid (85% v/v) and 0.4% triethylamine was used. The flow rate was maintained at 0.6 ml/min and chromatography was performed with a column temperature maintained at 20±2°C. The detection wavelength was 278 nm and the spectra were acquired in the 250-380 nm range.

The drug standards were prepared by dissolving HPLC grade (≥98.0%) ENR and CIP (Sigma-Aldrich) in 1:4 NaOH (0.1 N) and acetonitrile solution. The drug-free plasma samples were spiked with different concentrations of stock solutions and subjected to the same treatment as the sample to obtain standard chromatograms (Fig 1 and Fig 2) and calibration curves.

Following data acquisition, various pharmacokinetic parameters of enrofloxacin and its metabolite ciprofloxacin were determined by employing a menu-driven add-in program for Microsoft Excel called “PK Solver v2.0” as per the method developed and standardized by Zhang *et al*. (2010).

RESULTS AND DISCUSSION

The pharmacokinetic parameters following a single dose (7.5 $mg.kg^{-1}$) intravenous administration of enrofloxacin in Badri cows is depicted in Table 1 and the chromatogram of

the plasma sample with both ENR and CIP is depicted in Fig 3. The plasma concentration of enrofloxacin after 0.083 hours (5 minutes) of drug administration was observed to be 1.83± 0.04 µg.mL^{-1}. The drug concentration declined to 0.86±0.03 µg.mL^{-1} at 1 hour (60 minutes). Thereafter, the plasma drug concentration decreased to a minimum of 0.095±0.03 µg over a period of 24 hours (1440 minutes) (Table 2).

The compartmental analysis of the plasma drug concentration-time profile of enrofloxacin (Fig 4) was satisfactorily predicted using two-compartment model. This is in agreement with the models predicted by Ruennarong *et al.* (2016), Verma *et al.* (2003) and Sharma *et al.* (2003) but it is in contrast with the three compartmental model as predicted by Idowu *et al.* (2010) in bovines.

In the present study, the value of the zero-time intercept of distribution phase (A) was estimated to be 1.58±0.08 µg.ml^{-1}, whereas the zero-time intercept of elimination phase (B) in the present study was calculated

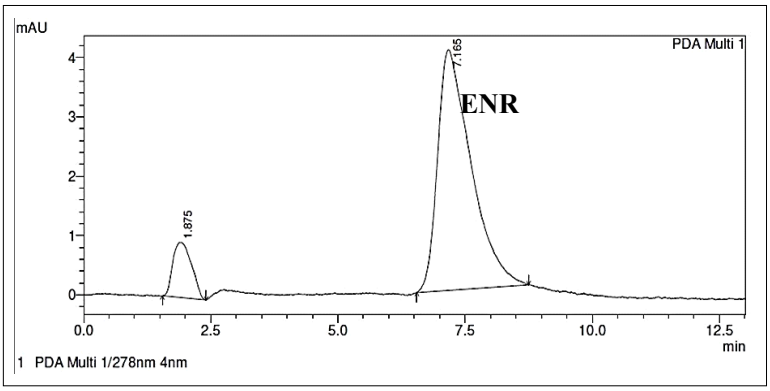


Fig 1: Chromatogram showing peak of enrofloxacin with a retention time of 7.165 minutes.

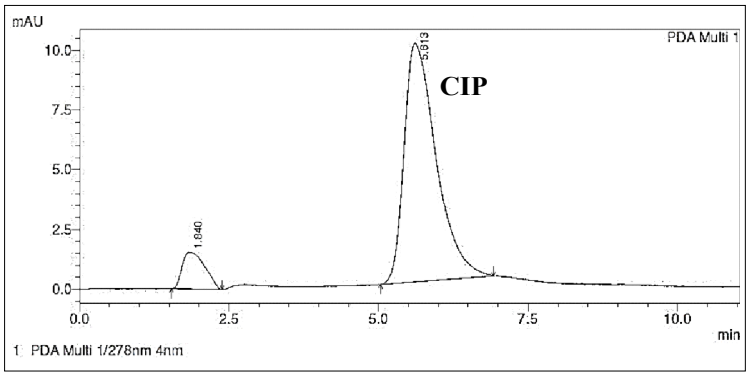


Fig 2: Chromatogram showing peak of ciprofloxacin with a retention time of 5.613 minutes.

