

## SOME PHARMACOKINETIC DATA AND DOSAGE REGIMEN OF DANOFLOXACIN AFTER INTRAVENOUS ADMINISTRATION IN NILI-RAVI AND KUNDHI BUFFALOES

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### ABSTRACT

Danofloxacin is a fluoroquinolone antibiotic which is extensively used against a variety of bacterial infection in animals worldwide except in Pakistan. The optimal dosage regimen of danofloxacin was necessary in order to use this drug therapeutically and to minimize the emergence of bacterial resistance in local buffalo breeds in Pakistan. For this, the present study aimed to investigate the pharmacokinetics behavior and optimal dosage regimen of danofloxacin in 16 adult healthy buffaloes, 8 each of Nili-Ravi and Kundhi breed following single intravenous administration at the dose of 2.5 mg/kg body weight. Blood samples (5 ml) were drawn from jugular vein in heparinized plastic centrifuge tubes at zero and 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 24 h after injection and drug concentrations in plasma were measured by HPLC method. The distribution of drug was rapid whereas elimination was low in both breeds. Volume of distribution at steady state ( $V_{ss}$ ) was significantly ( $P<0.05$ ) higher in Kundhi buffaloes indicating the extensive

distribution of danofloxacin in Kundhi than Nili-Ravi buffaloes. However, the total body area covered by drug was remarkably lower in Kundhi than Nili-Ravi buffaloes as indicated by lower values of area under concentration time curve (AUC) in Kundhi than Nili-Ravi buffaloes. Total body clearance ( $Cl_b$ ) was significantly ( $P<0.05$ ) faster in Kundhi buffaloes than Nili-Ravi buffaloes. The mean residence time (MRT) was noted to be (Mean $\pm$ SD) 4.78 $\pm$ 0.52 and 4.44 $\pm$ 0.37 h in Nili-Ravi and Kundhi buffaloes respectively. On the basis of pharmacokinetic parameters, suitable intravenous dosage regimens for danofloxacin in Nili-Ravi and Kundhi buffaloes would be 6.5 and 8.3 mg/kg to be repeated after 12 h intervals. The present study is the foremost pharmacokinetics study of danofloxacin in the local species which would provide the valuable contribution in the local manufacturing of danofloxacin in Pakistan in future.

**Keywords:** buffaloes, *Bubalus bubalis*, pharmacokinetics, danofloxacin, dosage regimen, plasma protein binding

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## INTRODUCTION

Danofloxacin (DNF) is a synthetic antibacterial agent of fluoroquinolone group, developed specifically for veterinary use. DNF possesses great potential against a variety of microorganisms by concentration-dependent killing mechanisms (Sarasola *et al.*, 2002). In field experiments, DNF was found to be highly effective in the treatment of bovine respiratory diseases associated with *Pasteurella* and *Mannheimiasis* species (Giles, 1996). *Pasteurella multocida* is the primary cause of Haemorrhagic septicemia (HS), which is a common disease of buffaloes and cattle causing high mortality in many countries of Asia, Middle East and sub-Saharan Africa (Verma and Jaiswal, 1998). Moreover, DNF displayed great potential against respiratory as well as many other infections caused by *Mycoplasma* species in cattle, pigs and poultry (Cooper *et al.*, 1993).

For the successful therapy, optimal dose of any drug should be strictly followed during the treatment. Wiuff *et al.* (2003) suggested the suboptimal dosage regimens of fluoroquinolones to be an important risk factor for the emergence of resistance in experimental studies. One clinical approach to prevent the resistance is to adjust the antibiotic dosage regimens upwards to the mutant prevention concentrations. Thus, the pharmacokinetic study of a drug is required in the species in which the drug is going to be used clinically for the selection of optimal dosing schedule.

Many drugs at the same dose rate showed variable pharmacological effects due to the diverse pharmacokinetics behavior among several species. Sidhu (2001) have determined such differences in fluoroquinolones. In addition, many studies revealed that the pharmacokinetics behavior,

