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Pharmacokinetics of difloxacin in pigs and broilers following intravenous, intramuscular, and oral single-dose applications

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Pharmacokinetics of difloxacin, a fluoroquinolone antibiotic, was determined in pigs and broilers after intravenous (i.v.), intramuscular (i.m.), or oral (p.o.) administration at a single dose of five (pigs) or 10 mg/kg (broilers). Plasma concentration profiles were analyzed by a compartmental pharmacokinetic method. Following i.v., i.m. and p.o. doses, the elimination half-lives ($t_{1/2\beta}$) were 17.14 ± 4.14, 25.79 ± 8.10, 16.67 ± 4.04 (pigs) and 6.11 ± 1.50, 5.64 ± 0.74, 8.20 ± 3.12 h (broilers), respectively. After single i.m. and p.o. administration, difloxacin was rapidly absorbed, with peak plasma concentrations (C_{max}) of 1.77 ± 0.66, 2.29 ± 0.85 (pigs) and 2.51 ± 0.36, 1.00 ± 0.21 μg/mL (broilers) attained at t_{max} of 1.29 ± 0.26, 1.41 ± 0.88 (pigs) and 0.86 ± 0.4, 4.34 ± 2.40 h (broilers), respectively. Bioavailabilities (F) were (95.3 ± 28.9)% and (105.7 ± 37.1)% (pigs) and (77.0 ± 11.8)% and (54.2 ± 12.6)% (broilers) after i.m. and p.o. doses, respectively. Apparent distribution volumes($V_{d(area)}$) of 4.91 ± 1.88 and 3.10 ± 0.67 L/kg and total body clearances(Cl_B) of 0.20 ± 0.06 and 0.37 ± 0.10 L/kg/h were determined in pigs and broilers, respectively. Areas under the curve (AUC), the half-lives of both absorption and distribution($t_{1/2ka}$, $t_{1/2\alpha}$) were also determined. Based on the single-dose pharmacokinetic parameters determined, multiple dosage regimens were recommended as: a dosage of 5 mg/kg given intramuscularly every 24 h in pigs, or administered orally every 24 h at the dosage of 10 mg/kg in broilers, can maintain effective plasma concentrations with bacteria infections, in which MIC_{90} are <0.25 μg/mL and <0.1 μg/mL respectively.

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INTRODUCTION

Difloxacin is a fluoroquinolone exclusively used in preventive and therapeutic treatments in animals. It has high antimicrobial activity *in vitro* against a wide variety of Gram-negative and Gram-positive bacterial and mycoplasma such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella sp.*, *Salmonella sp.*, etc. (Eliopoulos *et al.*, 1985; Fernandes *et al.*, 1986; Smith *et al.*, 1986; Bansal & Thadepalli, 1987; Digranes & Dibb, 1988). In veterinary medicine, difloxacin seems to have a great potential for treating infections caused by bacteria (Olchowy *et al.*, 2000; Zeng *et al.*, 2000, 2001). With its broad spectrum of antibacterial activity and good distribution in most tissues and body fluids as well as low incidence of adverse effects, difloxacin may be used in many types of infections in China. Successful therapeutic application of difloxacin requires detailed information on pharmacokinetic properties in those food-producing animals. To date, several studies have been published regarding pharmacokinetics of difloxacin in animals (Frazier *et al.*, 2000; Heinen, 2002; Fernandez-Varon *et al.*, 2006; Boothe *et al.*, 2006; Marin *et al.*, 2007). However, there is a paucity of systematic information in the literature regarding pharmacokinetics of difloxacin in pigs or broilers. The objective of this paper is to describe the absorption, distribution and elimination of difloxacin after intravenous (i.v.), intramascular (i.m.), or oral (p.o.) administration in pigs and broilers. With the pharmacokinetic parameters determined from the studies, reasonable multiple dosage regimens for difloxacin can be designed, which can be recommended for clinical treatment.

