Comparative studies on pulse and continuous oral norfloxacin treatment in broilers and turkeys

Géza Sárközy

Department of Pharmacology and Toxicology
Faculty of Veterinary Science
Szent István University
Budapest 2002

Supervisor: **Dr. Gábor Semjén, Ph.D.**

Department of Pharmacology and Toxicology
Faculty of Veterinary Science
Szt. István University Budapest
1078 Budapest, István u. 2.

Special advisor: **Dr. Péter Laczay, Ph.D.**

Department of Pharmacology and Toxicology
Faculty of Veterinary Science
Szt. István University Budapest
1078 Budapest, István u. 2.

Board members: **Dr. Károly Vörös, Ph.D.**

Department of Internal Medicine
Faculty of Veterinary Science
Szt. István University Budapest
1078 Budapest, István u. 2.

Dr. Imre Klebovich, Ph.D.

Department of Pharmacokinetics
EGIS Pharmaceuticals Co. Ltd.
1106 Budapest, Keresztúri út. 30-38.

I. CONTENTS

II. ABBREVIATIONS

AGDP	agar gel diffusion probe
AUC	area under the concentration-time curve
AUIC	area under the inhibitory concentration curve
BHI	Brain Heart Infusion
C_{max}	maximum plasma concentration
C_{pss}	steady-state plasma concentration
CFU	colony forming unit
CNS	central nervous system
DNA	deoxyribonucleic acid
DSA	Dextrose-Starch Agar
IBV	Infectious Bronchitis Virus
LD_{50}	lethal dose for the 50% of animals
MIC	minimum inhibitory concentration
MIC_{90}	minimum inhibitory concentration for the 90% of bacteria
MBC	mean bactericidal concentration
µg/ml	microgram per millilitre
NDV	Newcastle Disease Virus
mg/kg b.w.	milligram per bodyweight kilogram
mg/L	milligram per liter
ng/ml	nanogram per milliliter
PAE	post-antibiotic effect
RNA	ribonucleic acid
TSA	Trypto-Casein Soy Agar
$t_{1/2}$	biological half-life
τ	dosing interval

III. SUMMARY

Norfloxacin is a third generation fluoroquinolone that was first introduced for treating urinary tract infections in humans. Later it became popular in the veterinary medicine as well and so it was approved for use in poultry in 1990. The most attractive characteristics of norfloxacin are good absorption when given orally, and maintenance of effective serum and tissue levels against a broad range of pathogens causing systemic infections. A pulse-dose regimen of fluoroquinolones has been suggested and widely used with the aim of achieving high peak concentrations and exploiting the post-antimicrobial effect. The lack of information on the continuous dosing treatment, together with the high importance of the poultry pathogen Escherichia coli and Pasteurella multocida infections, led us to compare the pharmacokinetic properties and efficacy of pulse dosing oral norfloxacin treatment with that of an established medication of continuous dosing, in broiler chickens and turkeys.

I. Study No.1. (**Article No. II**) Comparative efficacy study of pulse- and continuous dosing treatment of norfloxacin in an experimentally induced *Pasteurella multocida* infection in chickens and turkeys.

Experimental fowl cholera was induced in 60, healthy 10-week-old broiler chickens and 8 week old turkeys by intramuscular inoculation with approximately 80 CFU of *Pasteurella multocida* (X-73 strain) and with approximately 70 CFU of *Pasteurella multocida* (P-1059 strain), respectively. This method of infection proved to be useful for evaluating the efficacy of antimicrobial medication, by measuring mortality, daily weight gain, pathological responses, daily clinical score, and frequency of reisolation of *Pasteurella multocida*. Using this model, the treatment efficacy of pulse- and continuous dosing was compared.

Chickens and turkeys were allotted into three experimental groups: infected and untreated controls (n=20, Group 1); infected and continuous dosing treated with norfloxacin 100 mg/L (n=20, Group 2); infected and pulse dosing treated with norfloxacin 15 mg/kg (n=20, Group 3). Each bird in all groups of chickens and turkeys were injected with 1 ml diluted broth culture containing approximately 80 CFU/ml X-73 (A:1) and 70 CFU/ml P-1059 (A:3), respectively.

Norfloxacin was given to Group 2 and 3 in drinking water for 5 days, starting on the day of infection and one hour later of inoculation. The animals were clinically examined and the body weights were measured each day before the treatment.

The efficacy was evaluated on the basis of daily weight gain, daily clinical score, post-mortem scores and mortality rates of the animals, and recovery of bacteria from the liver, lungs, heart, spleen, bone marrow, joint, brain and the pectoral muscle of inoculation.

Norfloxacin administered as continuous- and pulse dosing to drinking water for 5 days was proved to be significantly superior as compared to the control group in both chickens and turkeys. Acute pasteurellosis developed in the infected, non-medicated group within 24 hours after infection, as evidenced by the mortality rate (100%, all birds died within 6 days), clinical signs (1.71±1.34), post-mortem lesions (20.95 ±1.81) and the recovery of the challenge strain (110 out of 120 samples) from all inoculated controls.

In chickens there were no convincing differences in daily weight gain between the two medicated groups (0.10±0.07 for continuous- and 0.09±0.04 for pulse-dose). There was slight variation in mortality (0% and 15% for continuous- and pulse-dose, respectively) and well characterized alteration in postmortem scores (0.20±0.62 and 2.40±4.71 for continuous- and pulse-dose, respectively), daily clinical scores (0.12±0.06 and 0.37±0.20 for continuous- and pulse-dose, respectively) and recovery of bacteria (0 and 20 out of 120 samples). It should be considered that the inoculum was approximately 80 CFU X-73 (A:1), close to its LD_{50} (19.6 CFU) value. In turkeys, where 15 fold (approximately 70 CFU P-1059 (A:3)) of the LD_{50} (4.67 CFU) was used, significant alteration was observed between the two different treatment schedule in all examined parameters, except the average daily weight gain.

Based on the results obtained from this study it can be concluded that continuous dosing in chickens and pulse dosing in turkeys were significantly more effective in treating *Pasteurella multocida* infection. These results were exactly the opposite as was expected by the pharmacokinetic properties described in the literature.

II. Study No.2. (**Article No. III**) Comparative efficacy study of pulse- and continuous dosing treatment of norfloxacin in an experimentally induced *Escherichia coli* infection in broiler chicks and turkey poults.

Experimental colibacillosis was produced in 40, healthy, 7-day-old broiler chickens and turkeys by intratracheal injection of $1X10^8$ cfu/chick and $1.23X10^9$ cfu/poult bacteria of *Escherichia coli* 260 (O1:F11), respectively. Two days before *E. coli* challenge all chicks

were vaccinated with a live attenuated strain of bronchitis virus (H-52). This model of infection proved to be useful for evaluating the efficacy of antimicrobial medication, by recording mortality, weight gain, pathological alterations and frequency of reisolation of *E. coli*.

The chickens were allotted to 3 groups: infected and untreated controls (n=10, Group 1); infected and continuous dosing treated with norfloxacin (n=15, Group 2); infected and pulse dosing treated with norfloxacin (n=15, Group 3). Two days prior the start of the examination chickens were vaccinated through drinking water with Bronchovac II, infectious bronchitis virus H-52 attenuated strain (Ceva-Phylaxia, Budapest). Each bird in all groups was injected with 0.2 ml broth containing $1X10^8$ cfu for chickens and $1,23X10^9$ cfu for turkeys, respectively.

Norfloxacin treatments to Group 2 and 3 were initiated immediately after infection in drinking water and it was given for 5 days. Group 2 received continuous medication at 100 mg/L dose and Group 3 was given pulse dosing medication at 15 mg/kg. The body weight of animals was measured each day and the dosage was adjusted accordingly.

The birds were weight and clinically examined daily. The efficacy was evaluated on the basis of daily weight gain, daily clinical score, post mortem scores and mortality rates of the animals, and recovery of bacteria from the liver, spleen, lungs, air sac, heart and bone marrow.

Due to experimental infection, acute colibacillosis occurred in the infected, non-medicated controls with 40% mortality and 60% morbidity (clinical score of 1.77±1.27). Both treatment procedures effectively reduced the experimental colibacillosis in chickens as evidenced by the markedly decreased mortality (0% in both treated groups), the less pronounced clinical scores (0.25±0.11 continuous- and 0.18±0.08 pulse dosing) and pathological lesions (9.00±4.57 for the control and 1.67±2.32 and 0.87±1.55 for continuous- and pulse dosing, respectively), the better weight gains (12.40±6.32, 23.48±9.50 and 22.52±6.71 for control, continuous- and pulse dosing, respectively) and the low bacterial infestation (40 out of 60 samples in the control group, while 0 out of 90 samples for both treated groups), while there were no convincing differences between the two treatment regimens in turkeys. There was no significant difference between continuous- and pulse dosing concerning their effects on daily weight gain (22.54±9.07 and 20.46±5.75), daily clinical scores (0.14±0.15 and 0.16±0.07), and mortality (0 for both

groups), but well characterized alteration in postmortem scores (1.13±1.64 and 0.47±1.06 for continuous- and pulse dosing, respectively) for the benefit of pulse dosing.

In summary, the results of the experiment clearly indicate that norfloxacin administered in the water at 15 mg/kg pulse dosing was more efficacious then norfloxacin administered in the water at 100 mg/L, continuous dosing in chickens. Results confirmed earlier observations on pharmacokinetic properties of norfloxacin in chicks and turkeys.

III. Study No.3. (**Article No. IV**) The plasma profile of norfloxacin in broiler chickens and in turkeys.

Failure to respond to antibiotics is a multifactorial problem involving complex interactions among host defence, the bacterium causing the infection and its suspectibility, and the concentration profile of antibiotic chosen for treatment. Unfortunately, little can be done to alter the clinical factors, such as long-standing disease, compromise of the immune system, or age. We have, therefore, focused a substantive effort on the factor affecting outcome which can be optimized, the dosage regimen for the antimicrobial agent employed. We contend that the clinical and bacteriological outcomes can be significantly improved by optimizing the regimen according to the MIC for the pathogens and the poultry's norfloxacin pharmacokinetics. In birds, both the magnitude of exposure (peak concentration) and the duration of exposure (time above the MIC) are important for an optimal antibacterial effect.