optimal dosage, renal clearance and urinary excretion of the investigated drugs were different under indigenous conditions when compared with the values given in the literature or in the product inserts supplied by the manufacturers (Muhammad, 1997). The change in the genetic makeup has an influence on the biodisposition and fate of drugs in a population (Muhammad *et al.*, 2003). Such geonetical influences have been reported for blood and urine pH, blood proteins, drug metabolism and kidney function in buffaloes, cows, sheep and goats (Nawaz *et al.*, 1988). In veterinary field, DNF is being used successfully in many parts of the world except in Pakistan. The pharmacokinetics of danofloxacin has been studied in cattle, sheep and goats (McKellar *et al.*, 1998; Aliabadi and Lees, 2001; Sarasola *et al.*, 2002). Recently, Sappal *et al.* (2009) examined the kinetic behavior of DNF in buffalo calves, but no study exists describing the pharmacokinetics of DNF in adult buffaloes. Therefore, the present project has been planned to determine the pharmacokinetics and optimal dosage regimen of DNF following intravenous administration in adult local buffaloes of two breeds (Nili-Ravi and Kundhi) and to explore the interspecies variation between them. In Pakistan, this is the first study regarding the pharmacokinetics behavior of DNF in local buffaloes. The study will facilitate the pharmaceutical companies/manufacturers in the local manufacturing of DNF in Pakistan.

## MATERIALS AND METHODS

### Experimental animals

The study was performed in sixteen healthy adult female buffaloes, eight each of Nili-Ravi and Kundhi breed, weighting between 350 to 430 kg

and 310 to 370 kg respectively. The animals were 2 to 3 years of age of both breeds. The animals were kept at Livestock Experimental Farm, Institute of Nutrition and Feed Technology, University of Agriculture, Faisalabad and examined for any apparent clinical condition for 2 weeks prior start of the experiment. All the buffaloes were housed in a separate pen provided by wire mesh barriers to maintain social contact. The animals were fed on seasonal green fodder and water was provided ad libitum. The research protocol was critically reviewed and approved by the Institutional Animal Ethics Committee (IAEC). The animals were cared for according to the national animal welfare guidelines and technical supervision was provided at all the times.

### Methodology

The animals were weighted individually and restrained in standing position before the start of each experiment. One of the jugular veins of each animal was cannulated under strict aseptic conditions with plastic cannula No. 90 (Protex Ltd., England) for the collection of blood samples. A commercial preparation of danofloxacin mesylate (CAS 119478-55-6) in powdered form was procured from Hanqzou Thick Chemical Co. Ltd. China. An injectable preparation of danofloxacin (10%) was prepared and injected intravenously in the jugular vein at dose rate of 2.5 mg/kg body weight to each animal.

The blood samples (5 ml) were drawn from jugular vein in heparinized plastic centrifuge tubes at zero and 0.083, 0.167, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 24 h post administration of drug. The plasma was separated and stored at -20°C until analysis.

### Analytical procedure

Danofloxacin concentrations in plasma were determined by High Performance Liquid Chromatographic (HPLC) method as described by Soback *et al.* (1994) with certain modifications.

### Chemicals

All the chemicals used were analytical grade. Danofloxacin standard (Danofloxacin mesylate, 99%) was used as external standard, while the paracetamol (Paracetamol 99.7%) was used as internal standard (IS); these standards were requested from the Ferozsons Pvt. Ltd., Pakistan. Methanol and acetonitrile (Fisher Scientific Limited, UK) and phosphoric acid (Merk, Germany) were used.

### Instrumentation and chromatographic conditions

The HPLC system contained quarternary solvent delivery module, a UV- and visual-light variable-wavelength detector (Sykam, S-3210) and an integrator (HP 1050; Hewlett-Packard, Waldbron, Germany). A reversed-phase base deactivated column packed with YMC pack A-312 (Thermo Hypersil-Keystone, BDS-C<sub>18</sub> with 250 × 4.6 mm dimensions and 5 µm particle size) was used. The column was protected with a pre-column (Guard-Pak™) filled with a µBondapak™ C<sub>18</sub> cartridge (Thermo Hypersil, England). The mobile phase consisted of a mixture of 800 ml of 14 ml/L phosphoric acid (Merk, Germany) and 200 ml of acetonitrile (Fisher Scientific Limited, UK) per liter at pH 6.0. The flow rate was set at 1 ml/min. The detector wavelength was adjusted to 275 nm. The lower limit of detection (LOD) was 0.005 µg/ml while the limit of quantitation (LOQ) was 0.25 µg/ml. The output of the detector was monitored with computer software (Peak Simple Chromatography

Data System, Buck Scientific Inc., East Norwalk). Separation of danofloxacin was achieved at 37°C, using an isocratic mode.

### Stock solutions and standards

Danofloxacin and paracetamol (IS) were made up as 1 g/L stock solutions in methanol and distilled water (1: 10, by volume). Danofloxacin was diluted with distilled water to make additional working stocks of 10 mg/L for the plasma assay. Paracetamol was diluted with mobile phase to make a single working IS stock solution of 1 mg/ml. Plasma calibrators (0.5 to 32 µg/ml) for the calibration curve were prepared in drug-free control plasma. The working solutions were used to supplement the drug free matrices.

