Bulgarian Journal of Veterinary Medicine (2004), 7, No 3,139–148

PHARMACOKINETICS AND PK/PD MODELLING OF ENROFLOXACIN IN *MELEAGRIS GALLOPAVO* AND *GALLUS DOMESTICUS*

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Summary

Haritova, A., H. Djeneva, L. Lashev, P. Sotirova, B. Gyurov, V. Dyankov and M. Stefanova, 2004. Pharmacokinetics and PK/PD modelling of enrofloxacin in *Meleagris gallopavo* and *Gallus domesticus. Bulg. J. Vet. Med.*, **7**, No 3, 139–148.

The pharmacokinetics of enrofloxacin and the PK/PD parameters $AUC_{0\text{-}24h}/MIC_{90}$ and C_{max}/MIC_{90} of the drug were determined in healthy turkeys (*Meleagris gallopavo*) (n=6) and cocks and hens (*Gallus domesticus*) (n=6). The phamacokinetic parameters were calculated on the basis of enrofloxacin concentrations and its active metabolite ciprofloxacin using a microbiological assay. The elimination half-life ($t_{1/2\beta}$), the area under the concentration-time curve during a 24-hour dosing period ($AUC_{0\text{-}24h}$) and the total body clearance of intravenously administrered enrofloxacin at the dose of 2.5 mg/kg in turkeys and in cocks and hens were 6.05±1.83 h and 5.54±1.07 h; 5.00±0.99 μg.h/mL and 3.90±1.08 μg.h/mL; 8.36±2.03 mL/min.kg and 11.11±3.26 mL/min.kg respectively. Following oral administration at the dose of 5 mg/kg, $t_{1/2\beta}$, $AUC_{0\text{-}\infty}$ and the bioavailability in turkeys and in cocks and hens were 5.98±2.03 h and 4.92±0.94 h; 6.74±1.60 μg.h/mL and 6.22±2.10 μg.h/mL; 69.85±30.85 % and 87.94±49.53 % respectively. No statistically significant differences were observed between pharmacokinetic parameters of both avian species. The minimal inhibitory concentrations (MIC_{90}) of avian *Salmonella spp*. and *Escherichia coli* abattoir isolates were 0.5 μg/mL, whereas the $AUC_{0-24\ h}/MIC$ and C_{max}/MIC_{90} values were 11.5 h and 14.9 h; 1.6 and 2.0 in turkeys and in cocks and hens respectively. The used dose was assumed to be adequate for treatment of infections caused by very sensitive microorganisms with MIC 0.015 μg/mL.

Key words: enrofloxacin, pharmacokinetics, poultry

INTRODUCTION

During the last years, antibacterial drugs including quinolones, are among the most commonly used medicines for treatment of infections in poultry. The data for the activity of enrofloxacin against *Escherichia coli* and *Salmonella spp*., isolated in birds in several European countries showed that the majority of *E. coli* strains were inhibited by concentrations between 0.015–0.06–1 μg/mL (Scheer *et al.*, 1997a; Knoll *et al.*, 1999; Pirro *et al.*, 2000). The resistance of *E. coli* strains

isolated from chickens and parent flocks against enrofloxacin was from 3.8 to 47 % (Burch, 2000; Pirro *et al.*, 2000; Chen *et al.*, 2003). The frequent use of enrofloxacin in turkeys was associated with a higher degree of resistance to ciprofloxacin (49%) among *E. coli* organisms isolated from this avian species (Saenz *et al.*, 2001). A retrospective study on the resistance of 952 *Salmonella spp.* chicken isolates (1992–1998) revealed that the resistance to enrofloxacin was under 7.9% (threshold MIC≥2.0 μg/mL) (Pirro, 2000). MIC_{90} of ciprofloxacin and enrofloxacin for *Salmonella spp*. strains (n=994), isolated from broiler chickens were 0.06 and 0.03–1 μg/mL respectively (Scheer *et al.*, 1997b; Pirro *et al.*, 2000).

