

Pharmacokinetics, urinary excretion and plasma protein binding of ofloxacin in water buffalo calves (*Bubalus bubalis*)

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Pharmacokinetics and urinary excretion of an intravenous dose of 5 mg.kg⁻¹ ofloxacin were investigated in water buffalo calves. Plasma concentrations of ofloxacin were determined by high-performance liquid chromatography. Ofloxacin was rapidly distributed from the central to the peripheral compartment as evidenced by a short distribution half-life (0.09 h ± 0.003 h) and high K₁₂ (4.7 h⁻¹ ± 0.1 h⁻¹), and was detected in plasma for 8 h. The large volume of distribution (2.48 L.kg⁻¹ ± 0.18 L.kg⁻¹) obtained in this study indicated high distribution of ofloxacin in water buffalo calves. The elimination half-life, the area under the plasma drug concentration–time curve and total body clearance were 2.11 h ± 0.13 h, 6.20 µg.mL⁻¹ ± 0.23 µg.mL⁻¹.h and 0.81 mL.kg⁻¹.h⁻¹ ± 0.03 mL.kg⁻¹.h⁻¹, respectively. About 18.7% of administered drug was bound to plasma proteins and approximately 32.5% of the administered dose was recovered in urine within 48 h. The results of the study indicated a favourable pharmacokinetic profile of ofloxacin in water buffalo calves, which suggests that ofloxacin may be effective against urinary pathogens in this species.

Introduction

Fluoroquinolones have gained widespread acceptance in veterinary medicine thanks to their broad spectrum of activity against Gram-positive and Gram-negative aerobes, mycoplasma and rickettsia (Brown 1996). These antibacterials are currently used for the treatment of invasive and systemic bacterial infections, such as upper and lower respiratory infections, gonorrhoea, bacterial gastroenteritis, skin and soft tissue infections and urinary tract infections that occur in humans and animals (Duan & Yuan 2001). Ofloxacin, (±)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4 benzoxazine-6 carboxylic acid (Lode *et al.* 1987), a synthetic fluoroquinolone, was introduced in the late 1980s (Andersson & MacGowan 2003). It possesses a broad spectrum of activity against a variety of Gram-positive and Gram-negative bacteria, including some anaerobes. Pharmacokinetic studies of ofloxacin after intravenous administration have been conducted in humans (Bethell *et al.* 1996), dogs (Yoshida *et al.* 1998), swine (Son *et al.* 2000), goats (Baruah *et al.* 2004), cattle (Gaur *et al.* 2004), chicken (Kalaiselvi *et al.* 2006) and rabbits (Ahmad *et al.* 2008). However, there is little information available on the pharmacokinetics of ofloxacin in buffalo species. In view of the marked variation in the kinetic behaviour of antimicrobial drugs amongst species, the present study was undertaken to determine the pharmacokinetics, *in vitro* plasma protein binding and urinary excretion following a single intravenous administration of ofloxacin in water buffalo calves.

Materials and methods

The study was conducted in six healthy male water buffalo calves aged between 6 and 12 months and weighing between 75 kg and 120 kg. The animals were obtained from the university dairy farm and acclimatised in the departmental animal shed under standard conditions of management for two weeks prior to the commencement of the study. They were maintained on green fodder and water *ad libitum*. Ofloxacin was administered at a dose of 5 mg.kg⁻¹ body weight into the left jugular vein.

To conduct the pharmacokinetic study, the animals were kept in metabolic stalls of standard size. Blood samples (5 mL) were withdrawn from the contralateral jugular vein into heparinised glass centrifuge tubes before administration of the drug and again at 1 min, 2.5 min, 5 min, 7.5 min, 10 min, 15 min, 20 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 16 h and 24 h after administration of the drug. Plasma was separated by centrifugation at 1300 g for 15 min at room temperature and kept at -20 °C until analysis, usually on the day after collection. Urine samples were collected from each animal at predetermined time intervals (2 h, 4 h, 6 h, 8 h, 12 h, 16 h, 24 h, 36 h and 48 h) after administration of the drug. The metabolic stalls are designed in such a way

that the total amount of urine excreted by the animals within a period is automatically collected without contamination or spillage in containers placed beneath the stalls. At the end of a given time interval, the total volume of urine collected in the container was measured for each animal and, after filtration, 2 mL samples were extracted for analysis.

