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Disposition kinetics and bioavailability of danofloxacin in Mehsana buffalo calves after parenteral administration

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Abstract
The present study was undertaken to investigate the pharmacokinetics of danofloxacin following single-dose intravenous (IV) and intramuscular (IM) administration at 5.0 mg/kg body weight in Mehsana buffalo calves (n = 6). Plasma concentrations of danofloxacin were determined using a validated high-performance liquid chromatography (HPLC) method, and pharmacokinetic parameters were derived by non-compartmental analysis. Following IV administration, danofloxacin exhibited rapid systemic distribution with a high initial plasma concentration (9.55±0.49 µg/ml at 0.033 h) and a biphasic decline thereafter. The mean elimination rate constant (β) was 0.13±0.00 h^{-1}, corresponding to an elimination half-life ($t_{½\beta}$) of 5.24±0.15 h. The mean $AUC_{0-\infty}$ was 12.69±0.23 µg·h/ml, mean residence time (MRT) was 5.77±0.07 h, steady-state volume of distribution (Vdss) was 2.28±0.02 L/kg, and total body clearance (Cl_B) was 0.39±0.01 L/h/kg, indicating extensive tissue distribution and moderate elimination. Following IM administration, danofloxacin was rapidly absorbed, achieving a mean maximum plasma concentration (Cmax) of 2.09±0.14 µg/ml at a mean Tmax of 0.83±0.11 h. The elimination half-life (6.92±0.57 h) and mean residence time (10.08±0.89 h) were longer than those observed after IV administration, indicating prolonged systemic persistence. The mean $AUC_{0-\infty}$ following IM administration was 17.04±1.53 µg·h/ml, resulting in a high apparent systemic bioavailability (127%), suggestive of near-complete absorption from the injection site. The pharmacokinetic profile observed in Mehsana buffalo calves demonstrates rapid absorption, extensive tissue distribution, and sustained systemic exposure of danofloxacin, supporting its suitability for intramuscular administration

Keywords: Danofloxacin pharmacokinetics intravenous administration

Introduction
Buffaloes (*Bubalus bubalis*) constitute a vital component of the livestock sector in India, contributing substantially to milk and meat production. Bacterial infections, particularly those involving the respiratory system, continue to cause significant economic losses in buffalo production systems. Effective antimicrobial therapy, based on sound pharmacokinetic principles, is essential for optimizing therapeutic outcomes and minimizing antimicrobial resistance.

Danofloxacin is a third-generation fluoroquinolone developed exclusively for veterinary use and exhibits potent bactericidal activity against major Gram-negative respiratory pathogens (Sartini *et al*., 2021; Stipkovits & Szathmary, 2012) [10, 11]. Its favourable pharmacokinetic properties, including high lipid solubility, extensive tissue penetration, and prolonged post-antibiotic effect, make it suitable for use in food-producing animals. However, pharmacokinetic behaviour may vary among species and breeds, necessitating breed-specific evaluation.

The present study was therefore designed to characterize the pharmacokinetics of danofloxacin following intravenous and intramuscular administration in Mehsana buffalo calves to support rational dosage optimization in this breed.

Materials and Methods
Experimental Animals
The study was conducted on six clinically healthy male Mehsana buffalo calves (*Bubalus bubalis*), aged 6-10 months and weighing approximately 100-150 kg, procured from the

Livestock Research Station (LRS), Kamdhenu University, Sardarkrushinagar. The experimental work was approved by the Institutional Animal Ethics Committee (Reg. No. 734/GO/Re/SL/03/CCSEA) under protocol number VETCOLL/IAEC/2024/23/PROTOCOL-02 dated 21/12/2024. The trial was carried out at the Large Animal Experimental Facility of the College of Veterinary Science and Animal Husbandry, located in Banaskantha district of North Gujarat (24.323272°N, 72.297212°E; 154.52 m AMSL), which experiences a tropical semi-arid climate with temperatures ranging between 25-35°C during the study period. Animals were managed as per standard husbandry practices, provided with adequate green fodder, dry roughage, concentrate feed, and clean drinking water ad libitum. They were acclimatized and closely monitored for 10 days before the experiment, confirmed healthy through clinical examination, and individually identified using ear tags for the entire study duration.