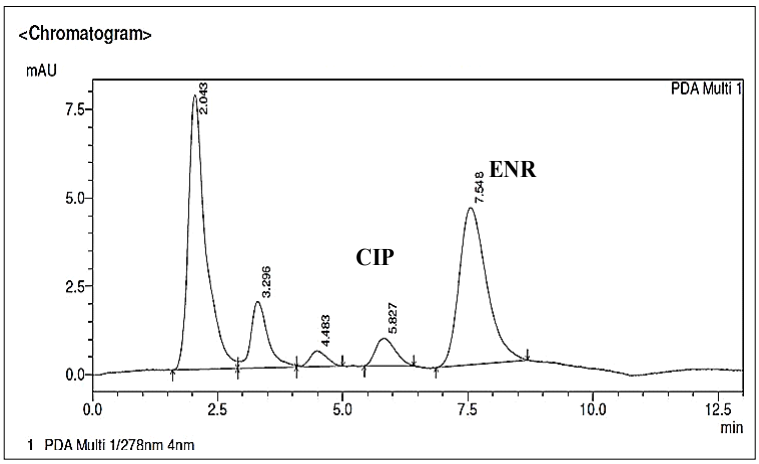


Fig 3: Chromatogram of plasma sample from Badri cow after intravenous injection of ENR. (10% v/v @ 7.5 mg.kg^{-1} b.wt.).

to be 0.96±0.30 μg.ml^{-1}. The distribution rate constant (α) was estimated as 6.95±0.61 h^{-1} with a distribution half-life ($t_{1/2\alpha}$) of 0.11±0.01 h. The elimination rate constant (β) was estimated to be 0.16± 0.01 h^{-1} with an elimination half-life ($t_{1/2\beta}$) of 4.27±0.20 h.

The initial or back extrapolated plasma drug concentration (C_0) was estimated as 2.53±0.10 μg.mL^{-1} which is 25 times higher than the minimum inhibitory concentration of 0.05-0.1 μg.mL^{-1} suggested for enrofloxacin (Prescott and Yeilding, 1990). Ruennarong *et al.* (2016) reported the plasma concentration at the initial time (C_0) to be 23.56±5.23 μg.mL^{-1} in Thai swamp buffalo following intravenous administration which was higher than the C_0 values reported in present study. Higher initial plasma concentration can be correlated with the longer distribution half-life of 6.12±0.86 h which is greater than the $t_{1/2\alpha}$ estimated in this study. While, $t_{1/2\alpha}$ of enrofloxacin reported in current investigation was in agreement with the values reported by Verma *et al.* (2003) in cross-bred Jersey cows and Sharma *et al.* (2003) in male buffalo calves, the β value of 0.28±0.02 h^{-1} reported by Verma *et al.* (2003) was higher when compared with current estimate. Sharma *et al.* (2003) reported a β value of 0.004±0.00 min^{-1} which is less than the β value reported in this study. The initial decline in plasma concentration in present investigation can be attributed to a higher distribution rate constant (α) which also correlates with shorter distribution half-life ($t_{1/2\alpha}$) of 0.11 hr or 6.6 minutes in this study (Buxton, 2017).

The apparent volume of the central compartment (V_c) in present study was calculated as 2.99±0.13 L.kg^{-1}.

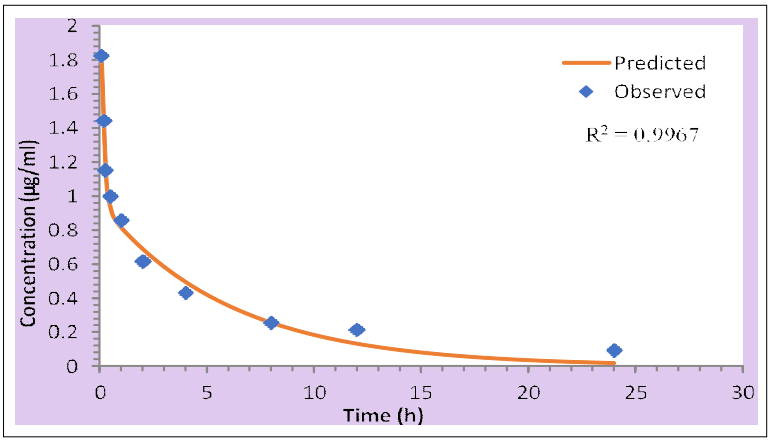


Fig 4: Predicted vs. observed linear concentration time curve of enrofloxacin.

Table 1: Pharmacokinetic parameters of enrofloxacin in plasma following single dose (7.5 mg.kg^{-1}) intravenous administration in Badri cows (n=6).