The plasma profile of norfloxacin was studied in 12 healthy, broiler chickens and 12 turkeys, 7 weeks of age. Norfloxacin was administered to 2 groups of chickens and turkeys (6 birds/group) at a dose of 15 mg/kg, as pulse dosing and 100 mg/L as continuous dosing for 5 consecutive days in drinking water. Blood samples were taken from each animal before treatment and then 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hours after the start of the medication during continuous dosing. During pulse dosing, on the first day blood samples were drawn 6 times at 2, 4, 6, 8, 12 and 22 hours after the drug administration. Then, blood samples were taken 3 times a day, 2 hours prior the start of pulse dosing treatment (22 hour) and 2 (6 hour) and 8 (12 hour) hours after the finishing of pulse dosing. Plasma concentrations of norfloxacin were measured using high-performance liquid chromatography.

Medicated water was readily consumed providing average daily norfloxacin intakes of 15.16 and 15.36 mg/kg in chickens and turkeys, respectively during continuous medication. Pulse dosing gave average daily norfloxacin intake of 15 mg/kg for both

species. Steady-state plasma concentrations of norfloxacin during continuous treatment were reached by 36 h after the start of treatment in both avian species with means of 776.7±33.23 ng/ml in chickens and 682.5±28.55 ng/ml in turkeys. Maximum steady-state plasma concentrations varied with means of 835.0±28.81 ng/ml in chickens and 750.0±37.42 ng/ml in turkey, whereas minimum steady-state plasma concentrations were 715.0±54.68 ng/ml in chickens and 616.7±32.04 ng/ml in turkeys. During pulse dosing the plasma norfloxacin concentrations at 2 h after the first administration were 949.8±52.81 ng/ml in chickens and 810.8±40.99 ng/ml in turkeys. By 22 h, the plasma concentrations were 127.9±15.4 and 106.5±12.9 ng/ml in chickens and turkeys, respectively. The average steady-state plasma concentrations of norfloxacin were 365.32±39.31 ng/ml in chickens and 306.03±32.26 ng/ml in turkeys. The lower steady-state concentrations in turkeys compared to chickens are in good agreement with the previous finding of Laczay et al., who concluded that a reduced bioavailability due to lower extent of absorption and/or pronounced first pass metabolism or quicker elimination might contribute to the different plasma concentration-time profile in turkeys.

Results of pulse dosing were compared to those that were published in a previous publication of the authors, in which single oral norfloxacin administration to chickens, turkeys and geese were investigated. The pharmacokinetic data obtained from the present study compares considerably with the article statistics (in chickens T_{calc}: 1.04, T_{cri}: 2.31; in turkeys T_{calc}: 0.18, T_{cri}: 2.31). The plasma norfloxacin concentrations increased rapidly and reached the MIC_{90}, 250 ng/ml in the first 20 minutes in both chickens and turkeys and remained above for 8 hours in chickens and 6 hours in turkeys. Therefore even with an 8 hour PAE the antimicrobial concentration is not sufficient to keep the microorganisms under continuous antibacterial pressure. Data of the daily pulse dosing demonstrated that every administration of the drug corresponds to a single, daily, repeated bolus administration. However pulse dosing achieved higher plasma concentrations more readily then continuous dosing.

Considering that the AUIC (AUC/MIC) can be used as an indicator of the pharmacodynamic potency of fluoroquinolones against pathogens, high AUIC exerts an exposure of longer duration to less sensitive strains. However, existing ideas are that C_{max} is more closely related to reducing resistance and a C_{max}/MIC ratio of over 2-3 could be sufficient. In our previously described, published, artificial disease models of *Escherichia coli* and *Pasteurella multocida* infection in chickens and turkeys the comparative efficacy

of continuous- and pulse dose treatment was evaluated. In both reports the C_{max} exceeded the MIC over 5-10 times and the AUIC was over 100 for the strains used, however substantial differences were obtained between dosing schedules. The published reviews and our findings support the view that low pathogenicity bacterial infection gives better recovery with continuous dosing, while severe bacterial infection shows improving effect following pulse dosing schedule.

In conclusion, administration strategies such as continuous dosing at 100 mg/L or pulse dosing medication at 15 mg/kg, may provide rational guidance to the veterinarian in the choice of appropriate chemotherapy, however, we recommend to treat bacterial infections of either high or low pathogenicity starting with pulse dosing for 4 hours and then maintaining continuous oral medication for 3-5 consecutive days. Nevertheless manufacturers recommend using fluoroquinolones in a pulse-dosing manner throughout the whole treatment period. Our recommended method of administration should help in preventing both the severe and less serious bacterial infections and could prevent the emergence of resistance against the fluoroquinolones.

IV. RESUME

Norfloxacin est un fluoroquinolone de la troisième génération qui a été présenté la première fois pour des infections de traitement d'appareil urinaire chez l'homme. Plus tard il est devenu populaire en médecine vétérinaire ainsi il était approuvé pour l'usage dans la volaille en 1990. Les caractéristiques les plus attrayantes du norfloxacin sont les suivantes: bonne absorption données oralement après une seule fois, l'entretien du sérum et du tissu efficaces niveaux contre une large gamme des microbes pathogènes causé par des infections systémiques. Un régime d'impulsion-dose des fluoroquinolones a été suggéré et largement répandu dans le but de réaliser des concentrations maximalements élevées et d'exploiter l'effet poteau-antimicrobien. Le manque d'information sur le traitement de dosage continu, ainsi que l'importance élevée les infections des volailles de microbe pathogène Escherichia coli et de Pasteurella multocida, nous a menés à comparer les propriétés et l'efficacité pharmacocinétiques du traitement oral du dosage de norfloxacin d'impulsion à celle d'un médicament établi du dosage continu, en poulets et dindes.

I. Étude No.1. (Article No. II) Étude comparative d'efficacité du traitement continu d'impulsion et de dosage du norfloxacin dans une infection expérimentalement induite de *Pasteurella multocida* chez les poulets et les dindes.

Le choléra expérimental de volaille a été induit en 60, poulets de 10 semaines sains et dindes de 8 semaines par inoculation intramusculaire avec approximativement 80 CFU de *Pasteurella multocida* (contrainte X-73) et avec approximativement 70 CFU de *Pasteurella multocida* (contrainte P-1059). Cette méthode d'infection s'est avérée utile pour évaluer l'efficacité du médicament antimicrobien, en mesurant la mortalité, du gain quotidien de poids, des réponses pathologiques, des points cliniques quotidiens, et de la fréquence de la reisolation du *Pasteurella multocida*. En utilisant ce modèle, l'efficacité de traitement du dosage d'impulsion et continu a été comparé.

Les poulets et les dindes ont été répartis dans trois groupes expérimentaux: commandes infectés et non traitées (n=20, groupe 1); dosage infecté et continu traité avec le norfloxacin 100 mg/l (n=20, groupe 2); dosage infectée et d'impulsion traité avec le norfloxacin 15 mg/kg (n=20, groupe 3). Chaque oiseau dans tous les groupes de poulets et

de dindes ont été injectés avec 1 ml de culture diluée de bouillon contenant approximativement 80 CFU/ml X-73 (A:1) et 70 CFU/ml P-1059 (A:3).

Norfloxacin a été donné au groupe 2 et 3 en eau potable pendant 5 jours, commençant le jour l'infection et une heure plus tard d'inoculation. Les animaux ont été médicalement examinés et les poids corporels ont été mesurés chaque jour avant le traitement.

L'efficacité a été évaluée sur la base du gain quotidien de poids, des points cliniques quotidiens, des points post mortem et des taux de mortalité des animaux, et du rétablissement des bactéries du foie, des poumons, du coeur, de la rate, de la moelle, du joint, du cerveau et du muscle pectoral de l'inoculation.

On s'est avéré que Norfloxacin administré en tant que continu et impulsion dosant à l'eau potable pendant 5 jours est sensiblement supérieur par rapport au groupe de commande chez des poulets et des dindes. Le pasteurellosis aigu s'est développé dans le groupe infecté et non-médicamenté dans un délai de 24 heures après infection, comme démontré par le taux de mortalité (100%, tous les oiseaux sont morts dans les 6ème jours), les signes cliniques (1.71±1.34), les lésions post mortem (20.95 ±1.81) et le rétablissement de la contrainte de défi (110 sur 120 échantillons) de toutes les commandes inoculées.

Chez les poulets il n'y avait aucune différence persuasive dans le gain quotidien de poids entre les deux groupes médicamentés (0.10±0.07 pour continu et 0.09±0.04 pour l'impulsion-dose). Il y avait légère variation de la mortalité (0% et 15% pour continu et l'impulsion-dose) et du changement bien caractérisé des points post mortem (0.20±0.62 et 2.40±4.71 pour continu et l'impulsion-dose), des points cliniques quotidiens (0.12±0.06 et 0.37±0.20 pour continu et l'impulsion-dose) et du rétablissement des bactéries (0 et 20 sur 120 échantillons). Il devrait considérer que l'inoculum était approximativement 80 CFU X-73 (A:1), près de sa valeur de LD50 (19,6 CFU). Chez les dindes, où 15 fois (approximativement 70 CFU P-1059 (A:3)) du LD50 (4,67 CFU) a été employé, on a observé le changement significatif entre les deux programmes différents de traitement dans tous les paramètres examinés, à moins qu'on puisse conclure le gain quotidien de poids moyen, basé sur les résultats obtenus à partir de cette étude.

Le dosage continu chez les poulets et l'impulsion dosant chez les dindes étaient sensiblement plus d'efficace dans l'infection de traitement de *Pasteurella multocida*. Ces résultats étaient exactement l'opposé comme il a été prévu par les propriétés pharmacocinétiques décrites dans la littérature.