In vitro binding of ofloxacin to plasma proteins was determined according to the equilibrium dialysis technique (Lode *et al.* 1987) to yield protein binding constants. Various concentrations of ofloxacin, ranging from 1 $\mu\text{g}\cdot\text{mL}^{-1}$ to 20 $\mu\text{g}\cdot\text{mL}^{-1}$, were prepared in pooled plasma sampled from untreated animals. Each dialysis bag (pore size = 4 Å), filled with 5 mL plasma containing a known concentration of ofloxacin, was then immersed in a separate tube containing 5 mL phosphate buffer (0.2 M; pH = 7.4). The tubes were incubated at 37 °C for 24 h, with occasional shaking. At the end of the incubation period the buffer and contents of the dialysis bags were analysed separately to determine the concentration of ofloxacin present. Dialysis of each prepared ofloxacin concentration was analysed in triplicate. The extent of *in vitro* plasma protein binding of ofloxacin was calculated according to:

$$\text{Ofloxacin (\% bound to plasma protein)} = \frac{CP^1 - CB}{CP} \times 100 \quad [\text{Eqn 1}]$$

where CP^1 is the concentration of ofloxacin in plasma after incubation, CB is the concentration of ofloxacin in phosphate buffer after incubation and CP is the concentration of ofloxacin in plasma before incubation. Binding capacity of the plasma protein to ofloxacin (β_1) and the dissociation rate constant of the protein drug complex (K_p) were calculated according to the method of Pilloud (1973).

The concentration of ofloxacin was analysed using high-performance liquid chromatography (HPLC) (Kalaiselvi *et al.* 2006). Briefly, 300 μL of plasma was added to 440 μL of acetonitrile (1:1.5). The mixture was vortexed for 15 s and centrifuged for 10 min at 900 g. The supernatant was transferred to a 2 mL microcentrifuge tube. Double the volume of HPLC-grade water was added. The aliquot was filtered through a 0.2 μm nylon membrane filter, after which 20 μL filtrate was injected into the HPLC system (Perkin Elmer Series 200, Massachusetts, USA) equipped with an autosampler, diode array detector, 200 μL loop size and a reverse-phase C-18 column (particle size = 5 μm ; column length = 150 mm \times 4.6 mm) and measured at 290 nm. The data were analysed with manufacturer-supplied software (TotalChrom Navigator version 6.3.1). The mobile phase comprised 0.1 M orthophosphoric acid (pH adjusted to 2.5 using 45% KOH) and acetonitrile, mixed in a ratio of 75:25. The flow rate of the mobile phase was 1.2 $\text{mL}\cdot\text{min}^{-1}$ and the retention time of ofloxacin was 1.55 min. The chromatogram of the ofloxacin standard prepared in plasma of untreated buffalo calves is shown in Figure 1. The validated limit of ofloxacin quantification for this method

was 0.03 $\mu\text{g}\cdot\text{mL}^{-1}$. Extraction recoveries were greater than 94% and the coefficient of variation was less than 10%. Linear calibration curves were obtained for a concentration range of 0.03 $\mu\text{g}\cdot\text{mL}^{-1}$ – 10.0 $\mu\text{g}\cdot\text{mL}^{-1}$ ($R^2 > 0.9999$). Intra-day precision was estimated from six replicates of three standard samples used for calibration curves (RSD < 10%). Inter-day precision was estimated from the analysis of standard samples on three separate days (RSD < 10%).

Pharmacokinetic parameters were calculated manually using regression analysis (Gibaldi & Perrier 1982). The means of pharmacokinetic variables were obtained by averaging the variables calculated for drug disposition after intravenous drug administration to each animal.

Ethical considerations

The experimental protocol followed the ethical guidelines on the proper care and use of animals and was approved by the institutional animal ethics committee (IAEC Reg. no. No.497/01/a/CPCSEA dt. 18.2.2002 vide approval no. VPS/2008/874-885 Dt.15.5.08).