Drug, Chemicals and Reagents

Danofloxacin 98% (CAS# 119478-55-6) was procured from JK Chemicals, Vapi, Gujarat (India). Water, methanol, acetonitrile, perchloric acid (70%), and formic acid were HPLC grade and purchased from S. D. Fine Chemicals Ltd., Mumbai.

Experimental protocol

For the pharmacokinetic evaluation of danofloxacin in Mehsana buffalo calves, a solution was prepared by dissolving 500 mg of danofloxacin mesylate powder in 10 mL of sterile water for injection and administered at a dose of 5.0 mg/kg body weight for both intravenous (IV) and intramuscular (IM) routes. All injections were given using disposable 18G × 38 mm needles, with IV dosing through the jugular vein and IM administration into the trapezius muscle of the neck region. Blood samples (up to 3.0 mL) were collected into heparinized tubes from the contralateral jugular vein at predetermined intervals: after IV treatment at 0 min, 2 min (0.033 h), 5 min (0.083 h), 15 min (0.25 h), 30 min (0.5 h), and 1, 2, 4, 8, 12, 24, 36, and 48 hours; and following IM dosing at 0 min, 5 min (0.083 h), 15 min (0.25 h), 30 min (0.5 h), and 1, 2, 4, 8, 12, 24, 36, 48, and 72 hours. Plasma was separated by centrifugation at 2856 *g* for 5 minutes at 4°C, transferred carefully into labelled 2 mL polypropylene microcentrifuge tubes, and stored at −4°C to avoid degradation and freeze-thaw effects until chromatographic analysis.

Analytical Method

In this experiment, an Ultra-High Performance Liquid Chromatography (UHPLC) system (Dionex Ultimate 3000®, Thermo Fisher Scientific, Germany) was employed. The setup included a UV detector, a gradient solvent delivery pump, and a manual sample injector. Separation of analytes was achieved using a reverse-phase C-18 column (ODS-3V, GL Science Inc., Japan; 250 cm × 4.6 mm ID), operated at ambient laboratory temperature (20-30°C). A 20 µL injection loop was utilized for sample introduction, and the chromatographic results were analyzed using Chromeleon™ software (version 6.8).

Chromatographic Conditions

For chromatographic analysis, a mobile phase composed of 0.01 M formic acid buffer and acetonitrile (79:21, v/v) was utilized, with the flow rate set at 1.0 mL/min. The formic acid buffer (0.01 M) was prepared by adding 460 µL of formic acid to one litre of HPLC-grade water, followed by pH adjustment to 3.0 using 230 µL of triethylamine. The buffer was then filtered through a 0.47 µm membrane filter using vacuum filtration and subsequently degassed in an ultrasonic bath for 10 minutes (Frontline Ultrasonic Cleaner, Ahmedabad, India). The separation was performed under isocratic conditions with UV detection at 282 nm. Samples (20 µL) were injected manually using a Hamilton® syringe #710 (Sigma-Aldrich, USA). Each chromatographic run lasted for 15 minutes, and danofloxacin typically eluted at approximately 5.5±0.3 minutes**.**

Sample Extraction Procedure

The extraction of danofloxacin from plasma was performed using a modified protocol based on Abo-EL-Sooud *et al*. (2017) [1]. Plasma samples were first equilibrated to room temperature, after which 100 µL of each sample was pipetted into a 2 mL Eppendorf® microcentrifuge tube. To precipitate plasma proteins, 100 µL of 20% perchloric acid—prepared by combining 2.86 mL of 70% perchloric acid with 7.14 mL of chilled acetonitrile—was added. The mixture was vortexed for 2 minutes at 658 *g* and then centrifuged at 11,424 *g* for 10 minutes at 4°C using a refrigerated centrifuge. The resulting clear supernatant was carefully transferred into a fresh microcentrifuge tube, and a 20 µL portion of this extract was injected into the UHPLC system for quantification.

Pharmacokinetic Analysis

The plasma concentration-time profiles obtained from each buffalo calf were evaluated using a non-compartmental analysis approach to determine the pharmacokinetic (PK) parameters of danofloxacin. Parameters were calculated separately for both intravenous and intramuscular routes of administration, and the results were presented as mean±standard error (SE). Non-compartmental analysis was selected as it provides reliable PK estimates without the need to assume any specific compartmental model. The calculations were based on the area under the plasma concentration-time curve (AUC) and the area under the first-moment curve (AUMC), determined using the trapezoidal rule. Parameters included β, $t_{½β}$, AUC, AUMC, MRT, Vd(area), Vdss, Cl_B, Cmax, Tmax, and bioavailability (F).