The pharmacodynamics, in combination with pharmacokinetics has the potential to ensure an objective approach in the treatment with quinolones (Gunderson *et al.*, 2001). The parameters, characterizing adequately the correlation between the pharmacokinetic parameters and the clinical efficacy, are AUC/MIC, C_{max}/MIC и T>MIC (duration of time that plasma antibiotic concentration exceeds MIC) as well as MIC (Aliabadi & Lees, 2002; McKellar, 2002; Toutain, 2002; Toutain *et al.*, 2002). The fluoroquinolone doses, ensuring a ratio C_{max}/MIC > 3, result in 99% reduction in bacterial counts (Aliabadi and Lees, 2001). AUC/MIC values < 125 require a longer time for elimination of bacteria (Aliabadi and Lees, 2001). The aim in quinolone use is to achieve a high C_{max} for a short time. That is why the single administration of high doses per 24 h is recommended (Sarasola *et al.*, 2002). The risk of appearance of resistance in Gram-negative microorganisms is increased if the ratios AUC_{0-24}/MIC > 100 and C_{max}/ MIC > 8 are not attained (Schentag *et al.*, 2001). The information about PK/PD parameters and their validation is still based on human clinical experience and the data in domestic animals are few (Toutain, 2003). Considering the species-related features of birds (anatomical, raising technologies etc.) the direct application of PK/PD parameters established in other animal species is not advisable and requires investigation of optimal values in cocks, hens and turkeys (Lees and Aliabadi, 2002).

The pharmacokinetics of enrofloxacin is studied in chickens (Conzelman *et al.*, 1987; Anadon *et al.*, 1995; Garcia Ovando *et al.* 1999; Knoll *et al.*, 1999; Korchi *et al.*, 2003) and at a lesser extent in turkeys (Bailey *et al.*, 1997) following intravenous and oral administration. The data in turkeys are few and do not permit the performance of a satisfactory therapy. Therefore, the aim of the present study was to study the pharmacokinetics of enrofloxacin in turkeys and to determined the PK/PD parameters AUC_{0-24h}/MIC_{90} and C_{max}/MIC_{90} of enrofloxacin in healthy turkeys, cocks and hens.

MATERIALS AND METHODS

Drug

Enrofloxacin - 2.5 % injectable solution in 100 mL flasks (Syvaquinol 2.5% injectable solution, Syva Laboratorios, Leon, Spain) and substance, provided from Biovet Ltd – Peshtera, Bulgaria.

Experimental animals

The studies were performed in healthy animals: 3 cocks and 3 hens from the Erebati breed at the age of 6 months weighing 2.27–3.63 kg; 6 turkeys (BUT 9 hybrids) at the age of 4 months, weighing 5.5–8.4 kg. The birds were provided from

the Stara Zagora Experimental Base of the Animal Breeding Institute – Kostinbrod.

The experimental animals (marked with colour markers) were under uniform conditions of housing and feeding, according to the species' requirements one week prior to the trials and to the end of the study. The hens and cocks were housed in groups of 3 in cages, conforming to the standard conditions and providing the necessary space. The turkeys were housed in metal cages in groups of 6 under conditions identical to those in the turkey-breeding farm. The birds received a standard diet (not supplemented with coccidiostatic) and free access to drinking water.

Experimental design

All experiments were performed between 7:30 and 8:30 h AM. Each treatment was followed by a washout period of 15 days. A cross-over design was employed. The intravenous treatment was done in *v. brachialis*. The intraingluvial administrations was performed via an elastic tube. Blood samples were obtained from *v. brachialis* (in case of i.v. treament, from the respective contralateral vein).

The enrofloxacin was applied i.v. (at 2.5 mg/kg) and p.o. (at 5 mg/kg) in turkeys, cocks and hens as the 2.5 % injectable solution. Blood samples were collected prior to drug application and by post treatment hours 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 h. In the oral admistration trial, samples were obtained prior to the treatment and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10 and 24 h after the drug administration.

The blood samples of 1 mL were allowed to clot at room temperature for 2 h. The sera were separated via centrifugation at 1500 rpm for 15 min and stored in plastic tubes at –20 ^{o}C until analysis, that took place not later than 48 h after sampling.

Drug assay

The enrofloxacin concentrations and those of its active metabolite ciprofloxacin were determined as a sum via a microbiological assay. *Escherichia coli* ATCC 25922 was used as test-microorganism (nutrient medium –meat peptone agar, supplied by the National Research Institute of Infectious and Parasitic Diseases, Sofia). The limit of quantitative determination of the method in serum was 0.01 μg/mL. The concentrations of standard solutions of enrofloxacin were 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, 0.019 and 0.01 μg/mL. The r^2 of standard serum curves was 0.9952. The recovery percentage was 94.38 %, the intra-assay CV – 6.78 and the inter-assay CV – 9.72. The standard solutions were prepared with sera obtained from intact animals of the respective species.