MATERIALS AND METHODS

Animals

Two-month-old castrated cross-bred (Duroc × Landrace × Yorkshire) pigs (n = 7) were used for the studies. The average body weight of the pigs was 20.4 ± 1.9 kg (range of 18.5–23.0 kg). Pigs were housed indoor and fed daily with drug-free commercial pellet diet. Pigs had free access to drinking water. A total of 30 broilers were also used in the studies. The average body weight was 2.12 ± 0.22 kg (range of 1.56–1.87 kg). The broilers were provided a drug-free pelleted diet and given water *ad libitum*. All the pigs and chickens were in good health as determined by physical examination before drug administration. The animals were humanely handled according to the approved IACUC protocols in South China Agricultural University.

Drugs and chemical reagents

Difloxacin (Standard, 99.9 or 2.5% injectable, Lot # 0408 or 0312) was donated by Guangzhou Huihua Animal Health Products Co. Ltd. (Guangzhou, China). Acetonitrile from Fisher Scientific was high performance liquid chromatography (HPLC) grade. Other agents were A.R. grade and purchased in China.

Drug application/kinetic sampling

Pigs

The pharmacokinetic study of difloxacin in pigs was carried with a Latin Square design, which eliminates the effect of the body weight on the pharmacokinetic parameters. Over the study period, each pig received an i.v., an i.m. and a p.o. administration of difloxacin at a dosage of 5 mg/kg b.w. A 7-day washout period was allowed between different treatments. So there were seven pigs for each different administration route and the bioavailability was calculated using an intra-individual approach. The i.v. bolus doses of difloxacin were administered via the ear vein, and the i.m. doses of difloxacin were injected into the neck muscle. Blood samples were collected from the superior vena cava by venipuncture into tubes containing heparin before drug application and at 0.1, 0.25, 0.5, 0.75, 1, 2, 4, 6, 9, 12, 16, 24, 36 and 48 h after i.v. or i.m. administration. The p.o. administrations were carried out by gavage and blood samples were collected into tubes containing heparin prior to and at 0.1, 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12, 16, 24, 36 and 48 h after difloxacin administration. All blood samples were centrifuged 5at 930 ***g*** for 10 min at room temperature (25 °C). The separated plasma samples were kept at −20 °C until HPLC analysis.

Broilers

Pharmacokinetic experiment was carried out with the parallel design. The AUC for each chiken of different administration route were firstly estimated and then bioavailability of oral and intramuscular administration for individual were estimated using the ratio of AUC of each chiken to the average AUC of the i.v. administration. All broilers were weighed on the day of drug administration (at a dosage of 10 mg/kg b.w.) and randomly assigned to one of the three treatment (i.v., i.m., p.o.) groups. Ten animals were used in each group. Each individual broiler was administrated the drug only once and there was no significant difference between the average body weight of different groups. The i.v. injections of difloxacin were administered into the brachial vein of the birds in group I; the i.m. doses of difloxacin were injected into the pectoral musculature of birds in group II. Two milliliters of blood samples were taken from the brachial vein into tubes containing heparin at the following preset time points: 0, 0.1, 0.25, 0.5, 0.75, 1, 2, 4, 6, 9, 12, 16, 24 and 48 h after i.v. or i.m. administration. difloxacin was administered to birds in group III orally by gavage. Blood samples were collected and heparinized at the following preset time points: 0, 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12, 16, 24 and 48 h after difloxacin administration. All blood samples were centrifuged at 930 ***g*** for 10 min at room temperature (25 °C) and the collected plasma samples were stored at −20 °C until HPLC analysis as described below.