Parameter	Unit	Animal number						Mean±S.E.
		I	II	III	IV	V	VI	
C_0	μg.ml^{-1}	2.71	2.14	2.82	2.38	2.69	2.44	2.53±0.10
A	μg.ml^{-1}	1.66	1.31	1.85	1.45	1.70	1.49	1.58±0.08
B	μg.ml^{-1}	1.06	0.83	0.97	0.94	0.99	0.95	0.96±0.30
α	h^{-1}	9.13	4.81	8.08	6.24	6.93	6.51	6.95±0.61
β	h^{-1}	0.18	0.14	0.18	0.14	0.17	0.17	0.16±0.01
$t_{1/2\alpha}$	h	0.08	0.14	0.09	0.11	0.10	0.11	0.11±0.01
$t_{1/2\beta}$	h	3.78	4.98	3.94	4.78	3.98	4.13	4.27±0.20
V_c	L.kg^{-1}	2.77	3.50	2.66	3.15	2.79	3.07	2.99±0.13
$V_{d\ (area)}$	L. kg^{-1}	7.00	8.57	7.26	7.99	7.47	7.49	7.63±0.23
V_{ss}	L.kg^{-1}	6.69	8.23	7.14	7.47	6.99	7.29	7.30±0.22
CL_B	L.kg .h^{-1}	1.26	1.20	1.31	1.12	1.27	1.27	1.24±0.03
$AUC_{0-\infty}$	μg.h$_2$.ml^{-1}	5.95	6.25	5.74	6.70	5.90	5.89	6.07±0.14
AUMC	μg.h .ml^{-1}	31.54	43.03	31.38	44.66	32.50	33.73	36.14±2.47
MRT	h	5.30	6.88	5.47	6.67	5.51	5.73	5.93±0.28

Where, C_0= initial or back extrapolated plasma drug concentration; A= Zero-time plasma drug concentration intercept of regression line of distribution phase; B= Zero-time plasma drug concentration intercept of regression line of elimination phase; α= Distribution rate constant; β= Elimination rate constant; $t_{½\ \alpha}$ = distribution half-life; $t_{½\ \beta}$= elimination half-life; V_{ss}= Volume of distribution at steady state; V_c= Apparent volume of central compartment; $V_{d\ (area)}$= Volume of distribution; CL_B = Total body clearance; $AUC_{0-\infty}$= AUC from time zero to infinity; AUMC= Area under the moment curve; MRT= Mean residence time.

The volume of distribution $V_{d\ area}$ for enrofloxacin was determined as 7.63±0.23 L.kg^{-1}. The volume of distribution relates drug concentration in the plasma to the amount of drug in the body and is an important indicator of the tissue penetration of a drug (Toutain and Bousquet-M ´Elou, 2004). Since volume of distribution is a measure of the extravascular distribution of a drug. The higher $V_{d\ area}$ value reported in present study indicates that fluoroquinolones have a higher distribution and penetration to different organs in the body and thus, is advantageous for therapeutic purposes. The difference in $V_{d\ area}$ when compared to other studies may be attributed to the difference in age and body weight of this particular breed (Varma, 2008).

The plasma clearance (CL_B) in this study was calculated as 1.24±0.03 L.kg^{-1}.h^{-1}. The total body clearance reported in present study was in agreement with the values reported by Verma *et al.* (2003) but was less than the body clearance values reported by Sharma *et al.* (2003). The total body clearance is a better index of efficiency of drug elimination as it gives the clearance of drug from blood per unit time. The difference in the clearance observed in this study is attributed to the age and body weight differences between Badri and other breeds of cattle.

After IV administration, the half-life of a drug is measured and is a parameter that indicates the time required for the plasma concentration to decrease by fifty percent of the original concentration after a quasi-equilibrium of drug distribution has been established. The half-life calculated when the decrease in plasma concentration of the drug is due solely to elimination of the drug, then the term elimination half-life applies. The elimination half-life of enrofloxacin ($t_{1/2\beta}$) in present study was 4.27±0.20 h, which is higher when compared with those reported by Sharma *et al.* (2003) and Verma *et al.* (2003) but is in agreement with those reported by Idowu *et al.* (2010) for lactating dairy cows. The difference in the mean elimination half-life ($t_{1/2\beta}$) observed in this study as compared to those reported can be attributed to the larger volume of distribution observed in Badri cattle as compared to other cattle breeds.

The mean area under curve from zero to infinity ($AUC_{0-\infty}$) was calculated to be 6.07±0.14 µg.h.ml^{-1}. The AUC values were lesser than those reported by Ruennarong *et al.* (2016) but comparatively higher than those reported by Idowu *et al.* (2010) and Sharma *et al.* (2003). Based on the ratio between the AUCs of ciprofloxacin and enrofloxacin, the plasma levels of ciprofloxacin represented 11% of the parent substance.