### Sample preparation

In a centrifuge tube, 2 ml of acetonitrile was added to 1 ml of plasma or plasma calibrator. The mixture was vortexed for one minute and centrifuged for 30 minutes at 4000 rpm. The supernatant was transferred into a glass tube and evaporated to dryness in a water bath. The residue was then reconstituted in 10 µl of IS and 1 ml of 14 ml/L phosphoric acid. The final solution was again vortexed for 30 seconds; filtered through cellulose acetate membrane filter (Sartorius AG, Germany, 13 mm diameter) and 0.2 µl was injected into the HPLC system.

### Standard curve

Working standards having danofloxacin concentrations 0.5, 1, 2, 4, 8, 16 and 32 µg/ml were prepared in the drug free plasma of adult buffaloes. These working standards were analyzed using HPLC and concentrations versus peak area data were plotted on a graph to construct the calibration curves. The curves were linear over the range of

2-32 µg/ml for plasma ( $R^2 = 0.9968$ ;  $y = 48.709x + 0.2083$ ) as shown in Figure 1. The concentrations of danofloxacin in samples were determined by the regression equation:

$$Y = bx+a$$

Where, Y = Peak area for unknown concentration of danofloxacin determined through HPLC, a = Intercept, b = Slope of regression line, x = Concentration of danofloxacin.

### *In vitro* plasma protein binding

*In vitro* plasma protein binding was determined by ultrafiltration technique according to the method described by Bratton and Marshall (1939) which is based on the physical separation of free drug molecules in plasma water from drug bound to plasma proteins by filtering plasma samples through a semi permeable membrane under a positive pressure generated by centrifugation. Stock solutions of danofloxacin were used to prepare the drug concentration in plasma ranging from 50, 100, 150, 200 and 250 µg/ml. Ultrafiltrates of the plasma samples were prepared by ultrafiltration through a cellophane membrane with pore-size of 20-80°A which permit molecules with a molecular weight up to 5000 to pass through the membrane. After ultrafiltration, drug concentration in the ultrafiltrate was the concentration of the free drug in plasma and the fraction of unbound drug was measured using the equation below:

$$\text{Unbound drug} = \frac{\text{Drug (Ultrafiltrate)}}{\text{(Total drug in plasma)}}$$

### Pharmacokinetics and dosage regimen

Pharmacokinetic parameters were calculated by non-compartment and compartment

analyses with non linear least square regression method using a computerized program WinNonlin (Pharsight Corporation, Mountain View, version 6.3, CA, USA). For comparing the pharmacokinetic parameters obtained by two methods, Minimum Akaike Information Criteria Estimates (MAICE) in different models were applied to determine the best fit of model for each animal (Yamaoka *et al.*, 1978).

The maintenance dose (D) was based on the minimum effective concentration ( $C^{\circ}P$  (min))/peak desired concentration of the drug in plasma which is sufficient to eradicate the bacterial population in the body. The equation was given by:

$$D = C^{\circ}P (\text{min}). Vd (e^{\beta t}-1)$$

Where,  $C^{\circ}P$  represents the minimum effective concentration/peak desired plasma

drug concentration,  $Vd$  denotes the volume of distribution,  $e$  =exponential term,  $\beta$  = over all elimination rate constant,  $t$  = time at which dose was calculated.

The priming dose was obtained by omitting “-1” from the above equation.

## STATISTICAL ANALYSIS

Mean with standard deviation values for each parameter was calculated by Microsoft Excel version 2007. Two tailed Student’s T test was used to compare the pharmacokinetic parameters in Nilli-Ravi and Kundhi buffaloes.  $P \leq 0.05$  was considered as significant.