II. Étude No.2. (Article No. III) Étude comparative d'efficacité l'impulsion et de traitement de dosage continu de norfloxacin dans une infection expérimentalement induite d'*Escherichia coli* en poussins, poulets et dinde.

Le colibacillosis expérimental a été produit dans, 7 jours des poulets 40, sain et des dindes par l'injection intratrachéale des bactéries de $1X10^8$ cfu/poussin et de $1.23X10^9$ cfu/dinde d'*Escherichia coli* 260 (O1:F11). Pendant deux jours avant que le défi de *E. coli* tous les poussins ont été vaccinés avec une contrainte atténuée de phase du virus de bronchite (H-52). Ce modèle de l'infection s'est avéré utile pour évaluer l'efficacité du médicament antimicrobien, en enregistrant la mortalité, le gain de poids, les changements pathologiques et la fréquence du reisolation de *E. coli*.

Les poulets ont été répartis à 3 groupes: commandes infectées et non traitées (n=10, groupe 1); dosage infecté et continu traité avec le norfloxacin (n=15, groupe 2); dosage infectée et d'impulsion traité avec le norfloxacin (n=15, groupe 3). Pendant deux jours antérieurement le début des poulets d'examen ont été vaccinés par l'eau potable avec Bronchovac II, contrainte atténuée par H-52 infectieuse de virus de bronchite (Ceva-Phylaxia, Budapest). Chaque oiseau dans tous les groupes a été injecté avec 0.2 ml de bouillon contenant luxuriance le $1X10^8$ cfu pour poulets et $1.23X10^9$ cfu pour des dindes.

Des traitements de Norfloxacin au groupe 2 et 3 ont été lancés juste après l'infection en eau potable et il a été donné pendant 5 jours. Le groupe 2 a reçu le médicament continu à la dose de 100 mg/l et le groupe 3 a été indiqué l'impulsion dosant le médicament à 15 mg/kg. Le poids corporel des animaux a été mesuré chaque jour et le dosage a été ajusté en conséquence.

Les oiseaux étaient poids et tons les jouos médicalement examiné. L'efficacité a été évaluée sur la base du gain quotidien de poids, des points cliniques quotidiens, des points post mortem et des taux de mortalité des animaux, et du rétablissement des bactéries du foie, de la rate, des poumons, d'air de sac, du coeur et du moelle.

En raison de l'infection expérimentale, le colibacillosis aigu s'est produit dans les commandes avec la mortalité de 40% et la morbidité infectées et non-médicamentées de 60% (une vingtaine clinique de 1.77±1.27). Les deux procédures de traitement ont efficacement réduit le colibacillosis expérimental chez les poulets comme démontré par la mortalité nettement diminuée (0% dans les deux groupes traités), moins les points cliniques prononcés (0.25±0.11 continu et impulsion 0.18±0.08 dosant) et les lésions pathologiques (9.00±4.57 pour la commande et le 1.67±2.32 et le 0.87±1.55 pour continu et l'impulsion dosant), les gains meilleurs de poids (12.40±6.32, 23.48±9.50 et 22.52±6.71

pour la commande, continu et l'impulsion dosant) et la basse infestation bactérienne (40 sur 60 échantillons dans le groupe de commande, tandis que 0 sur 90 échantillons pour les deux groupes traités), alors qu'il n'y avait aucune différence persuasive entre les deux régimes de traitement chez les dindes. Il n'y avait aucune différence significative entre continu et l'impulsion dosant au sujet de leurs effets sur le gain quotidien de poids (22.54±9.07 et 20.46±5.75), les points cliniques quotidiens (0.14±0.15 et 0.16±0.07), et la mortalité (0 pour les deux groupes), mais le changement bien caractérisé des points post mortem (1.13±1.64 et 0.47±1.06 pour continu et l'impulsion dosant) au profit du dosage d'impulsion.

En résumé, les résultats de l'expérience indiquent clairement que le norfloxacin administré dans l'eau au dosage d'impulsion de 15 mg/kg était puis un norfloxacin plus efficace administré dans l'eau à 100 mg/l, dosage continu chez les poulets. Les résultats ont confirmé des observations plus tôt sur les propriétés pharmacocinétiques du norfloxacin dans l'infection d'ultocida de poussins et de dindes. Ces résultats étaient exactement l'opposé comme il a été prévu par les propriétés pharmacocinétiques décrites dans la littérature.

III. Étude No.3. (Article No. IV) Le profil de plasma du norfloxacin en poulets et chez les dindes.

Le manque de répondre aux antibiotiques est un problème multifactoriel impliquant des interactions complexes parmi la défense de centre serveur, la bactérie causant l'infection et son suspectibility, et le profil de concentration de l'antibiotique choisi pour le traitement. Malheureusement, on ne peut pas changer les facteurs cliniques, tels que la maladie de longue date, le compromis du système immunitaire, ou l'âge. Nous avons donc concentré un effort substantif sur le facteur affectant les résultats qui peuvent être optimisés, le schéma posologique pour l'agent antimicrobien utilisé. Nous affirmons que les résultats cliniques et bactériologiques peuvent être sensiblement améliorés en optimisant le régime selon le MIC pour les microbes pathogènes et la pharmacocinétique de norfloxacin de vollaille. Dans les oiseaux, l'importance d'exposition (concentration maximale) et la durée de l'exposition (temps au-dessus du MIC) sont importantes pour un effet antibactérien optimal.

Le profil de plasma du norfloxacin a été étudié chez 12 poulets sains, et 12 dindes, de 7 semaines. Norfloxacin a été administré à 2 groupes de poulets et de dindes (6 oiseaux/groupe) à une dose de 15 mg/kg, comme impulsion dosant et à 100 mg/l comme le dosage continu pendant 5 jours consécutifs en eau potable. Des échantillons de sang ont été

pris de chaque animal avant traitement et puis 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 et 144 heures après le début du médicament dépende le dosage continu. Pendant l'impulsion dosant, sur les premiers échantillons de sang de jour ont été dessinés 6 fois à 2, 4, 6, 8, 12 et 22 heures après administration de drogue. Puis, des échantillons de sang ont été pris 3 fois un jour, 2 heures d'antérieurs le début du traitement de dosage d'impulsion (22 heures) et 2 (6 heures) et 8 heures (de 12 heures) après le finissage du dosage d'impulsion. Des concentrations en plasma du norfloxacin ont été mesurées en utilisant la chromatographie liquide à rendement élevé.

L'eau médicamentée a été aisément consommée fournissant les prises quotidiennes de norfloxacin de moyenne de 15,16 et 15,36 mg/kg chez les poulets et les dindes, pendant le médicament continu. Le dosage d'impulsion a donné la prise quotidienne moyenne de norfloxacin de 15 mg/kg pour les deux espèces. Des concentrations équilibrées en plasma du norfloxacin pendant le traitement continu ont été atteintes par 36 h après le début du traitement dans l'espèce avienne avec des moyens de 776.7±33.23 ng/ml chez les poulets et le 682.5±28.55 ng/ml chez les dindes. Les concentrations équilibrées maximum de plasma ont changé avec des moyens de 835.0±28.81 ng/ml chez les poulets et le 750.0±37.42 ng/ml chez la dinde, tandis que les concentrations équilibrées minimum de plasma étaient 715.0±54.68 ng/ml chez les poulets et le 616.7±32.04 ng/ml chez les dindes. Pendant l'impulsion dosant les concentrations de norfloxacin de plasma à 2 h après la première administration étaient 949.8±52.81 ng/ml chez les poulets et le 810.8±40.99 ng/ml chez les dindes. Par 22 h, les concentrations de plasma étaient 127.9±15.4 et 106.5±12.9 ng/ml chez les poulets et les dindes. Les concentrations équilibrées moyennes en plasma du norfloxacin étaient 365.32±39.31 ng/ml chez les poulets et le 306.03±32.26 ng/ml chez les dindes. Les concentrations équilibrées inférieures chez les dindes comparées aux poulets sont en bon accord avec la conclusion précédente de Laczay et les autres. On a conclu qu'une disponibilité biologique réduite due à l'ampleur inférieure de l'absorption et/ou de la première élimination prononcée de métabolisme passe plus rapide pourrait contribuer au profil différent de concentration-temps de plasma chez les dindes.

Des résultats du dosage d'impulsion ont été comparés à ceux qui ont été édités dans une publication précédente, dans lesquels l'administration orale simple de norfloxacin aux poulets, aux dindes et aux oies ont été étudiées. Les données pharmacocinétiques obtenues à partir de la présente étude rivalisent considérablement avec les statistiques d'article (chez poulets T_{calc}: 1,04, T_{cri}: 2,31; chez les dindes T_{calc}: 0,18, T_{cri}: 2.31). Les concentrations de norfloxacin de plasma ont augmenté rapidement et ont atteint le MIC_{90}, 250 ng/ml dans les

20 premières minutes chez les deux poulets et les dindes et sont demeurées en haut pour 8 heures chez les poulets et après 6 heures chez les dindes. Par conséquent même avec des 8 heures PAE la concentration antimicrobienne n'est pas suffisante pour garder les microorganismes sous la pression antibactérienne continue. Données du dosage quotidien d'impulsion démontré que chaque administration de la drogue correspond à un simple, quotidiennement, l'administration répétée de bol. Cependant dosage continu plus élevé réalisé de dosage de concentrations de plasma d'impulsion plus aisément.