HPLC analysis

Difloxacin plasma concentrations were determined with an HP 1100 HPLC system using a method adapted from Nilsson-Ehle (1987). An aliquot of 0.5 mL plasma was deproteinized with 0.5 mL methanol, vigorously vortexed for 2 min, and then followed by centrifugation (20 620 ***g***). Fifty microlitres of the supernatant was injected into the HPLC system (Hewlett Packard 1100, PaloAlto, CA, USA) for analysis. Chromatography was carried out using a HYPERSIL BDS C18 Column (5 μm, 4.6 × 250 mm); the mobile phase consisted of acetenitrile and 0.0174 mol/L tetrabutylammonium bromide solution (95:905, v/v, pH 3.0) at 1 mL/min flow rate. The fluorescence detector operated at an excitation wavelength of 278 nm and an emission wavelength of 465 nm. Chromatogram peak areas were quantitated by the external standard technique using standard solutions of difloxacin . For calibration, both 0.5 mL blank pig plasma and chiken plasma was spiked with 20 μL of a series of diluted difloxacin working standard solutions and analysed as above. The concentration of difloxacin in the prepared standard samples were 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 μg/mL. The limit of quantitation, quantitation linearity and recovery of difloxacin from plasma were determined in pigs and broilers. Coefficients of variation (CV %) within and between HPLC runs were also calculated.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed on the plasma data for individual animals using the MCPKP software described elsewhere (Xian & Cheng, 1988). In order to get detailed pharmacokinetic parameters of difloxacin in pigs and broilers, a compartmental analysis was selected in the study. The software automaticly select the best compartmental model according to the F test and AIC (akaike's information criterion). The area under the plasma concentration-time curve extrapolated to infinity (AUC0-∞) was calculated by the software with the

corresponding equation for individual administration routes. The elimination half-life at β phase ($t_{1/2\beta}$) was calculated with the equation $t_{1/2\beta} = 0.693/\beta$, where β was the elimination rate constant calculated by the linear regression from the terminal linear portion of the plasma concentration-time curve. The body clearance (Cl_B) following i.v. administration was calculated as the total dose administered divided by the AUC_0-∞. The apparent volume of distribution ($V_{d(area)}$), the maximal plasma concentration (C_{max}), and the time to maximal concentration (t_{max}) after the extravascular routes were calculated accordingly. The pharmacokinetic parameters are reported as group mean ± SD. The mean for each pharmacokinetic variable were determined by averaging the calculated parameters for drug in each animal.

RESULTS

The method refined in this study was selective for the substance analysed (difloxacin, peak t_R = 6.80 min); no endogenous interference was observed on chromatograms. Assay standards were prepared separately for pig and chicken plasma and the LLOQ was the same regardless of species. The limit of quantitation was 0.05 μg/mL for difloxacin. Difloxacin quantitation was linear within a range of 0.05–10 μg/mL. The recoveries of difloxacin from plasma samples were 98.25 and 99.73% for pigs and broilers, respectively. Coefficients of variation were <6% for both within runs and between runs.

The plasma concentration vs. time curves and log-concentration vs. time curves of difloxacin are shown in Fig. 1 for pigs and Fig. 2 for broilers. The main pharmacokinetic parameters calculated from the plasma data are listed in Table 1 for pigs and Table 2 for broilers, respectively.

In pigs, it's best to fit the difloxacin concentration-time data to a two-compartment open model after single i.v. dosing. A two-compartment model with first order absorption best described the drug concentration-time data after single i.m. and p.o. administration. The main pharmacokinetic parameters were as in Table 1. The results of present studies showed that difloxacin was rapidly absorbed, extensively distributed and slowly eliminated in pigs. The drug was completely absorbed after single i.m. and p.o. administration and had a good bioavailability in pigs.

In broilers, it's best to fit the difloxacin concentration-time data to a two-compartment open model after single i.v. dosing in

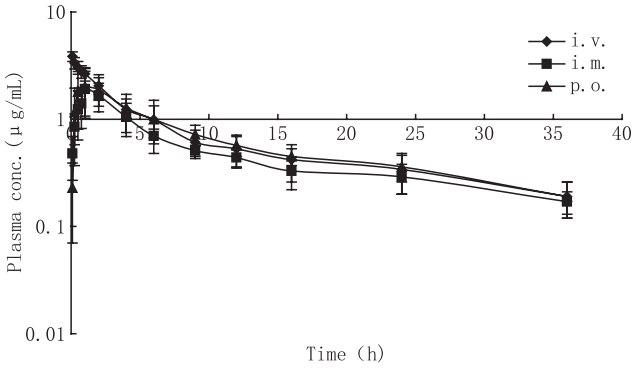

Fig. 1. Plasma concentration vs. time curves of difloxacin in pigs (n = 7) after intravenous (i.v.), intramuscular (i.m.), or oral (p.o.) administration at a single dose of 5 mg/kg b.w.