Results

The plasma concentration–time profile of ofloxacin is presented in Figure 2. At 1 min, the mean plasma concentration was 11.7 $\mu\text{g}\cdot\text{mL}^{-1} \pm 1.03 \mu\text{g}\cdot\text{mL}^{-1}$. The drug was detected in plasma for up to 8 h after dosing (0.16 $\mu\text{g}\cdot\text{mL}^{-1} \pm 0.02 \mu\text{g}\cdot\text{mL}^{-1}$). Evaluation of the results revealed that plasma disposition of ofloxacin followed a two-compartment open model, which is adequately described by the bi-exponential equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad [\text{Eqn 2}]$$

where C_p is the plasma level of ofloxacin at time t and e represents the base of the natural logarithm. A and B are the extrapolated zero-time intercepts of the distribution and elimination phases, respectively. The distribution and elimination rate constants are represented by α and β , respectively. The pharmacokinetic parameters that describe

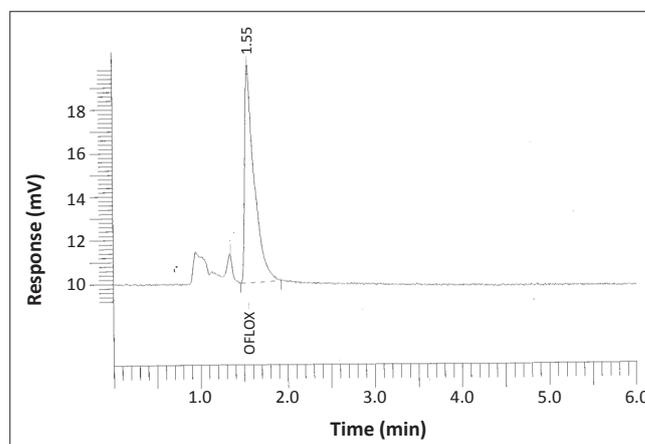


FIGURE 1: Chromatogram of extracted ofloxacin plasma standard (1.25 $\mu\text{g}\cdot\text{mL}^{-1}$) with a retention time of 1.55 min.

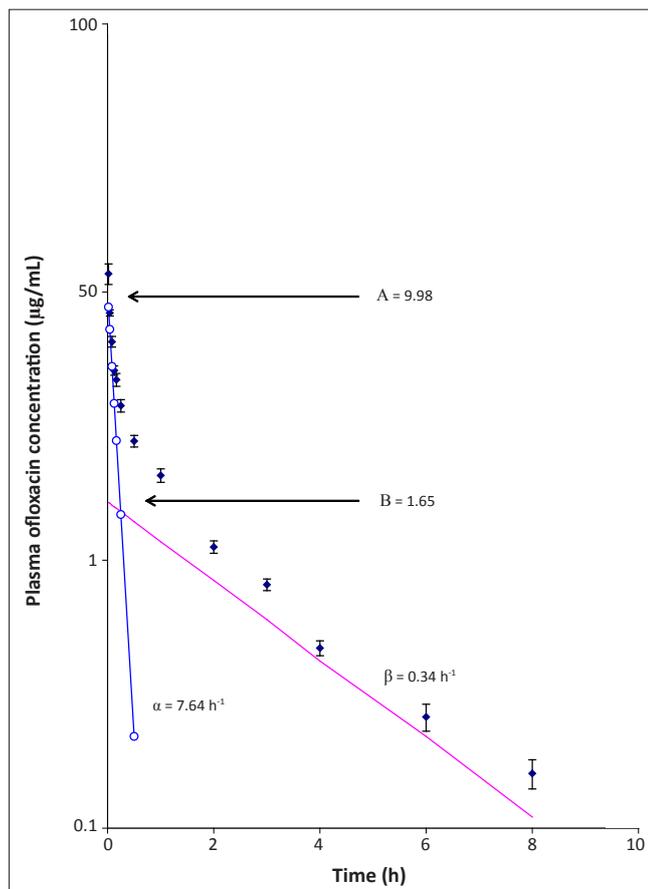
the disposition pattern of ofloxacin in water buffalo calves are presented in Table 1. A graphical representation of the mean urinary concentration of ofloxacin and the cumulative percentage of drug excreted in urine at different time intervals is shown in Figure 3. Table 2 shows the extent and kinetic constants of *in vitro* binding of different concentrations of ofloxacin to plasma proteins in water buffalo calves.