Considérant que l'AUIC (AUC/MIC) peut être employé comme indicateur du pouvoir pharmacodynamique des fluoroquinolones contre des microbes pathogènes et haut AUIC exerce une exposition d'une plus longue durée aux contraintes moins sensibles. Cependant, les idées existantes sont que Cmax plus étroitement est lié à réduire la résistance et un rapport de Cmax/MIC de plus de 2 ou 3 pourrait être suffisant. Dans nos modèles précédemment décrits, édités, artificiels de la maladie l'infection d'*Escherichia coli* et de *Pasteurella multocida* chez les poulets et les dindes l'efficacité comparative du traitement continu- et d'impulsion de dose a été évaluée. Dans les deux rapports le Cmax a excédé le MIC avec 5-10 fois et l'AUIC était plus de 100 pour les contraintes utilisées, toutefois des différences substantielles ont été obtenues entre les programmes de dosage. Les revues éditées et nos résultats soutiennent la vue que l'infection bactérienne de basse pathogénicité donne un meilleur rétablissement avec le dosage continu, alors que l'infection bactérienne montre gravement améliorer l'effet après le programme de dosage d'impulsion.

En conclusion, les stratégies d'administration telles que pour le dosage continu à 100 mg/l ou l'impulsion dosant le médicament à 15 mg/kg, peuvent fournir des conseils raisonnables au vétérinaire dans le choix de la chimiothérapie appropriée, cependant, nous recommandons de traiter des infections bactériennes de pathogénicité élevée ou basse commençant par l'impulsion dosant 4 heures et médicament oral continu alors de maintien pendant 3-5 jours consécutifs. Néanmoins les fabricants recommandent d'employer des fluoroquinolones d'une façon dedosage tout au long de toute la période de traitement. Notre méthode recommandée d'administration devrait aider en empêchant les infections bactériennes graves et moins sérieuses et pourrait empêcher l'apparition de la résistance contre les fluoroquinolones.

V. ÖSSZEFOGLALÁS

A norfloxacin harmadik generációs fluoroquinolon amelyet először a humángyógyászatban húgyúti dezinficiensként alkalmaztak. Később az állatorvosi gyakorlatban is elterjedt és 1990 óta baromfiban is engedélyezett készítmény. A norfloxacin legfigyelemreméltóbb tulajdonsága a jó felszívódás orálisan adagolva és a hatékony vér- és szövetszint fenntartása a kórokozók legszélesebb köre ellen még súlyos szisztémás fertőzések esetén is. Az állatorvosi gyakorlatban a pulzáló adagolás terjedt el azzal a céllal, hogy magas csúcskoncentrációt elérve használhassuk ki a posztantibiotikus hatást. A folyamatos kezelésről szóló beszámolók hiánya, valamint a baromfipatogén Escherichia coli és Pasteurella multocida fertőzések fontossága vezetett minket arra, hogy összehasonlítsuk az orális pulzáló norfloxacin adagolást a folyamatos gyógyszeritatással broiler csirkékben és pulykákban.

I. számú vizsgálat (**II. számú cikk**): A norfloxacin pulzáló- és folyamatos itatás hatékonyságának összehasonlító vizsgálata mesterségesen kialakított *Pasteurella multocida* fertőzés esetében csirkén és pulykán.

Mesterséges baromfikolera fertőzést hoztunk létre 80 CFU X-73 és 70 CFU P-1059 *Pasteurella multocida* pectoralis izomba való befecskendezésével 60 egészséges, 10 hetes broiler csirkében illetve 8 hetes pulykákban. Ez a mesterséges fertőzéses módszer hatékonynak bizonyult az antimikrobiális gyógykezelés hatékonyságának összehasonlítására a mortalitás, napi súlygyarapodás, klinikai pontszám, post mortem pontszám, valamint az eredeti *Pasteurella multocida* törzsek visszaizolálásának figyelembe vételével. Ezen modell felhasználásával összehasonlítottuk a pulzáló és folyamatos kezelés hatékonyságát.

Az akklimatizációs periódust követően a csirkéket és pulykákat randomizált módon 3, egyenlő számú állatot tartalmazó csoportba osztottuk: fertőzött, nem kezelt (n=20, 1. csoport, Kontroll csoport); fertőzött és folyamatos itatással, norfloxacinnal 100 mg/L adagban kezelt (n=20, 2. csoport); fertőzött és pulzáló itatással 15 mg/ttkg dózisban kezelt (n=20, 3. csoport) csoportok formájában. Minden csirke- és pulyka csoport összes madarát 1 ml higított leves kultúrával fertőztük, amely körülbelül 80 CFU/ml X-73 (A:1) illetve 70 CFU/ml P-1059 (A:3) tartalmazott csirkék, illetve pulykák esetében.

Norfloxacint adagoltunk a 2. és 3. csoportnak ivóvízbe 5 napon át a fertőzés napjától kezdve. A kezelés megkezdése előtt általános klinikai vizsgálatot végeztünk és lemértük testtömegüket.

A hatékonyságot a napi súlygyarapodás, klinikai pontszám, post mortem pontszám, az elhullások száma, valamint a baktériumok visszaizolálásának száma (máj, tüdő, szív, lép, csontvelő, izület, agy és a beadás helyén a pectoralis izom) alapján vizsgáltuk.

Az 5 napig tartó folyamatos- és pulzáló norfloxacin itatás szignifikánsan jobbnak mutatkozott a kontroll csoporthoz képest mind csirkékben, mind pulykákban. Akut pasteurellosis alakult ki a fertőzött, nem kezelt állatokban az első 24 órán belül, amit az elhullások száma (100%, minden állat elpusztult a 6. napig), klinikai tünetek (1,71±1,34), post mortem lelet (20,95±1,81) és a fertőzésre használt törzsek visszaizolálása (110 a 120 mintából) is alátámaszt.

Csirkében nem volt meggyőző különbség a napi súlygyarapodás tekintetében (0,1±0,07 folyamatos- illetve 0,09±0,04 pulzáló kezelés) a két kezelt csoport között. Enyhe, de szignifikáns különbség volt megfigyelhető az elhullások számában (0% folyamatos- illetve 15% pulzáló kezelés) és kifejezett eltérés a post mortem vizsgálat (0,20±0,06 folyamatos- illetve 0,37±0,020 pulzáló kezelés), napi klinikai pontszám (0,12±0,06 folyamatos- illetve 0,37±0,20 pulzáló kezelés) és a baktériumok reizolálása (0 a 120 mintából folyamatos- illetve 20 a 120 mintából pulzáló kezelés) tekintetében. Megfontolandó, hogy a fertőző dózis kb. 80 CFU X-73 (A:1) volt, amely csak négyszerese az LD_{50} (19,6 CFU) értéknek. Pulykában, ahol kb. 70 CFU P-1059 (A:3), az LD_{50} (4,67 CFU) 15-szörösével fertőztünk, a két kezelt csoport között minden vizsgált paraméterben szignifikáns különbség volt megfigyelhető. Csak a napi súlygyarapodásban nem volt különbség a két csoport között.

Ezen eredmények tükrében kijelenthetjük, hogy a folyamatos itatás csirkében, míg a pulzáló itatás pulykákban szignifikánsan hatékonyabbnak bizonyult a *Pasteurella multocida* kezelésében. Az eredmények az irodalmi adatokban közölt kinetikai vizsgálatok alapján vártakkal ellentétben alakultak.

II. számú vizsgálat (**III. számú cikk**): A norfloxacin pulzáló- és folyamatos itatás hatékonyságának összehasonlító vizsgálata mesterségesen kialakított *Escherichia coli* fertőzés esetében csirkén és pulykán.

Mesterséges colibacillosist alakítottunk ki $1X10^8$ CFU/csibe és $1,23X10^9$ CFU/pipe *Escherichia coli* 260 (O1:F11) intratracheális befecskendezésével 40 egészséges, 7 napos

broiler csirkékben illetve pulykákban. Ez a fertőzéses módszer használhatónak bizonyult az antimikrobiális gyógykezelés hatékonyságának összehasonlítására a mortalitás, napi súlygyarapodás, klinikai pontszám, post mortem pontszám, valamint az eredeti *E. coli* törzsek visszaizolálásának mérésével.

Az akklimatizációs periódust követően a csirkéket és pulykákat randomizált módon 3 csoportba osztottuk: fertőzött, nem kezelt (n=10, 1. csoport, Kontroll csoport); fertőzött és folyamatos norfloxacin itatással kezelt 100 mg/L adagban (n=15, 2. csoport); fertőzött és pulzáló norfloxacin itatással kezelt 15 mg/ttkg dózisban (n=15, 3. csoport) csoport formájában. Az *E. coli* fertőzés előtt 2 nappal minden csibét Bronchovac II, IBV H-52 attenuált törzsét tartalmazó vakcinával itattunk le. Minden csoport minden madarát 0,2 ml higított leves kultúrával fertőztük, mely $1X10^8$ CFU dózist tartalmazott csirkék illetve $1,23X10^9$ CFU pulykák esetében.

A kezelés megkezdése előtt általános klinikai vizsgálatot végeztünk és lemértük az állatok testtömegét. A 2. és 3. csoport állatainak a fertőzés napjától kezdve norfloxacint adagoltunk ivóvízbe 5 napon át. A madarakat minden nap megmértük és klinikailag megvizsgáltuk.

A hatékonyságot a napi súlygyarapodás, klinikai pontszám, post mortem pontszám, az elhullások száma, valamint a baktériumok visszaizolálása (máj, tüdő, szív, lép, csontvelő és légzsák) alapján vizsgáltuk.

A mesterséges fertőzés hatására akut colibacillosis alakult ki a fertőzött, nem kezelt csoportban 40% elhullással és 60% morbiditással (1,77±1,27 klinikai pontszám). Mindkét kezelés hatékonyan csökkentette a kísérletes colibacillosist csirkékben, mint az jól látható a határozottan alacsonyabb elhullásból (0% mindkét kezelt csoportban), a kevésbé kifejezett klinikai- (0,25±0,11 folyamatos-, 0,18±0,08 pulzáló itatás esetén) és kórbonctani (9,00±4,57 a kontrollban, 1,67±2,32 folyamatos-, 0,87±1,55 pulzáló itatás esetén) pontszámokból, a jobb testtömeg gyarapodásból (12,40±6,32 a kontrollban, 23,48±9,50 folyamatos-, 22,52±6,71 pulzáló itatás esetén) és az alacsony visszaizolálások számából (40 a 60 mintából a kontrollban, 0 a 90 mintából mindkét kezelt csoportban). Ezzel szemben, nem volt meggyőző különbség a két kezelési mód között pulykákban. Nem volt szignifikáns különbség a folyamatos- és pulzáló itatás között a napi súlygyarapodás (22,54±9,07 és 20,46±5,75 folyamatos, illetve pulzáló), napi klinikai pontszám (0,14±0,15 és 0,16±0,07) és mortalitás (0% mindkét csoportban) szempontjából, de jól látható eltérés

volt a post mortem pontszámokban (1,13±1,64 folyamatos- és 0,47±1,06 pulzáló itatás) a pulzáló adagolás javára.