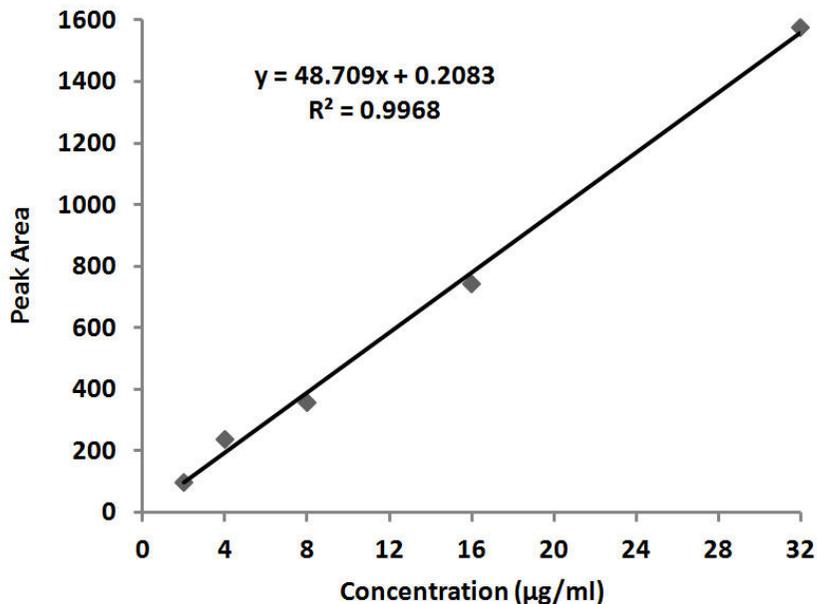


Figure 1. Standard curve of danofloxacin in plasma of buffaloes.

## RESULTS AND DISCUSSION

The chromatogram of danofloxacin standard in the plasma of buffaloes is presented in Figure 2. The Mean±SD plasma levels of danofloxacin versus different time intervals as a function of time following intravenous administration in Nili-Ravi and Kundhi buffaloes

are described as a semi-logarithmic plot (Figure 3). The pharmacokinetic parameters obtained are depicted in Table 1.

Data were best fitted by a two-compartment model with no lag time in all the buffaloes.

$$C(t) = Ae^{-at} + Be^{-bt}$$

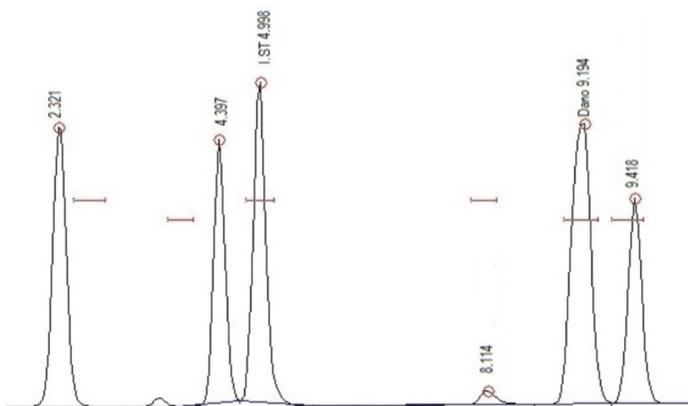


Figure 2. Chromatogram of 32 µg/ml danofloxacin standard in the plasma of buffalo.

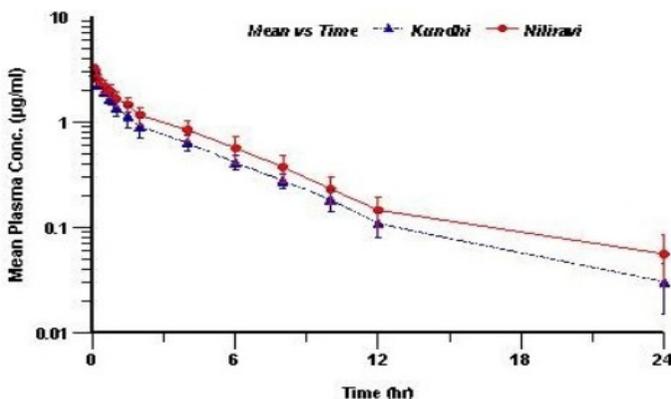


Figure 3. Semilogarithmic plot of danofloxacin concentrations (mean±SD) in plasma following (i.v.) administration at dose rate of 2.5 mg/kg in Nili-Ravi and Kundhi buffaloes.

Where  $C(t)$  is the plasma concentration of danofloxacin;  $t$  is the time after drug administration;  $A$  and  $\alpha$  are the intercepts and slope of the distribution phase;  $B$  and  $\beta$  are the intercepts and slope of the elimination phase; and  $e$  is the base of natural logarithm.