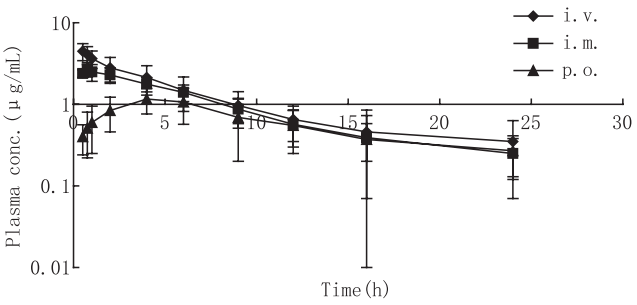

Fig. 2. Plasma concentration vs. time curves of difloxacin in broilers (n = 10) after intravenous (i.v.), intramuscular (i.m.), or oral (p.o.) administration at a single dose of 10 mg/kg b.w.

Table 1. Pharmacokinetic parameters (mean ± SD) obtained from plasma concentrations of difloxacin after single intravenous (i.v.), intramuscular (i.m.) or oral (p.o.) administration in pigs (5 mg/kg b.w., n = 7)

Pharmacokinetic parameters	i.v.	i.m.	p.o.
$t_{1/2ka}$ (h)		0.47 ± 0.22	0.54 ± 0.44
$t_{1/2a}$ (h)	1.58 ± 0.64	2.39 ± 1.52	2.04 ± 1.79
$t_{1/2\beta}$ (h)	17.14 ± 4.14	25.79 ± 8.10	16.67 ± 4.04
t_{max} (h)		1.29 ± 0.26	1.41 ± 0.88
C_{max} (μg/mL)		1.77 ± 0.66	2.29 ± 0.85
$V_{d(area)}$ (L/kg)	4.91 ± 1.88		
Cl_B (L/kg/h)	0.20 ± 0.06		
AUC (mg/L·h)	27.24 ± 8.12	24.98 ± 9.29	26.59 ± 5.30
F (%)		95.3 ± 28.9	105.7 ± 37.1

F, bioavailability (calculated using an intra-individual approach); the other abbreviations are defined in the text.

Table 2. Pharmacokinetic parameters (mean ± SD) obtained from plasma concentrations of difloxacin after single intravenous (i.v.), intramuscular (i.m.), or oral (p.o.) administration in broilers (10 mg/kg b.w., n = 10)

Pharmacokinetic parameters	i.v.	i.m.	p.o.
$t_{1/2ka}$ (h)		0.17 ± 0.12	1.46 ± 1.00
$t_{1/2\alpha}$ (h)	0.69 ± 0.43		
$t_{1/2\beta}$ (h)	6.11 ± 1.50	5.64 ± 0.74	8.20 ± 3.12
t_{max} (h)		0.86 ± 0.40	4.34 ± 2.40
C_{max} (μg/mL)		2.51 ± 0.36	1.00 ± 0.21
$V_{d(area)}$ (L/kg)	3.10 ± 0.67		
Cl_B (L/kg/h)	0.37 ± 0.10		
AUC(mg/L·h)	29.16 ± 8.04	22.62 ± 3.28	15.82 ± 3.67
F (%)		77.0 ± 11.8	54.2 ± 12.6

F, bioavailability (estimated using the ratio of AUC of each chicken to the average AUC of the i.v. administration); the other abbreviations are defined in the text.

healthy chickens. A one-compartment model with first order absorption best described the drug concentration-time data after single i.m. and p.o. administration in broilers. The main pharmacokinetic parameters were as in Table 2. The results of present studies showed that difloxacin was rapidly absorbed after intramuscular administration, extensively distributed and slowly eliminated in chickens. The drug was not completely absorbed after single i.m. and p.o. administration in broilers.