Pharmacokinetic analysis

A non-compartmental analysis was used. The pharmacokinetic parameters of enrofloxacin and its active metabolite, ciprofloxacin, were calculated with the WinNonlin 4.0.1 software (Pharsight Corporation, 800 West El Camino Real, Mountain View, CA, USA). The pharmacokinetic parameters were calculated individually for each animal and presetned as mean ± standard deviation.

The following parameters were determined: the hybrid rate constant for the elimination phase (β), obtained from the terminal part of the drug-concentration time curve; the elimination half-life ($t_{1/2\beta}$), the area under the drug concentration-time curve ($AUC_{0-\infty}$), calculated according to the linear trapezoidal rule with extrapolation to infinity. In cocks and hens the area

under the drug concentration-time curve was also calculated between 0 and 10 h after the oral drug administration, and in turkeys it was also calculated between 0 and 24 h and these values were used to estimate Pk/Pd surrogates. Also, the volume of distribution (Vd_{area}) (on the basis of the area under the curve), the total body clearance (Cl_B), the steady-state volume of distribution (V_{ss}), the maximum serum concentrations (C_{max}), the time of C_{max} (T_{max}), the mean residence time (MRT) were determined.

For the oral route of administration, the bioavailability (F) was calculated as

$$F_{abs}\% = (AUC_{im} \times D_{iv})/(AUC_{iv} \times D_{im}).100$$

Determination of minimal inhibitory concentrations (MIC)

The *in vitro* sensitivity to enrofloxacin was tested on 25 *Salmonella gallinarum pullorum,* 3 *Salmonella enteritidis* and 23 *Escherichia coli* strains. Strains were isolated via cultivation of faecal samples, obtained from turkeys and hens, on both selective and differentiating nutrient media: deoxycholate citrate agar, McConkey's agar and selenite broth (Bul Bio, Sofia, Bulgaria) The cultivation was done at 37 ^{o}C for 24–48 h. The colonies were biochemically identified via routine methods and the Sceptor – BBL automated system (Becton Dickinson, Sparcs, MD, USA) – Enteric Panel MIC/ID. The serological typization of serovars was done with the reaction agglutination, type Gruber, using agglutination serums (Bul Bio, Sofia, Bulgaria). The MIC was determined after serial dilutions in agar with enrofloxacin concentrations between 0.25 to 32 mg/L. The strains were determined as susceptible, intermediate and resistant according to NCCLS 2000 Guidelines (Murray *et al.*, 1995).

Statistical analysis

The data were statistically processed by the Wilcoxon's test (Statistica 6.1; StatSoft, Inc., USA, 2002).

RESULTS

Detectable serum concentrations of enrofloxacin and its primary metabolite – ciprofloxacin, were observed up to post i.v. administrations hour 24 in both avian species (Fig. 1 and Fig. 2). They had similar values in turkeys as well as in cocks and hens and following oral administration, serum concentrations were detected by hour 0.25 h. C_{max} were observed by post treatment hour 3 in turkeys (Fig. 1). In cocks and hens there was not a distinct peak and serum levels between hours 0.75 and 3 were comparable (Fig. 2). The pharmacokinetic parameters are presented in Table 1.

The minimal inhibitory concentrations (MIC_{90}) for *E. coli* (n=23) and *Salmonella spp*. (n=28) were 0.5 μg/mL. Six *E. coli* isolates that exhibited growth at concentrations ≥ 4 μg/mL were considered as resistant. No resistant *Salmonella spp.* were found out, considering , the levels of ≥1 μg/mL as break point for resistance. The C_{max}/MIC_{90} values, the area under the inhibitory concentrations (AUIC) and the time when serum enrofloxacin levels were higher that the MIC_{90} of *E. coli* and *Salmonella spp.*, calculated using with the pharmacokinetic parameters following single oral administration and by simulation following repeated administration, are presented in Table 2. The data for C_{max}/MIC_{90} ratios showed that following oral enrofloxacin administration, levels higher than MIC_{90} were maintained for 3.5 h and 5.5 h in turkeys and in cocks and hens,