The Mean $\pm$ SD plasma concentration of danofloxacin was significantly ( $P<0.05$ ) lower ( $3.00\pm 0.17$   $\mu\text{g/ml}$ ) in Kundhi buffaloes than Nili-Ravi buffaloes ( $3.30\pm 0.19$   $\mu\text{g/ml}$ ) obtained at 5 minutes after injection. The plasma concentration

declined in two phases, i.e. a rapid decline to  $1.16\pm 0.22$   $\mu\text{g/ml}$  and  $0.90\pm 0.20$   $\mu\text{g/ml}$  at 2 h, followed by a slow decline to  $0.056\pm 0.028$   $\mu\text{g/ml}$  and  $0.03\pm 0.015$   $\mu\text{g/ml}$  at 24 h in Nili-Ravi and Kundhi buffaloes respectively. The maximum concentration ( $C_p^0$ ) achieved after danofloxacin administration at zero time was considerably ( $P<0.05$ ) lower ( $3.32\pm 0.25$   $\mu\text{g/ml}$ ) in Kundhi buffaloes as compared to Nili-Ravi buffaloes ( $3.66\pm 0.74$   $\mu\text{g/ml}$ ). In the current study, danofloxacin was detected up to 24 h in plasma

Table 1. Pharmacokinetic parameters of danofloxacin after IV administration in 16 buffaloes (Mean  $\pm$  SD) using two compartment model.

Parameter/variable	Nili-Ravi buffaloes	Kundhi buffaloes
A ( $\mu\text{g mL}^{-1}$ )	1.72 $\pm$ 0.11	1.86 $\pm$ 0.32
B ( $\mu\text{g mL}^{-1}$ )	1.94 $\pm$ 0.21	1.46 $\pm$ 0.46*
$C_p^0$ ( $\mu\text{g mL}^{-1}$ )	3.66 $\pm$ 0.14	3.32 $\pm$ 0.25*
$t_{1/2\alpha}$ (hr)	0.25 $\pm$ 0.09	0.31 $\pm$ 0.10
$t_{1/2\beta}$ (hr)	3.26 $\pm$ 0.43	3.49 $\pm$ 0.87
$k_{12}$ (L.hr $^{-1}$ )	1.11 $\pm$ 0.28	1.02 $\pm$ 0.31
$k_{21}$ (L.hr $^{-1}$ )	1.63 $\pm$ 0.31	1.64 $\pm$ 0.32
AUC ( $\mu\text{g. h. mL}^{-1}$ )	9.80 $\pm$ 2.14	7.74 $\pm$ 0.98*
$Cl_B$ (Lkg $^{-1}$ h $^{-1}$ )	0.49 $\pm$ 0.21	0.32 $\pm$ 0.04*
$K_{el}$ (L.hr $^{-1}$ )	0.38 $\pm$ 0.06	0.43 $\pm$ 0.05
$\alpha$ (hr)	2.92 $\pm$ 0.59	2.48 $\pm$ 0.78
$\beta$ (hr)	0.21 $\pm$ 0.02	0.20 $\pm$ 0.04
$V_{darea}$ (L.kg $^{-1}$ )	2.28 $\pm$ 0.86	1.67 $\pm$ 0.54
$V_{ss}$ (L.kg $^{-1}$ )	1.14 $\pm$ 0.12	1.47 $\pm$ 0.37*
$V_c$ (L.kg $^{-1}$ )	0.68 $\pm$ 0.02	0.75 $\pm$ 0.05*

A,B = Y-axis intercept terms for distribution and elimination phases,

$C_p^0$  = Plasma drug concentration at the time of drug administration,

$t_{1/2\alpha}$ =Distribution half life,  $t_{1/2\beta}$  = Elimination half-life, AUC = Area under concentration,

$Cl_B$  = Total body clearance,  $K_{el}$  = Elimination rate constant,

$\alpha$  and  $\beta$  = Distribution and Elimination rate constants,

$V_{darea}$  = Volume of distribution at terminal phase of elimination,

$V_{ss}$  = Volume of distribution at steady state,  $V_c$  = Volume of central compartment.

\*Significant at ( $P < 0.05$ ).

after higher doses (2.5 mg/kg) in both breeds of buffaloes. Apley and Upson (1993) also reported the similar findings in cow calves. Similarly, some other investigators (Shem-Tov *et al.*, 1998; McKellar *et al.*, 1999; Shojaee Aliabadi and Lees, 2003) documented the DNF detection up to 24 h in various biological fluids. Hence, the results of present study are consistent with the results described by Apley and Upson (1993); Shem-Tov *et al.* (1998); McKellar *et al.* (1999); Shojaee Aliabadi and Lees (2003). On the other hand, DNF was quantified up to 4 h in goats and 12 h in cattle (Giles *et al.*, 1991; Atef *et al.*, 2001).

The distribution half-life ( $t_{1/2\alpha}$ ) was 0.25±0.09 and 0.31±0.10 h, whereas, elimination half-life ( $t_{1/2\beta}$ ) for DNF was 3.26±0.43 and 3.49±0.87 h in Nili-Ravi and Kundhi buffaloes respectively with no significant difference between them (Table 1). Sappal *et al.* (2009) determined this value to be 0.16±0.07 hours in buffalo calves. Similarly, rapid distribution of danofloxacin was also observed in cows, ewes and goats (Atef *et al.*, 2001; Shem-Tov *et al.*, 1997, 1998).