Összegezve, az eredmények azt mutatják, hogy a norfloxacin adagolás ivóvízben 15 mg/ttkg adagban, pulzáló itatás formájában hatékonyabb volt, mint a 100 mg/L folyamatos kezelés csirkében. Az eredmények alátámasztják az irodalmi adatokban közölt kinetikai vizsgálatok alapján várt eredményeket csirkében és pulykában.

III. számú vizsgálat (**IV. számú cikk**): A norfloxacin pulzáló- és folyamatos itatás farmakokinetikai összehasonlító vizsgálata broiler csirkékben és pulykákban.

Az antibiotikumokra adott válasz hiánya multifaktoriális probléma, mely magában foglalja a komplex interakciót a gazdaszervezet védekezése, a baktérium faja és érzékenysége valamint a választott antimikrobiális szer kinetikai és dinámiás tulajdonságai között. Sajnos, a klinikai faktorokra kevéssé vagyunk hatással, mint például a krónikus betegségek, az immunrendszer nem megfelelő működése, vagy a kor. Ezért arra a faktorra koncentráltunk, amely a végkifejletet alapjaiban befolyásolja és emellett optimalizálható is, az alkalmazott antimikrobiális szer adagolása, illetve adagolási formája. Meggyőződésünk szerint, mind a klinikai, mind a bakteriológiai végeredményt szignifikánsan javíthatjuk, ha az adagolást a patogén kórokozók MIC értékéhez és a norfloxacin baromfikban mért farmakokinetikájához igazítjuk. Madarakban, mind az antibakteriális szer csúcskoncentrációja, mind a készítmény időbeni hatása (az idő a MIC felett) fontos az optimális antibakteriális hatás eléréséhez.

A norfloxacin farmakokinetikáját tanulmányoztuk 12, 7 hetes, egészséges broiler csirkén és 12, 7 hetes pulykán. Az állatokat 2 csoportba osztottuk (6 madár/csoport), és ivóvízben 15 mg/ttkg adagban pulzáló itatás formájában, valamint 100 mg/L dózisban folyamatos itatásos elrendezésben norfloxacinnal kezeltük 5 napig. Vérmintákat vettünk minden csoportból a kezelés előtt, valamit a folyamatos itatás alkalmazását követő 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 és 144 óra múlva minden állat szárnyvénájából. A pulzáló itatás alkalmával, a kezelés megkezdését követően az első nap 6 alkalommal, 2, 4, 6, 8, 12 és 22 órakor, valamint minden egyéb nap 3 alkalommal, 2 órával a kezelés megkezdése előtt (22 óra), és 2 (6 óra) illetve 8 (12 óra) órával az adagolás befejezése után. A plazma norfloxacin koncentrációját HPLC rendszer segítségével határoztuk meg.

A folyamatos kezelés alkalmával a madarak készségesen fogyasztották el a medikált ivóvizet 15,16 mg/ttkg norfloxacin felvételével csirkében, illetve 15,36 mg/ttkg adagban pulykában. A pulzáló itatás alkalmával a napi gyógyszerfelvétel 15 mg/ttkg volt

mindkét fajnál. Folyamatos kezelés alkalmával a steady-state norfloxacin koncentrációt mindkét madárfaj a kezelés kezdete utáni 36. órára érte el, mely 776,7±33,23 ng/ml adódott csirkében és 682,5±28,55 ng/ml volt pulykában. A maximum steady-state koncentráció 835,0±28,81 ng/ml értéknek adódott csirkében, míg 750,0±37,42 ng/ml volt pulykában. A minimum steady-state koncentráció 715,0±54,68 ng/ml csirkében, valamint 616,7±32,04 ng/ml-nek mutatkozott pulykában. Pulzáló adagolás alkalmával a plazma norfloxacin koncentrációja 949,8±52,81 ng/ml csirkében és 810,8±40,99 ng/ml volt pulykában 2 órával az első beadást követően. A 22. órára a plazma koncentráció 127.9±15,4 ng/ml volt csirkében illetve 106,5±12,9 ng/ml pulykában. Az átlagos steady-state norfloxacin plazma koncentráció 365,32±39,31 ng/ml-nek adódott csirkében, míg 306,03±32,26 ng/ml volt pulykán. Az alacsonyabb steady-state koncentráció pulykánál a csirkéével összehasonlítva megfelel Laczay és mtsai. által leírtaknak, akik szerint a csökkent biológiai hasznosulás pulykában a rosszabb hatékonyságú felszívódásnak, és/vagy a kifejezettebb first-pass metabolizmusnak, vagy gyorsabb eliminációnak köszönhető.

A pulzáló adagolás eredményeit a szakirodalomban már megjelent cikk eredményeivel hasonlítottuk össze, amelyben az egyszeri, orális norfloxacin adagolás farmakokinetikáját vizsgálták csirkén, pulykán és libán. A jelen vizsgálatok adatai jól összevágnak a cikkben leírtakkal (csirkében a T_{calc}: 1,04, T_{cri}: 2,31; pulykában T_{calc}: 0,18, T_{cri}:2,31). A plazma norfloxacin koncentrációja gyorsan emelkedett, és a 20. percre elérte a 250 ng/ml, MIC_{90} értéket mind csirkében, mind pulykában, és további 8 órán át csirkében és 6 órán át pulykában a MIC_{90} érték felett maradt. Ennek következtében még a maximális, 8 órán át tartó PAE esetén is az antimikrobiális koncentráció nem elegendő a mikroorganizmusok folyamatos antibakteriális nyomás alatt tartásához. A pulzáló adagolás adatai azt bizonyítják, hogy a gyógyszer adagolása megfelel egy egyszeri, napi, folyamatos bólusz adagolásnak. Ugyanakkor a pulzáló adagolás sokkal gyorsabban, magasabb plazma koncentrációt ér el mint a folyamatos adagolás.

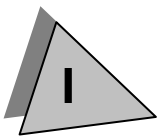
Figyelembe véve, hogy az AUIC-t (AUC/MIC) úgy használhatjuk, mint a fluoroquinolonok farmakodinámiás hatásának indikátorát a patogén kórokozók ellen, a magas AUIC hosszabb ideig gyakorol antibakteriális hatást a kevésbé érzékeny törzsekre. Csakhogy a jelenleg is széles körben elfogadott nézet szerint a C_{max} szorosabban összefügg a rezisztencia kialakulásának csökkentésével, és a 2-3 fölötti C_{max}/MIC arány elegendő. Az előzőekben tárgyalt, általunk publikált mesterséges fertőzéses *Escherichia coli* és

Pasteurella multocida modellekkel a pulzáló- és folyamatos adagolás hatékonyságának összehasonlító vizsgálatát végeztük el csirkében és pulykában. Mindkét vizsgálatban a C_{max} meghaladta 5-10-szeresen a MIC értékét és az AUIC értéke is 100 felett volt a használt törzsekre, azonban alapvető különbségeket véltünk felfedezni a két adagolási mód között. A publikált cikkek és saját vizsgálataink is azt a tényt támasztják alá, hogy az alacsonyabb patogenitású bakteriális fertőzések jobban gyógyulnak folyamatos adagolás hatására, míg a súlyos bakteriális infekciók jobban reagálnak a pulzáló kezelésre.

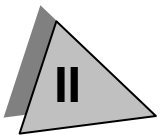
Összefoglalva, a norfloxacin adagolása mind 100 mg/L adagban folyamatos adagolás, mind 15 mg/ttkg dózisban pulzáló itatás során megfelelő hatékonysággal használható gyakorlati körülmények között is. Amennyiben a súlyos és az enyhe eseteket egyaránt közel azonos hatékonysággal szeretnénk kezelni, javaslatunk szerint a kezelést egy 4 órán át tartó pulzáló itatással célszerű elkezdeni, majd folyamatos itatással folytatni 3-5 napig. Jóllehet a gyártók a pulzáló adagolást javasolják a teljes kezelés ideje alatt, az általunk javasolt adagolási forma segít meggyógyítani mind a súlyos, mind az enyhe bakteriális fertőzéseket, valamint hozzájárul a fluoroquinolonok elleni rezisztencia kialakulásának késleltetéséhez.

VI. LIST OF ORIGINAL PUBLICATIONS

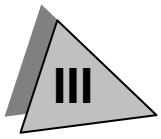
This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:



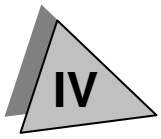
SÁRKÖZY G. (2002) QUINOLONES: A CLASS OF ANTIMICROBIAL AGENTS. *VETERINARY MEDICINE – CZECH*, **46** (9-10): 257-274.



SÁRKÖZY G., SEMJÉN G., LACZAY P. AND HORVÁTH E. (2002) TREATMENT OF EXPERIMENTALLY INDUCED *PASTEURELLA MULTOCIDA* INFECTIONS IN BROILERS AND TURKEYS - COMPARATIVE STUDIES ON DIFFERENT ORAL TREATMENT REGIMENS. *JOURNAL OF VETERINARY MEDICINE SERIES B*. **49**: 130-134.