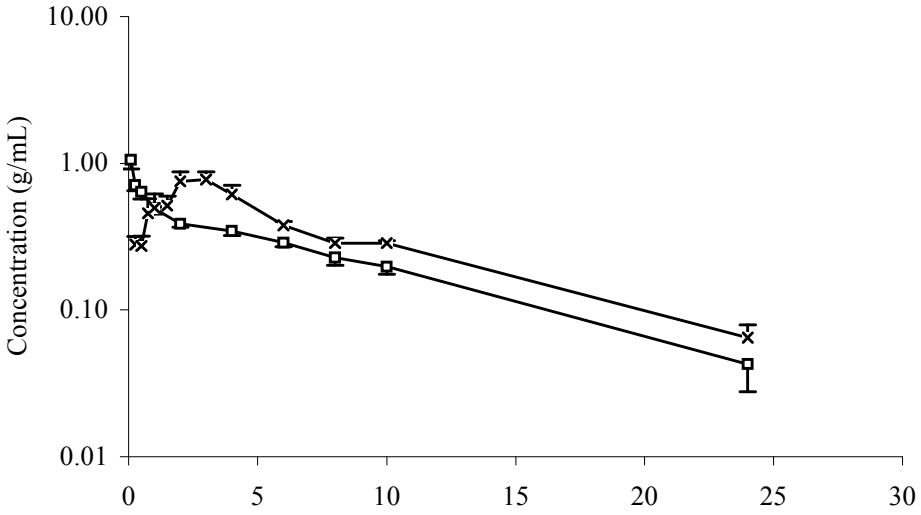

Fig. 1. Serum enrofloxacin concentrations (enrofloxacin and ciprofloxacin) in turkeys (n=6) following single i.v. administration at 2.5 mg/kg (–□–) and p.o. administration at 5 mg/kg (–×–).

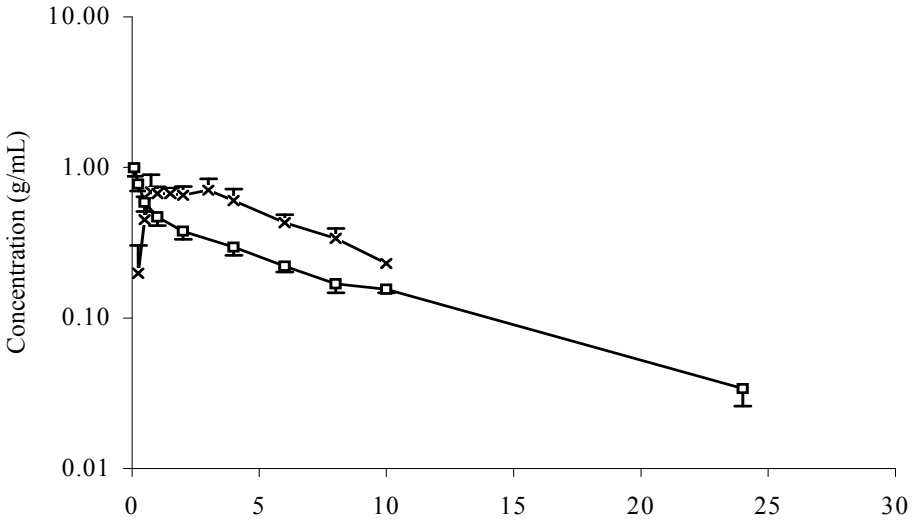

Fig. 2. Serum enrofloxacin concentrations (enrofloxacin and ciprofloxacin) in cocks and hens (n=6) following single i.v. administration at 2.5 mg/kg (–□–) and p.o. administration at 5 mg/kg (–×–).

Table 1. Pharmacokinetic parameters of enrofloxacin following intravenous (at 2.5 mg/kg) and oral (at 5 mg/kg) administration in turkeys (n=6) and cocks and hens (n=6)