The higher volume of distribution at steady state ( $V_{ss}$ ) suggests the good tissue penetration of DNF in adult local buffaloes. However, the value of  $V_{ss}$  was remarkably lower in Nili-Ravi buffaloes (1.14±0.12 L/kg) than Kundhi buffaloes (1.47±0.37 L/kg) indicating the best tissue penetration of DNF in Kundhi buffaloes than Nili-Ravi buffaloes, but at terminal phase ( $Vd_{area}$ ), these values were insignificant between them. However, the values of  $Vd_{area}$  in both breeds were lower than value of ( $Vd_{area} = 4.35±2.02$  L/kg) articulated by Sappal *et al.* (2009) in buffalo calves. The volume of the central compartment ( $V_c$ ) was also lower in Nili-Ravi buffaloes (0.68±0.02 L/kg) than Kundhi buffaloes (0.75±0.05 L/kg).

Area under the plasma concentration time

curve (AUC) was lower (7.74±0.98  $\mu\text{g}\cdot\text{h}\cdot\text{mL}^{-1}$ ) in Kundhi buffaloes as compared to Nili-Ravi buffaloes (9.80±2.14  $\mu\text{g}\cdot\text{h}\cdot\text{mL}^{-1}$ ) suggesting the large body area covered by DNF in Nili-Ravi than Kundhi buffaloes. Sappal *et al.* (2009), on the other hand, determined the lower value of AUC (1.79±0.028  $\mu\text{g}\cdot\text{hr}/\text{ml}$ ) than the current values (9.80±2.14 and 7.74±0.98  $\mu\text{g}\cdot\text{hr}/\text{ml}$ ) at lower doses (1.25 mg/kg) in buffalo calves. Kundhi buffaloes exhibited faster clearance (0.30±0.05  $\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) than Nili-Ravi buffaloes (0.23±0.05  $\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) in non compartment analysis (Table 2). The elimination half life ( $t_{1/2\beta}$ ) was comparable ( $P>0.05$ ) in both breeds of adult buffaloes in the current study, but shorter than ( $t_{1/2\beta} = 4.24±1.78$  h) in buffalo calves at lower doses (Sappal *et al.*, 2009). Aliabadi *et al.* (2003) determined this value to be 3.39 h following intravenous administration of DNF at dose rate of 1.25 mg/kg in sheep. The values of total body clearance ( $Cl_B = 0.23±0.05$  and  $0.30±0.05$   $\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ), suggested the low elimination of DNF in both breeds. To classify the drug clearance, Toutain and Bousquet-Melou (2004) determined the breakpoint clearance values as high (28 ml/kg/min), medium (12 ml/kg/min) or low (5.8 ml/kg/min), based on extraction ratios of 0.35, 0.15 and 0.05 respectively. DNF clearance (3.83 ml/kg/min) in Nili-Ravi and (5 ml/kg/min) in Kundhi buffaloes may be classified as low clearance in the current study. The mean residence time (4.78±0.52 and 4.44±0.37 h) values obtained by non-compartmental analysis were also in close agreement of low clearance as indicated by  $Cl_B$  values in Nili-Ravi and Kundhi buffaloes.

At plasma concentrations of 50 to 250  $\mu\text{g}/\text{ml}$ , the extent of plasma protein binding of DNF ranged from 23.6% to 30.4% with an overall mean of 27±2.83% in current study (Table 3). In general, the plasma protein binding of fluoroquinolones is

low and it directly affects the kinetic behavior and therapeutic efficacy of the drug. Sappal *et al.* (2009) determined relatively higher (36%) plasma protein binding for DNF in buffalo calves as compared to the current value (27%) in adult buffaloes. The low plasma protein binding along with other physicochemical properties of DNF may allow

its widespread penetration into intracellular and extra vascular sites, which was further supported by high values of  $V_{ss}$  ( $1.14 \pm 0.12$  and  $1.47 \pm 0.37$  L/kg) obtained in both breeds. The plasma protein binding of fluoroquinolones differ largely amongst species. Kaartinen *et al.* (1995) reported 36 to 45% protein binding for enrofloxacin in lactating

Table 2. Pharmacokinetic parameters of danofloxacin after IV administration in 16 buffaloes (Mean  $\pm$  SD) using non-compartment model.