Validation of analytical method

The UHPLC method developed for quantifying danofloxacin in buffalo plasma was validated by evaluating linearity, extraction recovery, accuracy, precision, and sensitivity in accordance with USFDA-CDER (1994) and ICH (2005) guidelines. The calibration curve for danofloxacin over the range of 0.1-8.0 µg/mL showed excellent linearity with an average R^2 value of 0.9988. The mean recovery at low, medium, and high concentrations was 84.94 %, 91.41 %, and 94.03 %, respectively, with an overall recovery of 90.12 %, confirming efficient analyte extraction. Accuracy values ranged from 91.66 % to 99.23 %, indicating reliable measurement of true analyte concentrations. Intra-day precision (%CV) ranged between 4.21-6.66 %, while inter-day precision ranged between 3.30-3.72 %, demonstrating good repeatability and reproducibility. The lower limit of quantification (LOQ) was established at 0.1 µg/mL within the validated linear range, ensuring reliable quantification of danofloxacin in all plasma samples analyzed.

Results and Discussion

Intravenous Administration

After a single intravenous (IV) dose of danofloxacin at 5.0 mg/kg body weight in Mehsana buffalo calves, the mean plasma concentration at the first sampling time point (0.033 h; 2 minutes) was 9.55 µg/ml, indicating rapid systemic availability immediately following IV administration. Thereafter, plasma concentrations declined sharply during the early post-administration period, with mean values of 5.27 µg/ml at 0.083 h and 3.43 µg/ml at 0.25 h, reflecting a rapid distribution of danofloxacin from the central compartment into peripheral tissues. This initial steep decline was followed by a more gradual reduction in plasma concentrations at later time points, with mean values of 2.95, 1.83, and 1.26 µg/ml at 0.50, 1, and 2 hours, respectively, suggesting a transition from the distribution phase to the elimination phase. Subsequently, plasma concentrations continued to decrease progressively, reaching 0.71, 0.43, and 0.26 µg/ml at 4, 8, and 12 hours, respectively, indicative of sustained elimination of the drug from systemic circulation. The lowest detectable plasma concentration (0.22 µg/ml) was observed at 12 hours in calf no. 3, while the average minimum concentration across all six calves at this time point was 0.26 µg/ml. Plasma drug levels were not detectable in any samples collected beyond 12 hours, including those obtained at 36 and 48 hours (Table1).

Table 1: Plasma concentrations of danofloxacin (5.0 mg/kg BW) following single dose intravenous administration in Mehsana buffalo calves (n=6)

Time point (h)	Plasma danofloxacin concentration (µg/ml)						Mean±SE (n=6)
	B1	B2	B3	B4	B5	B6	
0.033	9.6	8.15	11.42	9.95	9.82	8.33	9.55±0.49
0.0833	5.94	4.45	4.32	6.2	6.11	4.62	5.27±0.37
0.167	4.94	3.83	3.94	5.08	4.98	3.76	4.42±0.26
0.25	3.28	3.46	3.43	3.54	3.49	3.39	3.43±0.04
0.50	3.08	2.89	2.64	3.14	3.08	2.88	2.95±0.08
1	1.74	1.95	1.78	1.82	1.77	1.89	1.83±0.03
2	1.24	1.32	1.12	1.32	1.29	1.29	1.26±0.03
4	0.65	0.82	0.64	0.68	0.67	0.79	0.71±0.03
8	0.44	0.43	0.39	0.46	0.45	0.41	0.43±0.01
12	0.24	0.31	0.22	0.25	0.24	0.29	0.26±0.01
24	BDL						
36	BDL						
48	BDL						

BDL = Below Detection Limit

Table 2: Pharmacokinetic (PK) parameters of danofloxacin following single dose intravenous administration (5.0 mg/kg BW) in Mehsana buffalo calves (n=6)