Pharmacokinetic parameters	Turkeys	Cocks and hens
Non-compartmental model – i.v.		
β (h^{-1})	0.12 ±0.03	0.129 ±0.02
$t_{1/2\beta}$ (h)	6.05 ±1.83	5.54 ±1.07
$t_{1/2\beta}$ (h)*	5.66	5.39
MRT (h)	8.32 ±2.33	6.99 ±1.05
Vd_{area} (L/kg)	4.23 ±0.98	5.11 ±0.86
V_{ss} (L/kg)	4.06 ±0.94	4.55 ±1.08
Cl_B (mL/min.kg)	8.36 ±2.03	11.11 ±3.26
$AUC_{0-\infty}$ (μg.h/mL)	5.22 ±1.16	4.06 ±1.28
AUC_{0-24h} (μg.h/mL)	5.00 ±0.99	3.90 ±1.08
Non-compartmental model – p.o.		
β' (h^{-1})	0.13 ±0.06	0.144 ±0.03
$t_{1/2\beta}$ (h)	5.98 ±2.03	4.92 ±0.94
$t_{1/2\beta}$ (h)*	5.25	4.78
T_{max} (h)	2.29 ±0.90	1.71 ±1.12
C_{max} (μg/mL)	0.87 ±0.20	0.99 ±0.34
MRT (h)	8.67 ±2.45	7.59 ±1.35
$AUC_{0-\infty}$ (μg.h/mL)	6.74 ±1.88	6.22 ±2.10
$AUC_{0-24h \text{ or } 0-10h}$ (μg.h/mL)	6.40 ±1.60	6.22 ±2.10**
F (%)	69.85 ±30.85	87.94 ±49.53

β – terminal (elimination) rate constant; $t_{1/2\beta}$ – terminal elimination half-life; *$t_{1/2\beta}$ – harmonic mean; MRT – mean residence time; V_{darea}, and V_{ss} – apparent volume of distribution and steady-state volume of distribution; Cl_B – total body clearance; AUC – area under the serum concentration-time curves for serum from 0 to ∞ and from 0 to 24 h; C_{max} – maximum serum concentrations, T_{max} – time of C_{max}, F – bioavailability; ** AUC_{0-10h} – the AUC value in cocks and hens was calculated to the moment of determination of the last detectable blood concentrations, 10 h following p.o. administration of enrofloxacin.

Table 2. PK/PD parameters following single p.o. administration of enrofloxacin (at 5 mg/kg) in turkeys and cocks and hens and calculated on the basis of simulated repeated administration

PK/PD parameters	Turkeys		Cocks and hens	
	Single administration	Simulation of repeated administration	Single administration	Simulation of repeated administration
AUIC (h)	12.8	11.5	12.4	14.9
C_{max}/MIC_{90}	1.7	1.6	2.0	2.0
$Tmic_{90}$ (h)	ND	3.5	ND	5.5

AUIC (h) – area under the inhibitory concentration-time curve; C_{max}/MIC_{90} – maximal serum concentration-to-MIC_{90} ratio; $Tmic_{90}$ (h) –time during which enrofloxacin concentrations exceeded the MIC_{90}; ND – not determined.

respectively (Table 2). C_{max}/MIC_{90} values calculated on the basis of summarized enrofloxacin and ciprofloxacin levels following single application of enrofloxacin, were similar to those obtained after simulation of repeated administration. In cocks and hens they were insignificantly higher. AUIC values following single application of enrofloxacin in turkeys, cocks and hens were almost identical. The AUIC values in cocks and hens obtained via simulation after repeated application were increased.

DISCUSSION

The reported data of enrofloxacin pharmacokinetics following intravenous administration in fowls were obtained via microbiological and HPLC assays for serum concentrations determination. Therefore, the $t_{1/2\beta}$ values for i.v. administered enrofloxacin in chickens are considerably variable: when HPLC methdos were used (Anadon *et al.*, 1995; Garcia Ovando *et al.*, 1999; Knoll *et al.*, 1999) the $t_{1/2\beta}$ ranged between 6.7 and 10.3 h, whereas the microbiological assay yielded $t_{1/2\beta}$ values of 18.7 h (Conzelman *et al.*, 1987). The elimination half-life of i.v. administered enrofloxacin in turkeys was 4–6 h (Bailey *et al.*, 1997) – values, completely identical to our. The data about $t_{1/2\beta}$ in avian species, studied by us, were close to the lowest values calculated for this parameter and no statistical differences were present between turkeys, cocks and hens (p=0.46). Knoll *et al.* (1999) reported a Vd_{area} of 3.9 L/kg and total body clearance of 10.3 mL/min.kg in broiler chickens. Our data were similar. Lower Cl_B values were communicated in chickens (3.30 mL/min.kg) by Garcia Ovando *et al.* (1999).