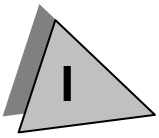


SÁRKÖZY G., SEMJÉN G., LACZAY P., HORVÁTH E. AND SCHMIDT J. (2002) PULSE AND CONTINUOUS ORAL NORFLOXACIN TREATMENT OF EXPERIMENTALLY INDUCED ESCHERICHIA COLI INFECTION IN BROILER CHICKS AND TURKEY POULTS. *ACTA VETERINARIA HUNGARICA*, **153**: 199-210.



SÁRKÖZY G., SEMJÉN G. AND LACZAY P. (2002) PHARMACOKINETICS OF NORFLOXACIN IN BROILERS AND TURKEYS AFTER DIFFERENT METHODS OF ORAL ADMINISTRATION. *JOURNAL OF VETERINARY PHARMACOLOGY AND TOXICOLOGY*, (PRESENTED FOR PUBLICATION).

VII. GENERAL INTRODUCTION



Quinolones: a class of Antimicrobial Agents [1]

ABSTRACT

The fluoroquinolones are a series of synthetic antibacterial agents that are used in the treatment of a variety of bacterial infections. These agents inhibit the DNA gyrase, abolishing its activity by interfering with the DNA-rejoining reaction. The inhibition of the resealing leads to the liberation of fragments that are subsequently destroyed by the bacterial exonucleases. All fluoroquinolones accumulate within bacteria very rapidly, so that within a few minutes a steady-state intrabacterial concentration is obtained. Resistance develops slowly and is usually chromosomal and not plasmid mediated. However, development of resistance and transfer between animal and human pathogens has become a fervently argued issue among microbiologists. A further concern regarding the use of new quinolones in the veterinary field is the possible detrimental effect on the environment. Still it seems unlikely that controlled use of veterinary quinolones will give rise to unfavorable effects on the environment.

Key words: fluoroquinolones, chemistry, pharmacokinetics, resistance, therapeutical use

INTRODUCTION

Older members of the quinolone class of synthetic antimicrobial agents, particularly nalidixic acid, have been available for the treatment of urinary tract infections in humans for many years. These drugs are of relatively minor significance because of their limited therapeutic utility and the rapid development of resistance (Goodman and Gilman, 1992).

Over the last two decades, research on 4-quinolone-3-carboxylates has led to the discovery of a family of 6-fluoro-7-piperazinyl-4-quinolones active against gram-negative and gram-positive bacteria in vitro (Hooper and Wolfson, 1985) as well as intracellular pathogens (Fitzgeorge et al., 1988) and trimethoprim/sulfonamide resistant microbes (Preheim et al., 1987); in addition

[1] This study was published in *Veterinární Medicína-Czech*, 2001 (9-10), **46**: 257-274.

these antimicrobials are also active against mycoplasmas (Braunius, 1987). Collectively, these compounds are called fluoroquinolones. Although dozens of fluoroquinolones have been synthesized and reported, the most notable ones being developed, or used, in veterinary medicine worldwide include (in alphabetical order) amifloxacin, benofloxacin, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, marbofloxacin, norfloxacin and norfloxacin nicotinate, ofloxacin, orbifloxacin and sarafloxacin. Other major fluoroquinolones in human medicine include enoxacin, ofloxacin, sparfloxacin, temafloxacin, and tosufloxacin. Enrofloxacin was the first fluoroquinolone introduced into veterinary medicine. All fluoroquinolones are bactericidal and all act on the same bacterial target: the bacterial DNA gyrase (type II topoisomerase). No plasmidic resistance against them has been demonstrated (Hooper and Wolfson, 1985). However, after in vitro experimental selection (Desgrandchamps, 1989), or clinical administration (Kresken and Wiedemann, 1988), resistant mutants have been isolated. These isolated mutants show cross reactivity for the different quinolones and fluoroquinolones but no cross reactivity with other antimicrobial families.

These fluoroquinolones share a great oral bioavailability in all monogastric species, a large volume of distribution and a low binding to plasma proteins that allows them to cross membranes and reach the most remote parts of the body at concentrations above the minimum inhibitory concentrations (MIC's) of most pathogens. Tissues and sites demonstrating high concentrations following systemic administration include the kidney, liver and bile plus the prostate, female genital tract, bone and inflammatory fluids (Montay et al., 1984). They are eliminated for the most part in the urine and reach levels 100 to 300 times more concentrated in the urine than in the serum (Montay et al., 1984). All the fluoroquinolones exhibit distributional and antimicrobial properties that make them potentionally useful in veterinary medicine.

CHEMISTRY

The 6-fluoroquinolones (also known as 4-quinolones or quinolones; **Figure 1**) are a series of synthetic antibacterial agents derived from, or related to, nalidixic acid and oxolinic acid.

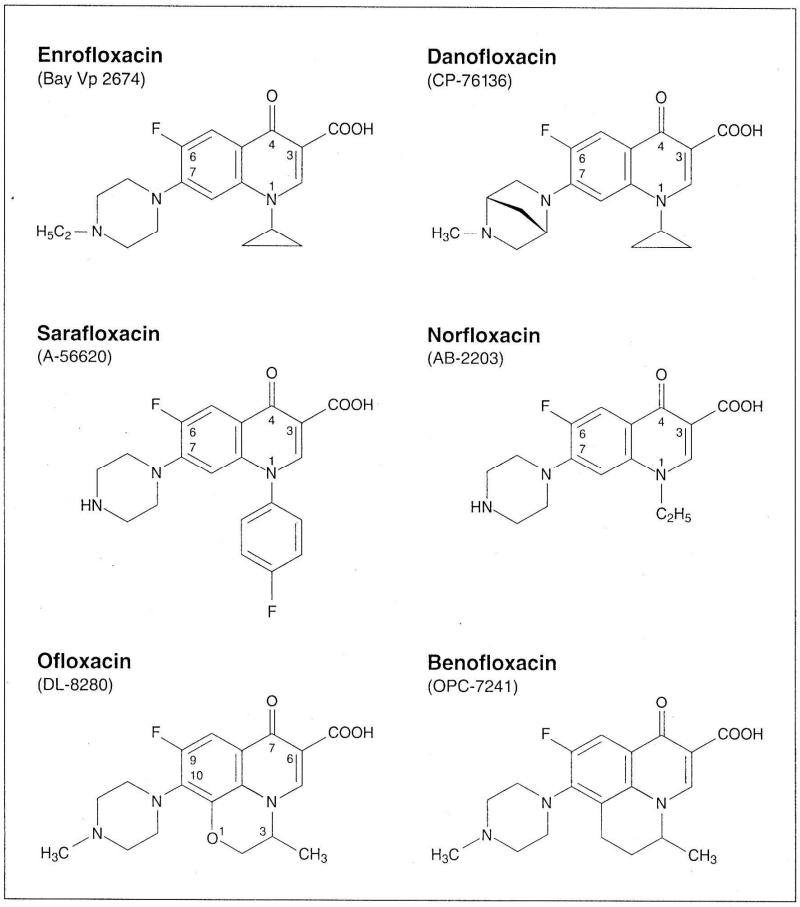


Figure 1. General chemical structure of some fluoroquinolones.

The 1 position is a nitrogen on the bicyclical aromatic ring structure, with an alkyl group (ethyl or perhaps cyclopropyl) often attached there. A carboxylic acid at position 3 is required for antimicrobial activity, as is a keto group at position 4. Many improvements on these early quinolone carboxylic acids have since been made based on systematic structure-activity studies. A fluorine atom at the 6 position on the quinolone carboxylic acid nucleus enhances the efficacy of these compounds against gram-negative pathogens and broadens the spectrum of activity against gram-positive pathogens: a basic nitrogen-containing moiety enhances tissue penetration and reduces central nervous system toxicity. Modifications at positions 2, 5 and 7 of the basic structure alter the pharmacokinetics of the compound. A carbon, nitrogen or oxygen atom occupies position 8 on the heterocyclic aromatic ring, depending on the quinolone. Nitrogen atoms at positions 1 and 8 produce naphthyridine carboxylic acids (e.g. enoxacin or nalidixic acid), whereas nitrogen at the 1 6 and 8 positions are called pyridopyrimidine carboxylic acids, which are not fluorinated at the 6 position (e.g. pipemidic acid). Because of the presence of a carboxylic acid and one or more basic amine functional groups, these antibacterial agents are amphoteric and

considered zwitterionic: however, between the pK_a of the acidic and the basic functional groups (between pH 6 and 8), these compounds are sufficiently lipid-soluble to be able to penetrate tissues. In octanol/water partition experiments conducted at pHs ranging from 2.9-7.6, ciprofloxacin, norfloxacin and enoxacin did not pass significantly into octanol: though nalidixic acid showed some increasing passage into the lipid layer from pH 7.6 to 6.4 (Ashby et al., 1985). However, these classic study methods are unable to determine the true partition coefficient unless the relative abundance of the four potential ion combinations (i.e. [0.0]. [+.0]. [0.-]. [+.-]) is known (Takács-Novák et al., 1990). It appears that the uncharged species (i.e. [0.0] is compared to a larger fraction of many quinolones in solution, this may account for the diffusion across membranes (Nikaido and Thanassi, 1993). Further complicating the issue is the possibility that initial interaction with membrane surfaces may be mediated by divalent cations (Nikaido and Thanassi, 1993), which may have effects that render classic octanol/water partitioning experiments not applicable. Water solubility at physiological pH varies across these compounds, depending on the substitutions on the quinolone carboxylic acid nucleus. Salt forms of the fluoroquinolones are freely soluble and are generally stable in an aqueous solution.

STRUCTURE-ACTIVITY RELATIONSHIP

As indicated above, the fluoroquinolones are based on the 4-quinolone ring (Figure 1). The structure of the ring has been largely modified to enhance the antimicrobial activity and to increase the volume of distribution of the molecule.

The substitution of a piperazinyl ring in position 7 has rendered the molecule active against Pseudomonas and the presence of an atom of fluorine at position 6 extends the activity of the molecule to some but not all gram-positive bacteria (Neer, 1988). Streptococcus can be resistant (Berg, 1988). Additions of alkyl chains on the para position of the piperazinyl ring, and on the nitrogen in position 1, increase the lipid solubility and the volume of distribution of the compounds. The substitution of hydrogen atoms by fluorines on the 8 position of the ring and on the methyl of the alkyl chain diminishes the rate of degradation and decreases the rate of elimination.