Parameter	Nili-Ravi buffaloes	Kundhi buffaloes
$AUC_{0-last}$ ( $\mu\text{g. h. mL}^{-1}$ )	10.3 $\pm$ 2.0	8.07 $\pm$ 1.21*
$AUC_{0-\infty}$ ( $\mu\text{g. h. mL}^{-1}$ )	10.96 $\pm$ 2.3	8.31 $\pm$ 1.39*
$AUMC_{0-\infty}$ ( $\mu\text{g. h}^2. \text{mL}^{-1}$ )	74.0 $\pm$ 30.6	44.9 $\pm$ 15.2*
$MRT_{0-last}$ (hr)	4.78 $\pm$ 0.52	4.44 $\pm$ 0.37
$\lambda_z$ ( $\text{L.hr}^{-1}$ )	0.11 $\pm$ 0.05	0.14 $\pm$ 0.043
$C_{max}$ ( $\mu\text{g.mL}^{-1}$ )	3.30 $\pm$ 0.19	3.00 $\pm$ 0.17*
$V_{ss}$ ( $\text{L.kg}^{-1}$ )	1.50 $\pm$ 0.34	1.59 $\pm$ 0.22
$Cl_B$ ( $\text{L.kg}^{-1} \text{h}^{-1}$ )	0.23 $\pm$ 0.05	0.30 $\pm$ 0.05*

$AUC_{0-\infty}$  = Area under plasma concentration-time curve,

$AUMC_{0-\infty}$  = Area under the first moment curve,

$MRT_{0-last}$  = Mean residence time to last sampling time,

$\lambda_z$  = Exponential constant for the terminal phase,  $C_{max}$  = Maximum plasma concentration,

$V_{ss}$  = Volume of distribution at steady state,  $Cl_B$  = Total body clearance.

\*Significant at (P<0.05).

Table 3. Mean $\pm$ SD *in vitro* danofloxacin binding to plasma proteins of adult buffaloes (Each data point is average of 3 determinants).

Total drug ( $\mu\text{g/ml}$ )	Drug concentration ( $\mu\text{g/ml}$ )		Percent drug	
	Free	Bound	Free	Bound
50	34.8 $\pm$ 1.65	15.2 $\pm$ 1.07	69.6 $\pm$ 1.58	30.4 $\pm$ 1.47
100	71.4 $\pm$ 0.98	28.6 $\pm$ 1.85	71.4 $\pm$ 2.41	28.6 $\pm$ 2.04
150	108.2 $\pm$ 1.84	41.8 $\pm$ 1.07	72.1 $\pm$ 0.97	27.8 $\pm$ 1.06
200	150.8 $\pm$ 0.67	49.2 $\pm$ 0.86	75.4 $\pm$ 1.19	24.6 $\pm$ 0.97
250	190.9 $\pm$ 1.04	59.1 $\pm$ 2.14	76.3 $\pm$ 2.87	23.6 $\pm$ 1.43

Overall mean  $\pm$  SD = 27.0 $\pm$ 2.83

cows, whereas, 70% binding of ciprofloxacin was observed in calves (Nouws *et al.*, 1988).

The optimal dosage regimens of DNF based upon the pharmacokinetic parameters were calculated in both breeds of buffaloes (Table 4). The calculations of priming and maintenance doses of DNF following intravenous administration were based upon the minimum effective concentration C<sup>o</sup>P (min) or minimum inhibitory concentration (MIC) of DNF in blood. The minimum effective concentrations of DNF C<sup>o</sup>P (min) 0.02, 0.03, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75 and 1 µg/ml have been used in the calculations for each time interval. The priming and maintenance doses of DNF in Nili-Ravi buffaloes at MIC=0.5 µg/ml were 7.08 and 6.51 mg/kg, and in Kundhi buffaloes 9.14 and 8.31 respectively; to be repeated after 12 h interval.

*In vitro*, DNF has great potential against a variety of microorganisms. Rowan *et al.* (2004) reported the MIC<sub>90</sub> of 0.25 and 0.06 µg/ml for DNF against *Mannheimia haemolytica* and *Pasteurella multocida*, respectively. Various pharmacokinetic/pharmacodynamic (PK/PD) indices have been proposed to predict the effectiveness of an antibiotic (Aliabadi and Lees, 2000). Three PK/PD indices are commonly used. These include T > MIC when the antibiotic is time