DISCUSSION

During the last decade, several fluoroquinolones such as enrofloxacin, danofloxacin, sarafloxacin and marbofloxacin have been carefully investigated for veterinary application in the treatment of a variety of bacterial infections. There are many papers published on the pharmacokinetics of these antimicrobial agents in various animal species. Our results from the i.v., i.m., and p.o. administrations of difloxacin in pigs and broilers show that difloxacin had quite similar pharmacokinetic characteristics as other fluoroquinolones, except for much longer half-life of elimination in pigs.

In pigs, difloxacin were adequately described by a two-compartment open model with a rapid absorption, distribution and a slow elimination phase. The pharmacokinetic variables for i.v. administration confirm the belief that difloxacin probably distributed out of the plasma compartment ($V_{d(area)}$ 4.91 ± 1.88 L/kg). The elimination half-lives of difloxacin in pigs by this study (17.17, 25.79, 16.67 h for i.v., i.m., and p.o. dosing respectively) were not only much longer than those obtained in goats, calves, horses, rabbits, dogs(Heinen, 2002; Abd El-Aty *et al.*, 2005; Fernandez-Varon *et al.*, 2006; Ismail, 2007; Marin *et al.*, 2007) (range 2.66–10.75 h) and chickens (this study) but also much longer than those of other fluoroquinolones in pigs(Anadon *et al.*, 1994; Zeng & Fung, 1997; Richez *et al.*, 1997; Fang *et al.*, 1999; Ding *et al.*, 2001) (range 3.15–7.20 h). In this study, the elimination half-life after i.m. administration was apparently longer than that obtained after i.v. administration. This difference is probably the result of continued absorption of difloxacin from the i.m. injection site during the elimination phase, thereby prolonging the $t_{1/2\beta}$ of the drug. Difloxacin was rapidly absorbed with a C_{max} of 1.77 being achieved 1.29 h after i.m. administration and a C_{max} of 2.29 being achieved 1.41 h after p.o. administration, respectively. The bioavailability of difloxacin was calculated to be 95.3% after i.m. administration, which is similar to that in goats, calves, horses, rabbits, pigs (Inui *et al.*, 1998; Abd El-Aty *et al.*, 2005; Fernandez-Varon *et al.*, 2006; Ismail, 2007; Marin *et al.*, 2007) (range 93.7–106.8%). After p.o. administration, the bioavailability of was 105.7%, more than that (68.62%) found by others in horses (Fernandez-Varon *et al.*, 2006). Because the methods for calculation were different. It is not suitable to make any comparisons between the bioavailability calculations in swine to that in broilers in this paper.

In broilers, difloxacin was also rapidly and extensively distributed into body fluids and tissues after i.v. administration. Distribution volume ($V_{d(area)}$) in broilers was calculated to be 3.10 L/kg, which is less than that in pigs. The $t_{1/2\beta}$ after i.v., i.m. and p.o. administration of difloxacin were estimated to be 6.11, 5.64, and 8.20 h respectively, shorter than those in pigs (this study). A C_{max} of 2.51 μg/mL after i.m. administration of difloxacin and a C_{max} of 1.00 μg/mL after p.o. administration in broilers were achieved at 0.86 and 4.34 h. The bioavailability were calculated to be 77.0 and 54.2% for i.m. administration and p.o. administration respectively, which are much less than those of difloxacin in other animals (Fernandez-Varon *et al.*, 2006; Ismail, 2007; Marin *et al.*, 2007). The results of the analysis after i.m. and p.o. administration showed that difloxacin was partially absorbed.

In conclusion, this study demonstrated that difloxacin was completely absorbed and slowly eliminated after single i.m. and p.o. administration in healthy pigs. In healthy broilers, the drug was incompletely absorbed and rapidly eliminated after single i.m. p.o. administration. So, in order to achieve a comparable peak plasma concentration, the effective administration of difloxacin in broilers should be higher than that in pigs.