It was widely believed that the 3-carboxylic acid and the 4-carbonyl were necessary for the antimicrobial activity of the compounds. However, Chu et al. (1988) showed that the transformation of existing molecules in 2,3,4,9-tetrahydroisothiazolo [5,4-b] quinoline-3,4-diones produces a significant increase in their biological activity.

The quinolones bear both an acidic group (carboxylic acid) and a basic group (tertiary amine). This association gives them amphoteric properties. Their solubility is low, except between pH 6 and 8. Within this range, they have low water solubility and are prone to precipitate under more acidic conditions (Jenkins and Friedlander, 1988). It is apparently due to this property that crystalluria has been observed in man and animals (Ball, 1986).

ANTIMICROBIAL ACTIVITIES

Bacteria possess a type II topoisomerase known as DNA gyrase: a tetrametric bacterial enzyme that folds and coils the 1.0-0.3 m of circular bacterial DNA so it can fit into the bacteria several thousand times shorter. Furthermore, the supercoiling of the DNA that is catalyzed by DNA gyrase aligns the DNA into a 'relaxed' form that has decreased susceptibility to fragmentation and increased ease of separation during strand replication (Fernandes, 1988). This is accomplished by coiling the DNA around an RNA core in a series of loops; each loop or domain is then negatively supercoiled by nicking both strands of the DNA and passing that broken strand 'behind' the accompanying double strand and then resealing the double nick. Quinolones inhibit the A subunit of DNA gyrase (produced by the *gyr* A gene) abolishing its activity, possibly by interfering with the DNA-rejoining reaction (Neu, 1988). The inhibition of the resealing leads to the liberation of fragments that are subsequently destroyed by the bacterial exonucleases (Smith, 1986).

DNA gyrase has also been described as working in a yin-yang mechanism with topoisomerase I. where fluoroquinolones inhibit DNA replication by stimulating topoisomerase I resulting from the inhibition of DNA gyrase. Coumermycin and novobiocin act on the B subunit of DNA gyrase (Fernandes, 1988), and coumermycin has shown synergy with the fluoroquinolones (Fernandes, 1988). In fact, fluoroquinolones most likely bind in a co-operative manner to a pocket of single strand DNA created by DNA gyrase.

Interestingly, a *gyr* B mutation (*gyr* B is the gene that codes for the B subunit of DNA gyrase) that changes amino acid 447 into a negatively charged amino acid confers hyper-susceptibility to the of DNA gyrase. Coumermycin and novobiocin act on the B subunit of DNA gyrase (Fernandes, 1988), and coumermycin has shown synergy with the fluoroquinolones with a positively charged piperazine substituent, suggesting that an electrostatic interaction between the fluoroquinolones and the gyrase B subunit may result in increased stability of quinolone binding to the complex, thereby increasing susceptibility (Smith, 1984).

Sigmoidal fluoroquinolone binding kinetics suggest that four molecules (two pairs with opposing orientation and stacked above or below each other) can stereochemically fit into the pocket, acting co-operatively to inhibit DNA gyrase (Hooper and Wolfson, 1991) in a similar fashion to the co-operative binding of four oxygen molecules to hemoglobin. The result is rapid bactericidal activity at relatively low concentrations. Rate of bacterial cell may be accelerated if the 7 substituent becomes a weaker base or if the carboxyl group becomes a stronger acid (Nikaido and Thanassi, 1993). One striking peculiarity of these antimicrobials is their biphasic concentration-response curve. The fluoroquinolones are considerably less effective against bacterial pathogens at concentrations much higher than, as well as lower than, their minimum inhibitory concentrations (MICs). In a first phase, the percentage of bacteria killed increases with concentration; in a second phase, a further increase in concentration causes a temporary decrease in the percentage of killed bacteria (Diver and Wise, 1986). This effect is only seen during short-term exposures. The percentage of bacteria killed after more than 1.5 hour exposure remains the same at any concentration above the MIC. Interestingly, the inhibition of the protein synthesis caused by the concomitant administration of chloramphenicol (inhibitor of protein synthesis) and fluoroquinolones decreases the percentage of bacteria killed by the fluoroquinolones. This is probably due to the inhibition of the de novo synthesis of exonucleases. It is unlikely that the accidental overdosage of a treated animal would cause a decreased action; however, neither overdosage, nor concomitant administration of a protein synthesis inhibitor is advisable. The specific and fundamental action on bacterial replication allows the fluoroquinolones to be active at very low concentrations and to show a post-administration activity. The concentration necessary to inhibit the mammalian replication enzymes is two orders of magnitude higher than the concentration inhibiting the corresponding enzymes in the bacteria (Oomori et al., 1988). This results in a favorable margin of safety for the fluoroquinolones.

Mammals have an enzyme that makes couple-stranded cuts in DNA, similar to DNA gyrase, but it does not supercoil the DNA, and is not affected by fluoroquinolones (Fernandes, 1988). However, increased activity of some fluoroquinolones at the mammalian topoisomerase II enzyme has been associated with genotoxicity (Hooper and Wolfson, 1991). Recent evidence suggest that an asymmetric barrier exist between mammalian topoisomerase II and bacterial DNA gyrase, with those fluoroquinolones with cis-3,5-dimethylpiperazine configurations on the C7 carbon conferring much more

selectivity for bacterial DNA gyrase than the *trans*-3,5-dimethyl analogue (Gootz et al., 1994).

DNA gyrase is an intracellular enzyme, so uptake of the fluoroquinolones by the bacteria is critically important. Entry into cells is via porins (Chapman and Georgopapadakou, 1988), with subsequent entry across the cytoplasmic membrane occurring depending on the fluoroquinolones physicochemical properties. All fluoroquinolones accumulate within bacteria very rapidly, so that within a few minutes a steady-state intrabacterial concentration is obtained (Piddock, 1994). Accumulation is antagonized by cations such as magnesium and calcium, perhaps by binding at the cell surface resulting from chelation with divalent cations (Kotera et al., 1991). For gram-positive bacteria, an energy-dependent efflux transport system, similar to the tetracycline pump mediated by the TetA protein, pumps the fluoroquinolones out of the bacterial cell.

Post-antibiotic effects (decreased or abnormal growth of bacteria after exposure to an antibacterial agent: PAE) lasting 4-8 hours have been seen in a number of strains including *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Neu et al., 1987). The PAE is associated with decreased adherence to cells as part of the phenomenon. Concentrations as low as 1000 fold less than the MIC have been shown to decrease adherence of *Staphylococcus aureus* bacteria to buccal cells (Desnottes et al., 1987) even though the PAE is concentration dependent (Gould et al., 1990). The active efflux mechanism described above is depressed during the post-antibiotic effect, and can be inhibited by carbonyl cyanide m-chlorophenylhydrazone, which dissipates energy (Piddock, 1994). Inhibition of the efflux mechanism results in an accumulation of fluoroquinolones inside the bacteria.

Recent data suggest that fluoroquinolones have a second intracellular target, DNA topoisomerase IV. This is a bacterial type II DNA topoisomerase that is also composed of two subunits. However, unlike the DNA topoisomerase IV cannot supercoil DNA. Instead it is involved in the ATP-dependent relaxation of DNA. It is a more potent decantenase than DNA gyrase (Hoshino et al., 1994). Topoisomerase IV may be the primary target of fluoroquinolones in *Staphylococcus aureus* and streptococci (Kaatz and Seo, 1998).

Fluoroquinolones are known to gain entry into phagocytic cells and remain microbiologically active inside the cells against bacterial pathogens such as *Legionella pneumophyla* (Carlier et al., 1990).

Microscopically, the morphologic alterations produced by the fluoroquinolones include decreased cell division, filamentation, and cellular lysis (Foerster, 1987).

Ultrastructurally, altered cell division is also evident, and bacterial cell 'ghosts', i.e. remnants of the outer bacterial cell wall without internal cell components are prominent after enrofloxacin treatment of bacterial cultures *in vitro* (Voight, 1987). These observations may be the result of the cascade of events resulting from inhibition of DNA gyrase leading to generalize bacterial cellular dysfunction, disruption of normal cellular replication and repair processes, and cell death.

BACTERIAL RESISTANCE

Resistance occurs primarily by alterations in bacterial cell wall penetration, with mutant forms of DNA gyrase occurring only rarely (Chamberland et al., 1989). Permeability changes occur either via decreased permeability of the hydrophilic pores (OMP) or through alteration of the active transport (efflux) pump (Kaatz et al., 1991), thereby decreasing the intracellular content of the fluoroquinolones. Enzymes that degrade the quinolone antibacterial agents have not been observed.

One of the major reasons nalidixic acid fell out of favor with physicians and veterinarians was the high level of resistance that quickly developed in the early 1960's (Neu, 1988). Although low-frequency chromosomal mutations are the primary source of bacterial resistance encountered against the fluoroquinolones to date, plasmid-mediated resistance to the older quinolones has only been encountered in a single isolate of *Shigella dysenteriae* in Bangladesh (Neu, 1988). Plasmid-mediated resistance has not been demonstrated for the fluoroquinolones (Fernandes, 1988). Bacteria that contain R-plasmids that carry resistance to other antibacterial agents remain sensitive to many of the fluoroquinolones. Cross-resistance with β-lactam antibiotics, aminoglycosides, tetracyclines, macrolide and polypeptide antibiotics, sulfonamides, diaminopyrimidines, and nitrofurans does not generally occur. However, certain mutations conferring resistance to the fluoroquinolones can also confer resistance to the cephalosporins, tetracyclines, and chloramphenicol, although other mutations conferring fluoroquinolone resistance can cause hypersusceptibility to β-lactams, aminoglycosides, and novobiocin (Piddock and Wise, 1989).