PK Parameters (Unit)	Calf Number						Mean±SE
	B1	B2	B3	B4	B5	B6	
B (Per h)	0.12	0.14	0.13	0.13	0.12	0.15	0.13±0.0
$t_{1/2\beta}$ (h)	5.59	4.85	5.15	5.54	5.55	4.76	5.24±0.15
$AUC_{0-\infty}$ (µg.h/ml)	12.55	13.26	11.65	13.02	12.77	12.87	12.69±0.23
AUMC (µg.h^2/ml)	73.52	78.78	63.11	75.55	74.21	74.49	73.28±2.17
MRT (h)	5.86	5.94	5.42	5.80	5.81	5.79	5.77±0.07
$Vd_{(area)}$ (L/kg)	3.21	2.64	3.19	3.07	3.14	2.67	2.98±0.11
$Vd_{(ss)}$ (L/kg)	2.34	2.24	2.32	2.23	2.28	2.25	2.28±0.02
Cl_B (L/h/kg)	0.40	0.38	0.43	0.38	0.39	0.39	0.39±0.01

(**Notations used:** - β: Elimination rate constant; $t_{1/2\beta}$: Elimination half-life; $AUC_{0-\infty}$: Area under curve; AUMC: Area under first moment of the plasma drug concentration; MRT: Mean Resident Time; $V_{d(area)}$: Apparent volume of distribution; $Vd_{(ss)}$: Volume of distribution at steady state; Cl_B: Total body clearance)

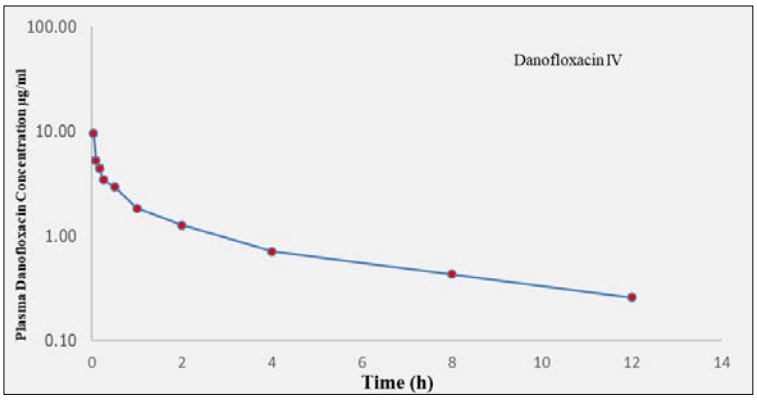

Fig 1: Semi-logarithmic plot of (mean±SE) plasma danofloxacin concentration *versus* time following single dose intravenous administration (5.0 mg/kg BW) in Mehsana buffalo calves (n=6)

In the present study, a comprehensive pharmacokinetic evaluation of danofloxacin was conducted following a single intravenous administration at a dose of 5 mg/kg body weight in clinically healthy Mehsana buffalo calves. The elimination rate constant (β) was determined to be 0.13±0.01 h^{-1}, demonstrating a moderate rate of drug removal from systemic circulation. This value is closely aligned with β estimates reported in Nili-Ravi (0.21±0.02 h^{-1}) and Kundhi buffaloes (0.20±0.04 h^{-1}) by Manzoor *et al.* (2017) [8], and comparable to that observed in cattle (0.13±0.01 h^{-1}) by Corum *et al.* (2019) [3], confirming that danofloxacin displays similar elimination kinetics across large ruminant species. The elimination half-life ($t_{½\beta}$) measured in this study (5.24±0.15 h) reflects sustained systemic persistence of danofloxacin, consistent with values reported in buffaloes by Sappal (5.98±0.42 h) and El-Gendy & Tohamy (3.80±0.31 h). Slightly shorter half-life values reported in Nili-Ravi and Kundhi buffaloes (3.26±0.43 h and 3.49±0.87 h, respectively) by Manzoor *et al.* (2017) [8] may be attributed to differences in physiological status, metabolic capacity, and hepatic microcirculation between geographically distinct buffalo breeds. Comparatively, large variability in half-life among ruminants—such as 4.67±0.45 h in goats (Aliabadi *et al*., 2003) [2] and 3.27 h in sheep (Escudero *et al*., 2007) [7]—reinforces species-dependent influences on disposition kinetics, while the markedly prolonged half-life reported in cattle (17.47±0.60 h) by Corum *et al.* (2019) [3] suggests lower total clearance and more extensive distribution in bovines. In this study, the mean residence time (MRT) was calculated to be 5.77 h, signifying that danofloxacin remained within the biological system for a considerable period before complete elimination. MRT results were in agreement with the obtained half-life and indicate favorable pharmacokinetic behavior for achieving therapeutic coverage in systemic infections. The area under the plasma concentration-time curve ($AUC_{0-\infty}$), an essential indicator of overall drug exposure and bioavailability, was 12.69±0.23 µg·h/mL. This value indicates sustained systemic availability following IV administration and is slightly higher than those documented in Nili-Ravi buffaloes (10.96±2.30 µg·h/mL) and Kundhi buffaloes (8.31±1.39 µg·h/mL) by Manzoor *et al.* (2017) [8]. Lower AUC values previously reported in buffaloes by Sappal *et al.* (2.35±0.52 µg·h/mL) and in sheep and goats (Escudero *et al*., 2007; Aliabadi *et al*., 2003) [7, 2] may be attributed to breed differences, age variation, dose regimens, plasma protein binding affinity, and metabolic activity. Conversely, the higher AUC reported in cattle (15.50±0.20 µg·h/mL) by Corum *et al.* (2019) [3] may reflect slower clearance and enhanced tissue distribution in that species.