The time-concentration profile of ciprofloxacin is shown in Fig 5 and the pharmacokinetic values describing the disposition kinetics of ciprofloxacin following single intravenous dose (7.5 mg.kg^{-1}) of enrofloxacin are presented in Table 3. Ciprofloxacin, is an active metabolite of enrofloxacin and the amount of ciprofloxacin produced from enrofloxacin varies between and even within the animal species and should not be expected to have a significant effect on the therapy (Walker, 2000).

In present study ciprofloxacin appeared in plasma at an initial concentration of 0.044±0.005 µg.mL^{-1} at 0.083 hours

Table 2: Observed plasma concentration (µg.mL^{-1}) of enrofloxacin following single dose (7.5 mg.kg^{-1}) intravenous administration in cows (n=6).

Time of collection (h)	0.083	0.166	0.25	0.5	1	2	4	8	12	24
Plasma drug concentration (µg.mL^{-1}) Mean±SEM (n=6)	1.83±0.035	1.44±0.009	1.16±0.020	1.00±0.016	0.86±0.025	0.62±0.122	0.43±0.006	0.26±0.004	0.22±0.007	0.095±0.03

(5 minutes) following administration of enrofloxacin. The concentration increased to 0.12±0.01 µg.mL^{-1} at 1 hour (60 minutes) and thereafter it decreased to a value of 0.02±0.001 µg.mL^{-1} at 8 hour (480 minutes) (Table 4). The plasma concentration time profile adequately fitted to one compartmental model. The zero-time intercept of elimination phase (B) was 0.15±0.01 µg.mL^{-1}. Metabolite rate forming constant (k_f) was 4.61±0.37 h^{-1} with the half-life of metabolite formation ($t_{1/2}k_f$) estimated as 0.16±0.01 h. The rate constant describing elimination of a drug (β) was calculated as 0.21 ±0.01 h^{-1} and the elimination half-life ($t_{1/2\beta}$) was 3.28±0.13 h. The elimination half-life of ciprofloxacin reported in current investigation was in agreement with Idowu *et al.* (2010) and Verma *et al.* (2003).

The peak plasma concentration (C_{max}) was calculated to be 0.12±0.01 µg.mL^{-1} which is less than the C_{max} value of 0.42±0.02 µg.mL^{-1} reported by Sharma *et al.* (2003). However, time required to achieve maximum concentration (T_{max}) calculated as 0.71±0.04 h was in agreement with the study conducted by Idowu *et al.* (2010) and Sharma *et al.* (2003).

Table 3: Pharmacokinetic parameters of ciprofloxacin in plasma following single dose (7.5 mg.kg^{-1}) intravenous administration of enrofloxacin in Badri cows (n=6).

Parameter	Unit	Animal number						Mean±S.E.
		I	II	III	IV	V	VI	
B	µg.ml^{-1}	0.13	0.16	0.15	0.13	0.14	0.16	0.15±0.01
k_f	h^{-1}	5.86	5.11	3.78	4.49	3.42	4.99	4.61±0.37
â	h^{-1}	0.21	0.19	0.20	0.23	0.24	0.21	0.21±0.01
$t_{1/2}k_f$	h	0.12	0.14	0.18	0.15	0.20	0.14	0.16±0.01
$t_{1/2\hat{a}}$	h	3.34	3.73	3.48	3.00	2.88	3.27	3.28±0.13
V/F	mL.kg^{-1}	7072.52	6635.50	7187.76	7426.40	7508.16	6137.27	6994.60±212.72
CL/F	mL.kg^{-1}.h^{-1}	1466.67	1232.59	1431.89	1716.45	1809.53	1302.1	1493.21±92.88
T_{max}	h	0.59	0.67	0.82	0.70	0.83	0.66	0.71±0.04
C_{max}	µg.ml^{-1}	0.11	0.14	0.12	0.10	0.11	0.13	0.12±0.01
$AUC_{0-"}$	µg.h.ml^{-1}	0.61	0.83	0.73	0.53	0.56	0.72	0.66±0.05
AUMC	µg.h^2.ml^{-1}	3.04	4.65	3.88	2.43	2.47	3.54	3.34±0.35
MRT	h	4.99	5.58	5.28	4.55	4.44	4.91	4.96±0.18

Where, B= Zero-time plasma drug concentration intercept of elimination phase; k_f= Metabolite rate forming constant; β= Elimination rate constant; $t_{1/2}k_f$= Half-life of metabolite formation; $t_{1/2\beta}$= Elimination half-life; V/F= Volume of distribution when fraction of dose absorbed is not known; CL/F= Clearance when fraction of dose absorbed is not known; T_{max}= Time to reach maximum plasma concentration; C_{max} = Maximum plasma concentration; AUC_{0-8}= AUC from time zero to infinity; AUMC= Area under the moment curve; MRT= Mean residence time.