The peak serum concentrations of enrofloxacin following p.o. administration were insignificantly lower in turkeys (p=0.46) and were attained statistically insignificantly later (p=0.47) compared to those in cocks and hens . The T_{max} values obtained by us were close to cited literature data (Anadon *et al.* 1995; Knoll *et al.* 1999). Following oral administration, the data about $t_{1/2\beta}$ in chickens were different again. The values calculated on the basis of concentrations determined via HPLC or microbiological assays, were between 6.6 and 14.2 h (Anadon *et al.*, 1995; Knoll *et al.*, 1999) and 14.9 h (Conzelman *et al.*, 1987), respectively. Our data were closer to the lowest among cited references. The $t_{1/2\beta}$ in turkeys, observed by us, was insignficantly longer than that in cocks and hens (p=0.35) and was within the range observed by Bailey *et al.* (1997): $t_{1/2\beta}$ from 4 to 6 h. The F values after oral application of 10 mg/kg enrofloxacin to chickens, published by Anadon *et al.* (1995) and Knoll *et al.* (1999) were betwen 64% and 89.2%. Our bioavailability value in cocks and hens (87.94 %) was within the cited range whereas the values in turkeys were lower, yet statistically insignificantly (69.85 %; p=0.35). Our data in this avian species were comparable to reprted ones and permit us to assume that the behaviour of enrofloxacin in turkeys and in cocks and hens was not quite different.

The effect of fluorochinolones is dose-dependent and therefore, C_{max}/MIC and AUIC are considered as the most important PK/PD parameters, being in the closest correlation with the efficacy (Toutain *et al.*, 2002; McKellar, 2002). The C_{max}/MIC_{90} values in turkeys and cocks and hens following p.o. adminsitration of enrofloxacin at the dose of 5 mg/kg showed that the applied dose was rather low for the treatment of *E. coli* and *Salmonella spp.* infections with MIC 0.5

μg/mL. The precondition C_{max}/MIC>3, that would result in 99% reduction of bacterial coutns was not accomplished (Aliabadi and Lees, 2001). Moreover, because of the fact that a ratio of C_{max}/MIC>8 is not attained, there is a risk of appearance of resistance in Gram-negative miroorganisms (Schentag *et al.*, 2001). This dose would be suitable for treatment of infections caused by very sensitive microorganisms with MIC 0.015 μg/mL, where C_{max}/MIC and AUC/MIC would range between 58–66 and 414.7– 426.7 h, respectively. In this case, the opinion of Anadon & Martinez-Larranaga (2002) that the oral administration of quinolones leads to potentially therapeutical plasma concentrations against Gram-negative bacteria such as *E. coli* and *Salmonella spp.* would be true. This should be valid for the oral enrofloxacin application in turkeys as well. The C_{max}/MIC_{90} values in cocks and hens and in turkeys were 2.0 and 1.6 respectively, thus presuming that a postantibiotic effect could be expected no more than one hour (Wang *et al.*, 2003).

The low AUC_{0-24}/MIC values in turkeys and cocks and hens allow to admit that the applied dose could not guarantee the elimination of pathogenic *E. coli* and *Salmonella spp*.. Because of the fact that attained AUC_{0-24}/MIC was not higher than 100–125, there is a risk of appearance of resistance in Gram-negative bacteria as well as of cross resistance (Schentag *et al.*, 2001). The *E. coli* infection in chickens and turkeys could commonly result in septicaemia (Sarkozy *et al.*, 2002). Therefore, the AUC_{0-24}/MIC ratio should better be over 125 h (Forrest *et al.*, 1993; Craig, 1998; Anadon *et al.*, 2001).

The data suggest that the non-validated use of quinolones as well as the inadequated dosage could contribute to the selection and distribution of resistant *E. coli* and *Salmonella spp*. strains. This is a possible explanation of the relatively high resistance of *E. coli* against enrofloxacin in turkeys. The sensitivity of the strains studied by us to enrofloxacin is low and therefore, its oral adminsitration at 5 mg/kg would be particularly insufficient for treatment of infections caused by organisms with MIC 0.5 μg/mL. On the basis of the formula for determination of the optimal dosage regimen, proposed by Toutain *et al.* (2002) – dose for 24 h = $(AUIC.Cl_{B24h}.MIC)/F.24$ h, exact doses could be proposed. The calculated dosage regimen for AUC/MIC=125 h and our PK/PD parameters was 45 mg/kg orally for 24 h in turkeys and 47 mg/kg orally for 24 h in cocks and hens. Because of the high MIC_{90} values, calculated doses are relatively high that supports once again the necessity of determination of MIC every time when antibacterial drugs, including quinolones, are to be administered as well as when the most suitable chemotherapeutic drug has to be chosen. The used approach of PK/PD integration requires a further development in order to use the obtained data in a prospect study in spontaneously diseased birds or after experimental infection. The data obtained from such experiments would enable the use of a rational choice of optimal dosage regimens.