The apparent volume of distribution (Vd(area): 2.98±0.11 L/kg) and volume of distribution at steady state (Vdss: 2.28±0.02 L/kg) observed in this study indicate that danofloxacin exhibits extensive tissue penetration in buffalo calves, consistent with its high lipid solubility and moderate plasma protein binding characteristics. These values are greater than those reported in Nili-Ravi and Kundhi buffaloes (Manzoor *et al*., 2017) [8] but lower when compared to the values recorded in cattle (Corum *et al*., 2019) [3], suggesting that distribution dynamics may vary depending on species-specific anatomical and physiological factors, including extracellular fluid volume, muscle-to-fat ratio, tissue perfusion rate, and receptor affinity in target tissues. Total body clearance (Cl_B) was measured to be 0.39±0.01 L/kg/h, indicating moderate systemic elimination capacity in Mehsana buffalo calves. This clearance estimate is close to that documented in buffaloes by El-Gendy & Tohamy (0.28±0.03 L/kg/h) and Manzoor *et al*. 0.23-0.30±0.05 L/kg/h), whereas higher clearance rates have been reported in goats and sheep (Aliabadi *et al*., 2003; Escudero *et al*., 2007) [2, 7], reflecting faster metabolic turnover and renal excretion in small ruminants. In contrast, cattle exhibit slower clearance (0.18±0.01 L/kg/h; Corum *et al*., 2019) [3], which contributes to their prolonged elimination half-life. Differences in hepatic enzyme activity, renal blood flow, physiological maturity, disease status, and genetic variability are key contributors to the wide variability in clearance values observed across species, as supported by Elazab *et al*. (2020) and De la Puente *et al*. (2024) [6, 4].

Intramuscular Administration

The plasma concentrations of danofloxacin following a single intramuscular dose of 5.0 mg/kg body weight in six buffalo calves, along with the corresponding mean values and standard error (SE), are presented in Table 3. The initial plasma samples collected 5 minutes after intramuscular administration exhibited a relatively high mean concentration of danofloxacin (0.97 µg/ml). The mean plasma drug concentration subsequently increased, reaching a peak value of 2.01 µg/ml at 1 hour, and then declined steadily, suggesting rapid distribution after achieving sufficient plasma levels. At later time points, plasma samples demonstrated a continuous decrease in drug concentration, with a low mean value of 0.22 µg/ml at 24 hours. No drug concentration was detected in plasma samples collected at 36 and 48 hours.