Table 4: Observed plasma concentration (µg.mL^{-1}) of ciprofloxacin (a metabolite of enrofloxacin) following single dose (7.5 mg.kg^{-1}) intravenous administration of enrofloxacin in cows (n=6).

Time of collection (h)	0.083	0.166	0.25	0.5	1	2	4	8
Plasma drug concentration	0.044±0.005	0.075±0.008	0.094±0.004	0.106±0.004	0.12±0.005	0.1±0.006	0.07±0.005	0.02±0.001

(µg.mL^{-1}) Mean±SEM (n=6).

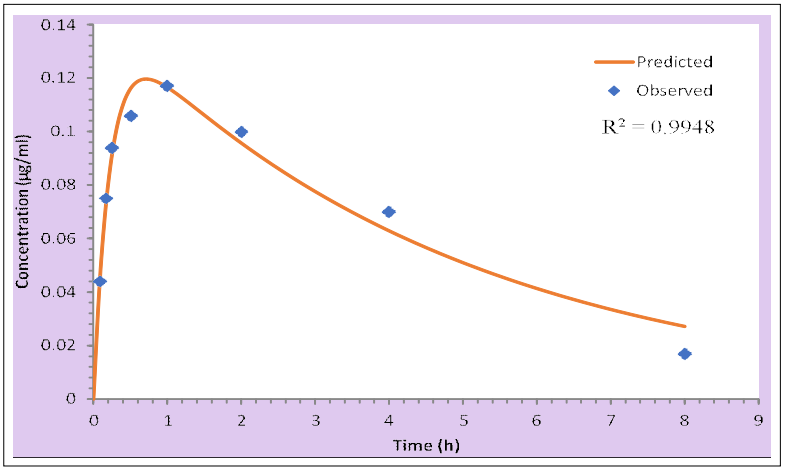


Fig 5: Predicted vs. observed linear concentration time curve of ciprofloxacin.

The mean area under curve from zero to infinity ($AUC_{0-\infty}$) was calculated to be 0.66±0.05 μg.h.ml^{-1} which was lesser when compared to those reported by Verma *et al.* (2003).

A therapeutic concentration of 0.1 μg.mL^{-1} (Prescott and Yeilding, 1990) was considered and based on the observations in the pharmacokinetic study the following intravenous dosage regimen was suggested for enrofloxacin. A priming dose (D) of 5 mg.Kg^{-1} and a maintenance dose of 4.5 mg.kg^{-1} for every 12 hr was estimated to maintain the therapeutic concentration. The minimum steady state concentration $C_{ss(min)}$ and the maximum steady state concentration $C_{ss(max)}$ were estimated as 0.16 and 1.15 μg.mL^{-1}, respectively. However, owing to the concentration dependent action of the fluoroquinolones and the post-antibiotic effects reported to last for 4-8 hr the recommended dosage regimen could be 4.5 mg at every 24 hr interval but a higher dosage can be used for pathogens with higher MIC. Proper care such as adherence to specified withdrawal periods is required for use of this drug in food producing animals.

CONCLUSION

Based on the study it can be observed that the breed specific variation in pharmacokinetic parameters exit in Badri cattle and therefore the underlying mechanisms for such difference should be further investigated. These differences must be accounted for during the preparation of dosage regimen of a drug. Furthermore, it is also recommended that as the Badri cattle showed lower distribution half-life, higher volume of distribution, longer elimination half-life and mean residence time than those reported in available literatures for other cattle breeds, the dosage regimen of enrofloxacin calculated in this investigation is recommended to be followed.

Authorship contribution statement

S. Ramanarayanan conceived and designed the analysis, collected the data, performed the analysis and wrote the manuscript. S.P. Singh was involved in the design of the experiment, analysis of the data and preparation of the manuscript. A.H. Ahmad was involved in the designing of the experiment, analysis of the data, interpretation of results and statistical analysis. Deeksha Maletha was involved in the collection of data and preparation of the manuscript.

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Conflict of interest

All authors declare that they have no conflict of interest.

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