Discussion

In accordance with these findings, a two-compartment open model for disposition of ofloxacin has also been reported in calves (Gaur *et al.* 2004; Parry *et al.* 2007). Minimum inhibitory concentration (MIC_{90}) values of ofloxacin against some of the common isolates of veterinary importance, including *Salmonella*, are reported to be as low as $0.06 \mu\text{g}\cdot\text{mL}^{-1}$ (Greene & Budberg 1993; Parry *et al.* 2007). Therefore, for purposes of this discussion the MIC_{90} of $0.06 \mu\text{g}\cdot\text{mL}^{-1}$ of ofloxacin has been taken into consideration. At 1 min after injection, the plasma level ($11.7 \mu\text{g}\cdot\text{mL}^{-1} \pm 1.03 \mu\text{g}\cdot\text{mL}^{-1}$) was approximately 195-fold higher than the MIC of ofloxacin. The drug was detected above the MIC level for up to 8 h after administration. Comparable plasma levels of ofloxacin ($14.76 \mu\text{g}\cdot\text{mL}^{-1}$) have been reported 1 min after a single

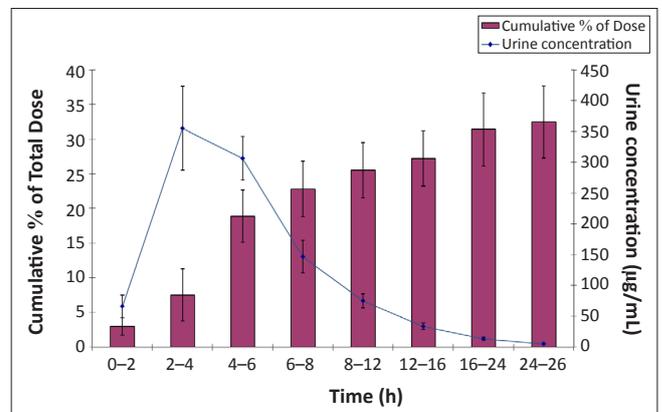
intravenous injection in goats (Baruah *et al.* 2004), but low initial plasma levels ($4.26 \mu\text{g}\cdot\text{mL}^{-1}$) were reported in calves (Gaur *et al.* 2004). Ofloxacin was rapidly transferred from the central to the peripheral compartment in water buffalo calves, as is evident from the short distribution half-life ($0.09 \text{ h} \pm 0.003 \text{ h}$) and high values of K_{12} ($4.7 \text{ h}^{-1} \pm 0.1 \text{ h}^{-1}$) and the K_{12}/K_{21} ratio (3.56 ± 0.32). Low values of $t_{1/2\alpha}$ in calves (0.12 h) and rabbits (0.7 h) and a high K_{12}/K_{21} ratio (1.15) in calves have also been reported after intravenous administration of ofloxacin (Ahmad *et al.* 2008; Mohan & Garg 2006).

The values of the area under the plasma drug concentration–time curve (AUC) and the area under the first moment of plasma drug concentration–time curve (AUMC) were $6.2 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h} \pm 0.23 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$ and $15.0 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}^2 \pm 0.93 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}^2$, respectively. In contrast, high values have been reported for AUC of ofloxacin in calves ($26.5 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$) and goats



Values (solid dots) are presented as mean \pm SE ($n = 6$). The data were analysed according to a two-compartment open model. Distribution (α) and elimination (β) phases are represented by least square regression lines. The calculated points (open circles) of distribution phase were obtained by the feathering technique.

FIGURE 2: Semilogarithmic plot showing the plasma concentration–time profile of ofloxacin following a single intravenous injection of $5 \text{ mg}\cdot\text{kg}^{-1}$ body weight in water buffalo calves.



Each point and bar represent the mean \pm SE of the results of six animals.

FIGURE 3: Urinary concentration and cumulative percentage of total dose of ofloxacin recovered in urine of water buffalo calves after a single intravenous dose of $5 \text{ mg}\cdot\text{kg}^{-1}$ body weight.

TABLE 1: Pharmacokinetic parameters of ofloxacin in water buffalo calves ($n = 6$) following a single intravenous dose of $5 \text{ mg}\cdot\text{kg}^{-1}$ body weight.