Table 3: Plasma concentrations of danofloxacin (5.0 mg/kg BW) following single dose intramuscular administration in Mehsana buffalo calves (n=6)

Time point (h)	Plasma danofloxacin concentration (µg/ml)						Mean±SE (n=6)
	B1	B2	B3	B4	B5	B6	
0.0833	1.02	0.58	1.02	1.17	1.01	1.04	0.97±0.08
0.167	1.20	0.82	1.23	1.29	1.40	1.26	1.20±0.08
0.25	1.32	1.15	1.37	1.49	1.60	1.39	1.39±0.06
0.50	1.47	1.55	1.50	1.60	1.85	1.52	1.58±0.06
1	2.34	1.23	2.38	2.08	1.66	2.36	2.01±0.19
2	1.73	1.16	1.66	1.57	1.36	1.68	1.53±0.09
4	1.64	0.92	1.01	1.06	0.99	1.05	1.11±0.11
8	1.02	0.60	0.59	0.68	0.79	0.61	0.72±0.07
12	0.57	0.33	0.29	0.33	0.40	0.31	0.37±0.04
24	0.31	0.21	0.20	0.19	0.19	BDL	0.22±0.02
36	BDL						
48	BDL						

BDL = Below Detection Limit

Table 4: Pharmacokinetic (PK) parameters of danofloxacin following single dose intramuscular administration (5.0 mg/kg BW) in Mehsana buffalo calves (n=6)

PK Parameter (Unit)	**Calf Number**						**Mean±SE**
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	
C_{max} (µg/ml)	2.34	1.55	2.38	2.08	1.85	2.36	2.09±0.14
T_{max} (h)	1.00	0.50	1.00	1.00	0.50	1.00	0.83±0.11
B (Per h)	0.09	0.08	0.11	0.10	0.10	0.15	0.10±0.01
$t_{1/2\beta}$ (h)	7.96	8.52	6.58	6.71	7.22	4.54	6.92±0.57
AUC_{0-t} (µg.h/ml)	20.74	12.58	14.15	14.61	14.92	11.49	14.75±1.31
$AUC_{0-\infty}$ (µg.h/ml)	24.27	15.12	16.01	16.42	16.91	13.50	17.04±1.53
AUMC (µg.h^2/ml)	284.51	188.50	156.55	160.47	177.60	84.10	175.29±26.46
MRT (h)	11.72	12.47	9.78	9.77	10.50	6.23	10.08±0.89
$Vd_{(area)}/F$ (L/kg)	2.36	4.07	2.96	2.95	3.08	2.42	2.97±0.25
Cl_B/F (L/h/kg)	0.21	0.33	0.31	0.30	0.30	0.37	0.30±0.02
F (%)	115	114	156	126	133	117	126.83±6.56

(**Notations used: -** Cmax: Observed peak plasma concentration; Tmax: Time at which Cmax was observed; β: Elimination rate constant; $t_{1/2\beta}$: Elimination half- life; $AUC_{0-\infty}$: Area under curve; AUMC: Area under first moment of the plasma drug concentration; MRT: Mean Resident Time; $Vd_{(area)}$: Apparent volume of distribution; Cl_B: Total body clearance; F: Bioavailability)

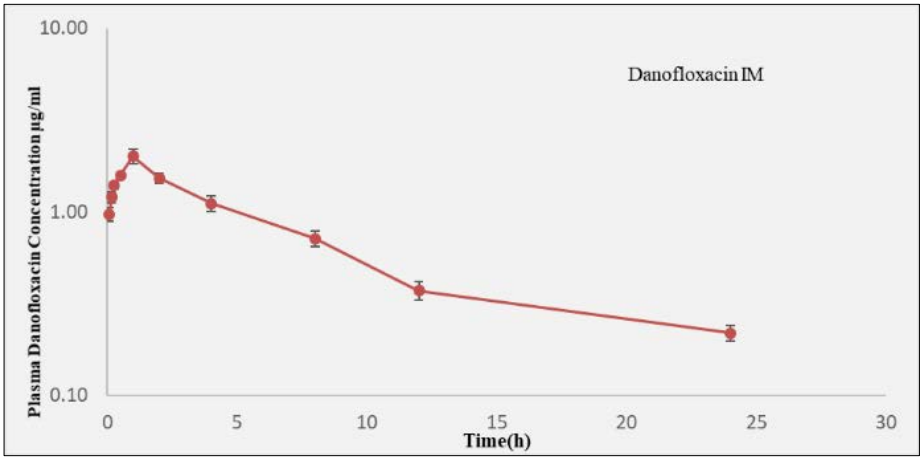

Fig 2: Semi-logarithmic plot of (mean±SE) plasma danofloxacin concentration *versus* time following single dose intramuscular administration (5.0 mg/kg BW) in Mehsana buffalo calves (n=6)