As the AUC to MIC ratio and C_{max} to MIC ratio were considered to be critical for fluoroquinolone efficacy (Brown, 1996; Shojaee Aliabadi & Lees, 1997; Adams, 2001), C_{max}/MIC ratio and AUC/MIC ratio can be used in designing reasonable dose regimens. Since a AUC/MIC ratio of 125–250 has been associated with the optimum antibacterial effect, according to this study, a dosage regimen was recommended. For pigs, a difloxacin dosage of 5 mg/kg i.m. with a 24 h dosing interval will provide effective plasma concentration to inhibit bacteria with MIC less than 0.2 μg/mL (AUC/MIC = 125 h) such as *E. coli* and *Salmonella sp.* In contrast, a dosage of 10 mg/kg administered orally at 24 h intervals could provide effective plasma concentration in chickens with bacteria infection in which MIC are <0.1 μg/mL (AUC/MIC = 150 h) such as *Mycoplasma gallisepticum* infection, *Salmonellosis avium* and *Colibacillosis*. Since difloxacin is forbidden or severely restricted to be used in food producing animals in the United States and European Union, the drug must be restricted to use by or on the order of a licensed veterinarian because professional expertise is judged to be critical in the diagnosis and proper treatment of bacterial infections.

Because of the economic trend towards globalization, the issues of antimicrobial resistance should be carefully considered when applying drugs such as fluroquinolones. As there was a paucity of MIC data for difloxacin and bacteria isolated from pigs and broilers, collected cultures and sensitivities is of practical importance in determining the optimal treatment regimens, and thus should deserve further investigations.

REFERENCES

Abd El-Aty, A.M., Goudah, A., Ismail, M. & Shimoda, M. (2005) Disposition kinetics of difloxacin in rabbit after intravenous and intramuscular injection of Dicural. *Veterinary Research Communication*, **29**, 297–304.

Adams, H.R. (2001) Veterinary pharmacology and therapeutics. In *Fluoroquinolone Antimicrobial Drugs*, 8th edn, Chapter 45. Eds Papich, M.G. & Riviere, J.M., pp. 907. Iowa State University Press, Ames, IA.

Anadon, A., Martinez, M.R., Diaz, M.J., Fernandez, R., Martinez, M.A. & Fernandez, M.C. (1994) Pharmacokinetic properties of norfloxacin in pigs. *The Congress of 6th EVAPA*, **17,** (Suppl. 1), 230–231.

Bansal, M.B. & Thadepalli, H. (1987) Activity of difloxacin (A-56619) and A-56620 against clinical anaerobic bacteria *in vitro*. *Antimicrobial Agents and Chemotherapy*, **31,** 619–621.

Boothe, D.M., Boeckh, A., Simpson, R.B. & Dubose, K. (2006) Comparison of pharmacodynamic and pharmacokinetic indices of efficacy for 5 fluoroquinolones toward pathogens of dogs and cats. *Journal of Veterinary Internal Medicine*, **20,** 1297–1306.

Brown, S.A. (1996) Fluoroquinolones in animal health. *Journal of Veterinary Pharmacology and Therapeutics*, **19,** 1–14.

Digranes, A. & Dibb, W.L. (1988) *In vitro* activities of A-56619 (difloxacin) and A-56620, two aryl-fluoroquinolones. *Chemotherapy*, **34,** 298–307.

Ding, H.Z., Zeng, Z.L., Fung, K.F., Chen, Z.L. & Qiao, G.L. (2001) Pharmacokinetics of sarafloxacin in pigs and broilers following intravenous, intramuscular, and oral single-dose applications. *Journal of Veterinary Pharmacology and Therapeutics*, **24,** 303–308.

Eliopoulos, G.M., Moellering, A.E., Reiszner, E. & Moellering, R.C. Jr (1985) *In vitro* activities of the quinolone antimicrobial agents A-56619 and A-56620. *Antimicrobial Agents and Chemotherapy*, **28,** 514–520.

Fang, B.H., Fung, K.F., Chen, Z.L. & Wang, Z.Q. (1999) Bioavailability and pharmacokinetics of ciprofloxacin in pigs. *Chinese Journal of Veterinary Science*, **19,** 588–590. (in Chinese)

Fernandes, P.B., Chu, D.T., Bower, R.R., Jarvis, K.P., Ramer, N.R. & Shipkowitz, N. (1986) *In vivo* evaluation of A-56619 (difloxacin) and A-56620: new aryl-fluoroquinolones. *Antimicrobial Agents and Chemotherapy*, **29,** 201–208.