Parameter	Unit	Mean \pm SE
C_p^0	$\mu\text{g}\cdot\text{mL}^{-1}$	11.6 ± 0.58
$t_{1/2\alpha}$	h	0.09 ± 0.003
$t_{1/2\beta}$	h	2.11 ± 0.13
AUC	$\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$	6.20 ± 0.23
AUMC	$\mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}^2$	15.0 ± 0.93
Vd_{area}	$\text{L}\cdot\text{kg}^{-1}$	2.48 ± 0.18
K_{12}	h^{-1}	4.70 ± 0.10
K_{21}	h^{-1}	1.40 ± 0.19
K_{12}/K_{21}	Not applicable	3.56 ± 0.32
K_{el}	h^{-1}	1.88 ± 0.08
Cl_B	$\text{mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$	0.81 ± 0.03
P/C	Not applicable	4.77 ± 0.49
AUIC	h	103.3 ± 3.87
V_c	$\text{L}\cdot\text{kg}^{-1}$	0.44 ± 0.02
MRT	h	2.42 ± 0.11
$t_{1/2\alpha}$	h	13.5 ± 0.80

K_{12} and K_{21} are rate constants of drug transfer from the central to the peripheral and from the peripheral to the central compartment, respectively.

AUC, area under the plasma concentration–time curve; AUIC, area under inhibitory concentration of drug; AUMC, area under the first moment of the plasma concentration–time curve; C_p^0 , plasma drug concentration at time zero after intravenous dose; Cl_B , total body clearance of drug; K_{el} , rate constant for elimination of drug from central compartment; MRT, mean residence time; P/C, ratio of drug present in peripheral and central compartment; $t_{1/2}$, duration of therapeutic effect; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, elimination half-life; V_c , volume of central compartment; Vd_{area} , apparent volume of distribution.

TABLE 2: *In vitro* binding of ofloxacin with plasma proteins and kinetic constants in water buffalo calves at various drug concentrations.

Experiment number	Extent of binding (%): Concentration of ofloxacin ($\mu\text{g}\cdot\text{mL}^{-1}$)					Association rate constant, β_1 ($\text{mol}\cdot\text{g}^{-1}$)	Dissociation rate constant, k_p (mol)
	1	2	5	10	20		
1	29.4	17.1	8.28	24.0	15.96	7.3×10^{-6}	8.3×10^{-6}
2	28.2	12.9	12.0	24.54	13.5	7.9×10^{-6}	8.2×10^{-6}
3	33.6	13.2	12.0	17.34	18.0	5.7×10^{-6}	8.1×10^{-6}
Mean \pm SE	30.4 ± 1.64	14.4 ± 1.35	10.8 ± 1.24	22.0 ± 2.32	15.8 ± 1.30	$8.2 \times 10^{-6} \pm 0.06 \times 10^{-6}$	$6.97 \times 10^{-6} \pm 0.66 \times 10^{-6}$

Overall (Mean \pm SE) extent of binding = $18.67\% \pm 1.94\%$.

($58.9 \mu\text{g}\cdot\text{mL}^{-1}\cdot\text{h}$) following a dose of $5\text{mg}\cdot\text{kg}^{-1}$ (Baruah *et al.* 2004; Gaur *et al.* 2004). The large $V_{d_{\text{area}}}$ ($2.48 \text{L}\cdot\text{kg}^{-1} \pm 0.18 \text{L}\cdot\text{kg}^{-1}$) measured in these buffalo calves indicated extensive distribution of ofloxacin. In accordance with this observation, large volumes of distribution have been reported for ofloxacin in calves ($1.4 \text{L}\cdot\text{kg}^{-1}$), goats ($2.18 \text{L}\cdot\text{kg}^{-1}$), swine ($2.06 \text{L}\cdot\text{kg}^{-1}$), rabbits ($4.47 \text{L}\cdot\text{kg}^{-1} \pm 0.11 \text{L}\cdot\text{kg}^{-1}$) and poultry ($1.76 \text{L}\cdot\text{kg}^{-1}$) after intravenous injection (Ahmad *et al.* 2008; Baruah *et al.* 2004; Kalaiselvi *et al.* 2006; Mohan & Garg 2006; Son *et al.* 2000). Furthermore, a high P/C ratio (4.77 ± 0.49) observed in the present study indicated good penetration of the drug. Similar values have also been reported for ofloxacin in calves (4.32) after intravenous administration (Gaur *et al.* 2004).