Following intramuscular administration of danofloxacin at 5.0 mg/kg body weight in Mehsana buffalo calves, the pharmacokinetic assessment revealed a mean maximum plasma concentration (Cmax) of 2.09 µg/mL, attained at a mean Tmax of 0.83 h, indicating rapid absorption from the injection site. Individual variations were observed, with four calves achieving Cmax at 1.0 h and two at 0.5 h. The Cmax obtained in the present study is consistent with the dose-dependent pharmacokinetic behavior of danofloxacin and is higher than that reported at lower dose levels in buffaloes by Sappal *et al*. (2009) [9] and El-Gendy & Tohamy (2007) [5]. Lower Cmax values have also been documented in goats and sheep (Aliabadi *et al*., 2003; Escudero *et al*., 2007) [2, 7], highlighting interspecies differences in systemic exposure, which may be attributed to variation in muscle perfusion, absorption rates, and species-specific physiology.

The elimination rate constant (β) following IM administration was 0.10 h^{-1}, with a corresponding elimination half-life ($t_{½\beta}$) of 6.92 h, which was longer compared to the half-life observed after intravenous administration in this study (5.24 h). Such prolongation supports a slower elimination phase and suggests possible flip-flop kinetics, where the absorption rate becomes the rate-limiting determinant for overall drug disposition. The $t_{½\beta}$ value observed in this study is within the range reported for buffaloes by Sappal *et al*. (2009) [9] and reflects moderate persistence of danofloxacin in systemic circulation. Comparatively shorter half-lives reported in sheep and goats (Escudero *et al*., 2007; Aliabadi *et al*., 2003) [7, 2] indicate more rapid elimination in smaller ruminants, whereas the substantially prolonged half-life in cattle (Corum *et al*., 2019) [3] may reflect slower metabolic and renal clearance in that species. The mean residence time (MRT) of 10.08 h further supports the prolonged systemic exposure achieved via the intramuscular route, contributing to extended antimicrobial action.

The extent of systemic exposure quantified through $AUC_{0-\infty}$ was 17.04 µg·h/mL and the corresponding AUMC was 175.29 µg·h²/mL. These values were notably higher compared to those reported in Nili-Ravi and Kundhi buffaloes by Manzoor *et al*. (2017) [8], as well as those documented in goats and sheep (Aliabadi *et al*., 2003; Escudero *et al*., 2007) [2, 7], demonstrating superior exposure in Mehsana buffalo calves and emphasizing the influence of species and breed on danofloxacin disposition. Differences in AUC values among studies may be associated with variations in dose rates, physiological status, plasma protein binding capability, tissue distribution, and elimination efficiency.

The apparent volume of distribution corrected for bioavailability [Vd(area)/F] was 2.97 L/kg, closely matching the value observed after intravenous administration (2.98 L/kg), indicating extensive tissue penetration regardless of route. These values demonstrate that danofloxacin is widely distributed beyond the vascular compartment, a characteristic desirable for managing infections in deep

tissues. Comparison with earlier reports in buffaloes, cattle, goats, and sheep (Manzoor *et al*., 2017; Sappal *et al*., 2009; Corum *et al*., 2019; Aliabadi *et al*., 2003; Escudero *et al*., 2007) [3, 9, 8, 2, 7] reveals considerable interspecies variability, primarily influenced by lipid solubility, tissue perfusion, extracellular fluid volume, and protein binding dynamics.

The mean apparent total body clearance scaled by bioavailability (Cl_B/F) was estimated at 0.30±0.02 L/kg/h, indicating a moderate rate of drug elimination from the body. This value showed close similarity to clearance reported for buffaloes by El-Gendy & Tohamy (2007) [5] and Manzoor *et al*. (2017) [8], suggesting that danofloxacin is efficiently eliminated without excessive accumulation risks. However, differences in clearance between species, particularly the lower clearance in cattle reported by Corum *et al*. (2019) [3], can be attributed to species-specific variations in hepatic metabolism, renal blood flow, and overall metabolic activity. These findings emphasize the relevance of pharmacokinetic profiling for dose optimization in food-producing animals to prevent drug residues.