dependent, AUC (AUC/MIC) and C<sub>max</sub>/MIC when antibiotic is concentration dependent Pickerill *et al.* (2000) proposed that AUC (AUC:MIC) is a better interpreter of antibacterial activity of fluoroquinolones as compared to the time during which plasma concentration exceeds the MIC level. The C<sub>max</sub>/MIC ratio has no meaning in terms of PK/PD index in this study being the intravenous administration. Brown (1996) suggested the AUC value of 125 h for DNF against *Mannheimia haemolytica* (MIC<sub>90</sub>=0.25 µg/ml) for obtaining optimal efficacy. In order to calculate the AUC in this study, the MIC<sub>50</sub> value of 0.06 µg/ml value has been used for DNF against *Pasteurella multocida*. The determined value of AUC was 163 and 129 h for a dosage of 2.5 mg/kg in Nili-Ravi and Khundhi buffaloes respectively. Thus, the current dose of 2.5 mg/kg/24 h of DNF is adequate against the *Pasteurella multocida* with (MIC<sub>50</sub>=0.06 µg/ml) in both breeds. But the optimal dose is the amount of drug that rapidly kills as well as inhibits the re-growth of bacteria without the support of host defense mechanisms (Toutain *et al.*, 2002). By incorporating the (MIC<sub>90</sub>=0.25 µg/ml) of DNF against *Pasteurella multocida*, the determined values of AUC were 39.2 h in Nili-Ravi and 30.9 h in Kundhi buffaloes. The values of the surrogate

Table 4. Intravenous dosage regimen of danofloxacin (mg/kg) to be repeated after 12 h for different dosing intervals and minimum effective concentrations in adult buffaloes.

Breed	Minimum Effective Concentration (µg/ml)	0.02	0.03	0.05	0.1	0.2	0.3	0.4	0.5	0.75	1
		Nili-Ravi	Priming dose	0.28	0.43	0.71	1.41	2.83	4.25	5.66	7.08
	Maintenance dose	0.26	0.39	0.65	1.30	2.60	3.90	5.21	6.51	9.7	13.02
Kundhi	Priming dose	0.36	0.54	0.91	1.82	3.66	5.49	7.31	9.14	13.7	18.3
	Maintenance dose	0.33	0.49	0.83	1.66	3.32	4.99	6.65	8.31	12.4	16.6

marker obtained by PK-PD integration in the present study fall below of suggestive values (AUC=125 h). Hence, it is supposed that the current dose of 2.5 mg/kg will promote the resistant mutants in the adult local buffaloes.

The optimal dosage regimen of DNF by means of pharmacokinetic parameters obtained in this study for the treatment of diseases caused by susceptible bacteria with MIC<sub>90</sub> of ≤0.50 µg/ml computed after intravenous administration at 12 h time interval are 6.5 mg/kg and 8.31 mg/kg body weight in Nili-Ravi and Kundhi buffaloes respectively. It is speculated that these dosage regimens might achieve the desired value of AUC=125 h; predictor of antibacterial activity. Further, the plasma concentrations will remain in mutant prevention window as a minimum for 75% of dosage time interval which is essential for fluoroquinolones to ensure the bacteriological eradication and emergence of resistance explained by Drlica (2003). In another study, the optimal dose of DNF was supposed to be 6 mg/kg on the basis of clinical reports. Brown (1996) demonstrated that the administration of DNF at dose rate of 6 mg/kg in European cattle achieved an AUC of 126 h and successful therapeutic efficacy of this dose was seen against natural outbreaks of calf respiratory disease.

This is the first study regarding the pharmacokinetics behavior of DNF in local adult buffaloes in Pakistan. The study will aid in the local manufacturing of DNF in the light of its pharmacokinetic profile in these species. However, further detailed pharmacodynamic studies of DNF in adult local buffaloes are required to identify an actual as opposed to an assumed MIC<sub>90</sub> for confirming the dose by generating sufficient data from field isolates in future studies before issuing final recommendations.

## CONCLUSION

The pharmacokinetics behavior of danofloxacin displayed some inter and intra species variations due to the difference in the environment and genetic makeup among species. The current dose of 2.5 mg/kg body weight may promote the bacterial resistance in Nilli-Ravi and Kundhi buffaloes. Hence, the suggested doses of 6.5 and 8.31 mg/kg/12 h calculated on the basis of PK/PD integration in the current study may be optimum doses for successful therapeutic outcome and prevention of mutant selection pressure. The computed dosage regimens will help in the local manufacturing of the drug and can be used to establish disposition kinetics of danofloxacin following extra vascular administration in buffalo species. However, there is further need to conduct detailed pharmacodynamic studies of danofloxacin against respiratory disease causing organisms in buffalo species in order to establish PK-PD inter-relationships.

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