Total body clearance of ofloxacin, which represents the sum of the metabolic and excretory processes, was $810 \text{mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1} \pm 3.0 \text{mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in the examined buffalo calves. Rapid clearance of ofloxacin, as indicated by high values of CL_{br} , has also been shown in calves ($430 \text{mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) goats ($135.6 \text{mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and poultry ($317.6 \text{mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) after intravenous administration (Baruah *et al.* 2004; Kalaiselvi *et al.* 2006; Mohan & Garg 2006).

The elimination half-life of ofloxacin in the present study was $2.11 \text{h} \pm 0.13 \text{h}$, which is comparable to that measured in calves (2.21h), rabbits (1.77h) and in broilers (4.46h) (Ahmad *et al.* 2008; Kalaiselvi *et al.* 2006; Mohan & Garg 2006). However, in contrast to the present findings, a very long elimination half-life for ofloxacin has been reported in goats (15.58h) after intravenous administration (Baruah *et al.* 2004). The value of MRT of ofloxacin was $2.42 \text{h} \pm 0.11 \text{h}$ in buffalo calves. In support of the present finding, high MRT values have been reported for ofloxacin in rabbits (2.18h) and chicken (5.48h) after intravenous administration (Ahmad *et al.* 2008; Kalaiselvi *et al.* 2006).

The concentration of ofloxacin in the urine of the water buffalo calves was very high (more than 85 times the MIC_{90}), even 48 h after administration and approximately 32.5% of the administered drug was recovered in the urine. Similar results have been reported in bovines after intravenous administration of levofloxacin, with 38.4% of the total administered dose being recovered in urine of calves within 24 h (Dumka *et al.* 2008 2008). These findings suggest that ofloxacin may be an appropriate drug for treating urinary tract infections in bovines. However, contrary to the aforementioned findings, high urinary recovery ($57.1\% - 74.2\%$) has been reported after intravenous administration of ofloxacin in swine (Son *et al.* 2000).

Binding of drugs to plasma proteins plays a major role in their distribution, elimination and therapeutic effectiveness. The extent of protein binding has been reported to affect the therapeutic efficacy of drugs directly (Craig & Kunin 1976). Within a plasma concentration range of $1 \mu\text{g}\cdot\text{mL}^{-1} - 20 \mu\text{g}\cdot\text{mL}^{-1}$, the extent of plasma protein binding of ofloxacin ranged from 10.76% to 30.4%, with an overall mean of $18.67\% \pm 1.94\%$. Protein binding of ofloxacin in buffalo calves was concentration independent. The association (β_1) and dissociation rate constant (K_p) ranges were measured as $5.7 \times 10^{-6} \text{mol}\cdot\text{g}^{-1} - 7.9 \times 10^{-6} \text{mol}\cdot\text{g}^{-1}$ and $8.1 \times 10^{-6} \text{mol}\cdot\text{g}^{-1} - 8.3 \times 10^{-6} \text{mol}\cdot\text{g}^{-1}$, respectively. The weak and low extent of binding, as evidenced by the lower association rate constant compared with the dissociation rate constant, indicated that ofloxacin bound to plasma proteins only to a moderate extent in water buffalo calves. In accordance with these findings, low plasma protein binding (15.28%) has also been reported in goats (Baruah *et al.* 2004).

Conclusion

The results of the study indicated a favourable pharmacokinetic profile of ofloxacin in water buffalo calves, which suggests that ofloxacin may be effective against urinary pathogens. The pharmacokinetic parameters such as mean elimination half-life, volume of distribution and body clearance need to be verified further in a larger population of this species.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

H.S.S. (Guru Angad Dev Veterinary and Animal Sciences University) was the project leader, V.K.D. (Guru Angad Dev Veterinary and Animal Sciences University) was responsible for experimental design, A.K.O. (Guru Angad Dev Veterinary and Animal Sciences University) performed most of the experiments and B.R. (Guru Angad Dev Veterinary and Animal Sciences University) was responsible for HPLC analysis of the samples. V.K.D. wrote the manuscript.

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