The intramuscular bioavailability (F) of danofloxacin in this study was calculated as 127%, indicating excellent systemic availability and suggesting prolonged absorption kinetics likely due to depot formation at the injection site. A similar high bioavailability trend has been observed in cattle (Corum *et al*., 2019) [3], supporting enhanced absorption and sustained systemic persistence in large ruminants. Interstudy variations in bioavailability may also depend on formulation properties, injection site factors, and breed-specific physiological characteristics.

Conclusion

The present study successfully characterized the pharmacokinetics of danofloxacin following single-dose intravenous and intramuscular administration in Mehsana buffalo calves. Danofloxacin displayed rapid systemic distribution and extensive tissue penetration after IV dosing, accompanied by moderate clearance and sustained therapeutic plasma levels. Intramuscular administration resulted in rapid absorption, high systemic exposure, prolonged elimination, and notably high bioavailability, indicating efficient uptake from the injection site. Overall, both administration routes demonstrated favourable pharmacokinetic behaviour, with IM administration offering practical advantages for field use due to excellent absorption and prolonged persistence. These findings support the suitability of danofloxacin for treating systemic and respiratory bacterial infections in buffalo calves and provide valuable pharmacokinetic data for rational dosage optimization in this important indigenous breed.

References

1. Abo-El-Sooud K, Soliman AM, Goudah A, Sobhy SF. Comparative serum concentrations and pharmacokinetics of levofloxacin and danofloxacin in broiler chickens. Poultry Science. Year not available;Volume(Issue):Page range not available.
2. Aliabadi FS, Landoni MF, Lees P. Pharmacokinetics, pharmacodynamics, and PK-PD integration of danofloxacin in sheep biological fluids. Antimicrob Agents Chemother. 2003;47(2):626-635. DOI:10.1128/AAC.47.2.626-635.2003.
3. Corum O, Altan F, Yildiz R, Ider M, Ok M, Uney K. Pharmacokinetics of enrofloxacin and danofloxacin in premature calves. J Vet Pharmacol Ther. 2019;42(6):624-631.
4. de la Puente R, Diez R, Diez MJ, Fernandez N, Sahagun AM, Rodriguez JM, Garcia JJ, Lopez C. Pharmacokinetics of meloxicam in different animal species: a comprehensive review. Vet Sci. 2024;11(11):519. DOI:10.3390/vetsci11110519.
5. El-Gendy AA, Tohamy MA. Effect of age on pharmacokinetics of danofloxacin in Egyptian buffalo calves. Kafrelsheikh Vet Med J. 2007;5(1):16-31. DOI:10.21608/kvmj.2007.115847.
6. Elazab ST, Elshater NS, Hashem YH, Park SC, Hsu WH. Tissue residues and pharmacokinetic/pharmacodynamic modeling of tiamulin against *Mycoplasma anatis* in ducks. Front Vet Sci. 2020;7:603950. DOI:10.3389/fvets.2020.603950.
7. Escudero E, Cárceles CM, Fernandez-Varon E, Marin P, Benchaoui H. Pharmacokinetics of danofloxacin 18% in lactating sheep and goats. J Vet Pharmacol Ther. 2007;30(6):572-577. DOI:10.1111/j.1365-2885.2007.00898.x.
8. Manzoor Z, Munawar SH, Iqbal Z, Abid MA. Pharmacokinetic data and dosage regimen of danofloxacin after intravenous administration in Nili-Ravi and Kundhi buffaloes. Buffalo Bull. 2017;36(2):335-347.
9. Sappal R, Chaudhary RK, Sandhu HS, Sidhu PK. Pharmacokinetics, urinary excretion, and plasma protein binding of danofloxacin following intravenous administration in buffalo calves (*Bubalus bubalis*). Vet Res Commun. 2009;33(7):659-667. DOI:10.1007/s11259-009-9215-6.
10. Sartini I, Łebkowska-Wieruszewska B, Lisowski A, Poapolathep A, Llewelyn V, Giorgi M. Single intravenous and oral pharmacokinetics of danofloxacin in goats. Small Rumin Res. 2021;200:106393. DOI:10.1016/j.smallrumres.2021.106393.
11. Stipkovits L, Szathmary S. Mycoplasma infection of ducks and geese. Poult Sci. 2012;91(11):2975-2983.