REFERENCES

Aliabadi, S. F. and P. Lees, 2001. Pharmacokinetics and pharmacodynamics of danofloxacin in serum and tissue fluids of goats following intravenous and intramuscular administration. *American Journal of Veterinary Research*, **62**, 1979–1989.

Aliabadi, S. F. and P. Lees, 2002. Pharmacokinetics and pharmacokinetic/pharmacodynamic integration of marbofloxacin in calf serum, exudate and transudate. *Jour-*

nal of Veterinary Pharmacology and Therapeutics. **25**, 161–174.

Anadón, A. and M. R. Martinez-Larranaga, 2002. Fluoroquinolone antibiotics in veterinary medicine. In: Proceedings of the International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM), Helsinki, Finland, pp.36–42.

Anadón, A., M. R. Martinez-Larranaga, M. J. Diaz, P. Bringas, M. A. Martinez, M. I. Fernandez-Cruz and R. Fernandez, 1995. Pharmacokinetics and residues of enrofloxacin in chickens. *American Journal of Veterinary Research*, **56,** 501–506.

Anadón, A., M. R. Martinez-Larranaga, J. Iturbe, M. A. Martinez, M. J. Diaz, M. T. Frejo and M. Martinez, 2001. Pharmacokinetics and residues of ciprofloxacin and its metabolites in broiler chickens. *Research in Veterinary Science,* **71**, 101–109.

Bailey, T. A., R. S. Sheen, J. H. Samour, A. Garner and D. Harron, 1997. Pharmacokinetics of enrofloxacin after intravenous, intramuscular and oral administration to houbara bustars (*Chlamydotis undulata macqueenii*). *Journal of Veterinary Pharmacology and Therapeutics*, **20** (Suppl. 1), 204–205.

Burch, D. G. S., 2000. Antimicrobial sensitivity patterns of UK chicken *E. coli* isolates. In: Proceedings of the 8th International Congress of the EAVPT (Abstracts), Jerusalem, Israeli, G 9.

Chen, Z. L., H. X. Jiang, C. M. Wu and X. P. Liao, 2003. A survey of resistance in *E. coli* to 21 antimicrobial agents and analysis of gyra and parc mutations in fluoroquinolone-resistance *E. coli* and *M. galisepticum*. *Journal of Veterinary Pharmacology and Therapeutics,* **26** (Suppl. 1), 219–220.

Conzelman, G. M., J. G. Babish, J. N. Davidson, R. A. McMillan and D. D. Copeland, 1987. Pharmacokinetic of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-ethyl-1-piperazinyl)-3-quinolone-carboxylic acid (Bay Vp 2674) in chickens. *Proceedings of the Western Pharmacology Society*, **30**, 393–395.

Craig, W. A., 1998. Pharmacokinetic/pharmacodynamic parameters: Rationale for antibacterial dosing of mice and man. *Clinical Infectious Diseases*, **26**, 1–10, 11–12.

Forrest, A., D. E. Nix, C. H. Ballow, T. E. Goss, M. C. Birmingham and J. J. Scheniag, 1993. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrobial Agents and Chemotherapy,* **37**, 1073–1081.

Garcia Ovando, H., N. Gorla, C. Luders, G. Poloni, C. Errecalde, G. Prieto and I. Puelles, 1999. Comparative pharmacokinetics of enrofloxacin and ciprofloxacin in chickens. *Journal of Veterinary Pharmacology and Therapeutics*, **22**, 209–212.

Gunderson, B. W., G. H. Ross, K. H. Ibrachim and J. C. Rotschafer, 2001. What do we really know about antibiotic pharmacodynamics? *Pharmacotherapy*, **21** (11s), 302–318.