Fernandez-Varon, E., Carceles, C.M., Marin, P., Martos, N., Escudero, E. & Ayala, I. (2006) Pharmacokinetics of difloxacin after intravenous, intramuscular, and intragastric administration to horses. *American Journal of Veterinary Research*, **67,** 1076–1081.

Frazier, D.L., Thompson, L., Trettien, A. & Evans, E.I. (2000) Comparison of fluoroquinolone pharmacokinetic parameters after treatment with marbofloxacin, enrofloxacin, and difloxacin in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, **23,** 293–302.

Heinen, E. (2002) Comparative serum pharmacokinetics of the fluoroquinolones enrofloxacin, difloxacin, marbofloxacin, and orbifloxacin in dogs after single oral administration. *Journal of Veterinary Pharmacology and Therapeutics*, **25,** 1–5.

Inui, T., Taira, T., Matsushita, T. & Endo, T. (1998) Pharmacokinetic properties and oral bioavailabilities of difloxacin in pig and chicken. *Xenobiotica*, **28,** 887–893.

Ismail, M. (2007) Disposition kinetics of difloxacin after intravenous, intramuscular and subcutaneous administration in calves. *Veterinary Research Communication*, **31,** 467–476.

Marin, P., Escudero, E., Fernandez-Varon, E. & Carceles, C.M. (2007) Pharmacokinetics and milk penetration of difloxacin after intravenous, subcutaneous and intramuscular administration to lactating goats. *Journal of Veterinary Pharmacology and Therapeutics*, **30,** 74–79.

Nilsson-Ehle, I. (1987) Assay of ciprofloxacin and norfloxacin in serum and urine by high-performance liquid chromatography. *Journal of Chromatography*, **416,** 207–211.

Olchowy, T.W., TerHune, T.N. & Herrick, R.L. (2000) Efficacy of difloxacin in calves experimentally infected with *Mannheimia haemolytica*. *American Journal of Veterinary Research*, **61,** 710–713.

Richez, P., Pedersen Morner, A., De Jong, A. & Monlouis, J.D. (1997) Plasma pharmacokinetics of parenterally administered danofloxacin and enrofloxacin in pigs. *Journal of Veterinary Pharmacology and Therapeutics*, **20** (Suppl 1), 41–42.

Shojaee Aliabadi, F. & Lees, P. (1997) Pharmacodynamic and pharmacokinetic inter-relationships of antibacterial drugs. *Journal of Veterinary Pharmacology and Therapeutics*, **20** (Suppl. 1), 14–17.

Smith, B.R., Lefrock, J.L., Donato, J.B., Joseph, W.S. & Weber, S.J. (1986) *In vitro* activity of A-56619 and A-56620, two new aryl-fluoroquinolone antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, **29,** 355–358.

Xian, W.J. & Cheng, Z.R. (1988) MCPKP- a microcomputer program specialized for pharmacokinetic compartment analysis. *Acta Pharmcologica Sinica*, **9,** 188–192. (in Chinese)

Zeng, Z. & Fung, K. (1997) Effects of experimentally induced Escherichia coli infection on the pharmacokinetics of enrofloxacin in pigs. *Journal of Veterinary Pharmacology and Therapeutics*, **20** (Suppl. 1), 39–40.

Zeng, Z.L., Liu, J.X., Chen, Z.L. & Feng, Q.H. (2000) A comparison of enrofloxacin, danofloxacin, difloxacin in the treatment of experimental of *Pasteurella multocida* infection of chickens [J]. *Journal of South China Agricultural University*, **21,** 82–84. (in Chinese)

Zeng, Z.L., Fang, B.H., Chen, Z.L., Feng, Q.H. & Peng, X.F. (2001) Pharmacodynamics of difloxacin against experimentally induced *Streptococcosis* and *Mycoplasmal pneumonia* in pigs [J]. *Acta Veterinaria et Zootechnica Sinica*, **32,** 175–180. (in Chinese)