Knoll, U., G. Glunder and M. Kietzmann, 1999. Comparative study of the plasma pharmacokinetics and tissue concentrations of danofloxacin and enrofloxacin in broiler chickens. *Journal of Veterinary Pharmacology and Therapeutics*. **22**, 239–246.

Korchi, G. El., C. Pratis, M. Giralt and B. Perez, 2003. Plasma levels of enrofloxacin and ciprofloxacin in different species after therapeutic administration. *Journal of Veterinary Pharmacology and Therapeutics.* **26** (Suppl. 1), 124–125.

Lees, P. and F. S. Aliabadi, 2002. Rational dosing of antimicrobial drugs: animal versus humans. *International Journal of Antimicrobial Agents*, **19**, 269–284.

McKellar, Q., 2002. How can knowledge of Pk/Pd interactions help limit the selection of antibiotic resistance? In: Proceedings of the International Conference on Antimicrobial Agents in Veterinary Medicine (AAVM), Helsinki, Finland, 28–29.

Murray, P. R., E. J. Baron, M. A. Pfaller, F. C.

Tenover and R. H. Yolken, 1995. Manual of Clinical Microbiology, 6th edn, eds. P. Murray, ASM Press, Washington, DC, USA.

Pirro, F., 2000. Antimicrobial susceptibility of current *Salmonella* strains isolated in Germany. In: Proceedings of the 8th International Congress of the EAVPT (Abstracts), Jerusalem, Israeli, G 26.

Pirro, F., R. Froyman, H.A. Greife, A. De Jong and M. Scheer, 2000. Enrofloxacin resistance monitoring in *E. coli* and *Salmonella* isolated from chickens: Eight years of experience. In: Proceedings of the 8th International Congress of the EAVPT (Abstracts), Jerusalem, Israeli, G 25.

Saenz, Y., M. Zarazaga, L. Brinas, M. Lantero, F. Ruiz-Larrea and C. Torres, 2001. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans. *International Journal of Antimicrobial Agents*, **18**, 353–358.

Sarasola, P., P. Lees, F. S. AliAbadi, Q. A. McKellar, W. Donachie, K. A. Marr, S. J. Sunderland and K. A. Rowan, 2002. Pharmacokinetic and pharmacodynamic profiles of danofloxacin administered by two dosing regimens in calves infected with *Mannhemia* (*Pasteurella*) *haemolytica*. *Antimicrobial Agents and Chemotherapy*, **46**, 3013–3019.

Sarkozy, G., G. Semjen, P. Laczay, E. Horvath and J. Schmidt, 2002. Pulse and continuous oral norfloxacin treatment of experimentally induced *E. coli* infection in broiler chicks and turkey poults. *Acta Veterinaria Hungarica*, **50**, 199–210.

Scheer, M., R. Froyman, A. De Jong, and P. Altreuther, 1997a. Antibacterial sensitivity monitoring of avian *Escherichia coli* isolates over 5 years. *Journal of Veterinary Pharmacology and Therapeutics,* **20** (Suppl. 1), 189.

Scheer, M., A. De Jong, R. Froyman and E. Heinen, 1997b. Antimicrobial activity in the digestive tract of broiler chickens treated orally with enrofloxacin. *Journal of Veterinary Pharmacology and Therapeutic,*. **20** (Suppl. 1), 201–202.

Schentag, J. J., K. K. Gilliland and J. A. Paladino, 2001. What have we learned from pharmacokinetic and pharmacodynamic theories? *Clinical infectious Diseases*, **32** (Suppl 1), S39–S46.

Toutain, P. L., 2002. Pharmacokinetic/pharmacodynamic integration in drug development ant dosage-regimen optimization for veterinary medicine, *AAPS PharmSci*, **4,** No 4, E38.

Toutain, P. L., 2003. Antibiotic treatment of animals – a different approach to rational dosing. *The Veterinary Journal*, **165**, 98–100.

Toutain, P. L., J. R. E. Del Castillo and A. Bousquet-Melou, 2002. The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. *Research in Veterinary Science*, **73**, 105–114.

Wang, Z. Q., Z. L. Chen, B. H. Fang and D. X. Tan, 2003. The postantibiotic effects of fluoroquinolones for veterinary use *in vitro* and *in vivo*. *Journal of Veterinary Pharmacology and Therapeutics,* **26** (Suppl. 1), 146–147.

Paper received 03.02.2004; accepted for publication 15.04.2004

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