Single-step resistance to the fluoroquinolones occurs in 10^{-7} – 10^{-10} bacteria (Watanabe et al., 1990), with mutations in certain bacteria (e.g. *Enterobacter cloacae* and *Serratia marcescens*) developing at higher frequencies than others (Watanabe et al., 1990). The frequencies of these mutations suggest a single mutation at a single locus (Piddock 1994). When resistance does occur, cross-resistance between fluoroquinolones is generally

observed to occur at higher frequencies for the older, and less potent, quinolones such as nalidixic acid and flumequine. Development of resistance generally means a 2-8-fold change in the minimum inhibitory concentration (MIC) (Fernandes, 1988). The decreased susceptibility caused by these mutations was not considered clinically significant in the mid-1990s, as the MIC values are so low, compared with clinical drug concentration ranges attainable in human beings (Smith, 1984). More recently, resistance has been reported most often for *Pseudomonas aeruginosa, Serratia marcescens*, and staphylococci in chronic infections or chronic bacterial exposure (e.g. indwelling venous catheter or urinary catheter). During oral administration to humans, aerobic fecal flora was almost entirely abolished, while anaerobic bacteria remained little affected: after a week without selective pressure, fecal flora returned to normal (Brumfitt et al., 1984). The MIC values increased in the anaerobes, although the anaerobic bacteria were not considered initially susceptible to the fluoroquinolones. Furthermore, Brumfitt's study did not identify or type the anaerobes, and it was not proven without doubt that the same bacterial populations had developed resistance. Similar results were seen with pefloxacin (Janin et al., 1987), in that the *Enterobacteriaceae* were eliminated, but streptococci and anaerobes were unaffected. In both studies, yeast overgrowth did not occur. Resistance has developed to some of the fluoroquinolones during clinical use in humans, as evidenced by an increased MIC observed in *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* isolates from human patients with chronic respiratory infections treated with enoxacin, pefloxacin (Maesen et al., 1987) or norfloxacin (Rowan et al., 1988). When the fluoroquinolones are used, data support that intensive therapy, such that maximum concentrations achieved at the site of infection greater than 4-8 times the MIC or areas under the curve (AUC) to MIC rations of greater than 125, are associated with the minimization of resistance development (Felmingham et al., 1988; Forrest at al., 1993). Conversely, therapy over prolonged periods of time (4-10 days) in human beings is associated with the emergence of resistant strains of bacteria (Bayer et al., 1988). Clearly, intermittent dosing regardless of the route of administration is one method of minimizing the development of bacterial resistance. Novel administration strategies, such as pulse dosing water medication, may provide a rational means of administering fluoroquinolones in herd or flock situations.

Serial passage of strains of *S. aureus* through broth containing either enrofloxacin or flumequine at 0.5 times the MIC resulted in increases in the MIC for the organism, although the cross drug resistance pressure (i.e. passage in media containing enrofloxacin on flumequine susceptibility, and vice versa) produced less development of resistance than

direct drug resistance pressure (Semjén and Blaskó, 1994). This suggests that development of resistance to a human fluoroquinolone will be less if different fluoroquinolones are used in veterinary medicine. In addition, Dijkstra et al., (1994) indicated that gut models of resistance development were less indicates of the emergence of bacterial resistance than broth cultures of sarafloxacin. In the Dijkstra et al., (1994) study, the susceptibility of isolates obtained from sarafloxacin-containing models was similar to those obtained from models without sarafloxacin, as well as being similar to the susceptibility of parent strains. There was no indication of emergence of resistance in their model. One of the major differences in the latter gut model compared with broth cultures is the presence of organic (fecal) material in the gut model, which is known and was shown to reduce the activity of the fluoroquinolones (Dijkstra et al., 1994). Greenwood et al., (1984) showed that surviving bacteria were no less susceptible to ciprofloxacin after over 24 h than to therapeutic concentrations in conditions simulating bacterial cystitis.

Development of resistance is the greatest source of debate and political fallout for the use of fluoroquinolones in animals. Because the fluoroquinolones are the drugs of choice for many refractory and/or nosocomial infections in human beings, there has been an attempt to minimize the development of resistance to them by the medical profession (Beam, 1994). Several multiyear surveys, particularly in Europe, have shown that resistance has developed slowly, coincidentally with the approval and increased use of fluoroquinolones in food-producing animals as well as in humans (Endtz et al., 1991; Pérez-Trallero et al., 1993). Increased prescription of fluoroquinolones by physicians also occurred during the same period of time, confounding any conclusions of a causal relationship between veterinary use of fluoroquinolones and the development of resistance to human fluoroquinolones. In addition, these same discussions regarding restriction of antimicrobial usage in veterinary medicine to minimize development of resistance occurred several years ago, when firstly the aminoglycosides and then the cephalosporins were the classes of drugs reserved for refractory or hard to treat nosocomial infections in human beings. The nature of antimicrobial research is such that, as classes of antimicrobial agents become lass effective, new classes are developed to address the problem infections.

It is clear that resistance to any class of antimicrobial agent increase as the level of use increases due to selective pressure. Furthermore, the injudicious use of antimicrobial agents at insufficiently high doses, for inappropriate durations of therapy, or for use in clinically ill patients who do not warrant such treatment, exacerbates the development of resistance. Both the medical profession and the veterinary profession need to prescribe

and/or administer agents like the fluoroquinolones more conscientiously to minimize the development of resistance. The lack of solid data, and the potentionally erroneous conclusions regarding the cause of the development of resistance to fluoroquinolones, speaks to the need for widespread and well-designed programs to monitor the development of bacterial resistance, not only to the fluoroquinolones but also to all antimicrobials, in bacterial populations from normal animals and human beings. Such a monitoring program should investigate all sources of resistance pressure and the persistence of resistance in specific populations once it has been identified.

SPECTRUM OF ACTIVITY

In general, the fluoroquinolones have excellent activity against *Enterobacteriaceae*, fastidious gram-negative bacteria and *Pseudomonas Aeruginosa*, good to moderate activity against staphylococci, mycobacteria, chlamydia, mycoplasma and ureaplasma: and little or no activity against streptococci (particularly group D streptococci), enterococci, and anaerobic bacteria. The post-antibiotic effect of the fluoroquinolones has been shown to be 4-8 h against *Escherichia coli, Klebsiella*, *Serratia*, and *Pseudomonas Aeruginosa* (Neu et al., 1987). Comparison of ciprofloxacin, norfloxacin, pefloxacin, pipemidic acid and a variety of nonquinolone antibacterial agents (nitrofurantoin, sulfamethoxazole, trimethoprim, cephradine and amoxicillin) demonstrated that ciprofloxacin had the broadest spectrum of activity against all gram-negative bacteria and streptococci tested, with the exception of *Enterococcus faecalis* and *Streptococcus pneumoniae* (Hoogkamp-Korstanje, 1984). Compared with rosoxacin, norfloxacin, nalidixic acid and oxolinic acid the activity of ciprofloxacin against *Chlamydia trachomatis, Mycoplasma hominis* and *Ureaplasma urealyticum* was found to be at least twice as active (Ridgway et al., 1984). Enrofloxacin has structural similarity to ciprofloxacin and has a similar antibacterial spectrum to ciprofloxacin against *Haemophilus sp., Pasteurella sp.* and *Actinomyces sp.* (Prescott and Yielding, 1990). Temafloxacin is more potent than either ciprofloxacin or ofloxacin against staphylococci and streptococci, but not *Haemophylus influenzae*. Improved oral activity of temafloxacin is a function of both improved potency and better oral bioavailability (Swanson et al., 1991). The MIC of danofloxacin against 90 % of the field isolates of *Pasteurella haemolytica, Pasteurella multocida*, and *Haemophylus somnus* was found to be < 0.125 μg/mL (Jackson et al., 1990), and the range of MICs against *Mycoplasma* species was 0.008-0.5 μg/mL (Cooper et al., 1993). Many gram-negative bacteria that have become resistant to other classes of antibacterial agents, such as

aminoglycosides, anti-pseudomonal penicillins, and third-generation cephalosporins, remain susceptible to the fluoroquinolones.

Newer fluoroquinolones (either in development or already marketed) such as difloxacin, sparfloxacin, temafloxacin, tosufloxacin, and several other fluoroquinolones have increased activities against staphylococci, streptococci, enterococci, *Corynebacterium* sp., *Listeria monocytogenes* and *Bacillus* sp. (Furet and Pechére, 1991). These also have activity against various anaerobic bacteria, including *Clostridium perfringens, Clostridium difficile*, and *Bacteroides fragilis*. Those containing a cyclopropyl group at position 1 have activity against *Mycobacterium leprae* (Furet and Pechére, 1991). Recently, pefloxacin, ofloxacin, and ciprofloxacin were found to be active against *Plasmodium, Trypanosoma cruzi*, and *Leishmnia donovan*i, although *Toxoplasma gondii* was not susceptible (Furet and Pechére, 1991). Many of the newer fluoroquinolones with increased activity against gram-positive bacteria have less activity against *Pseudomonas Aeruginosa* than older fluoroquinolones (Furet and Pechére, 1991).

Fluoroquinolones are more active in alkaline environments (pH > 7.4) for gram-negative bacteria (Blaser and Lüthy, 1988), but susceptibility of gram-positive bacteria to the fluoroquinolones is not affected by pH (Fernandes, 1988). Susceptibility is not affected by inoculum size (Fernandes, 1988), but activity is reduced by the presence of divalent cations (Blaser and Lüthy, 1988).

In general, aminoglycosides, β-lactams, imidazoles, macrolides, and lincosamides infrequently show synergy with the fluoroquinolones against Enterobacteriaceae, gram-positive bacteria and anaerobes: but rarely they do show antagonism (Neu, 1991). Antipseudomonal penicillins and imipenem are synergistic with fluoroquinolones in 20-50 % of the *in vitro* and *in vivo* models. Antagonism in streptococci and enterococci occur between the fluoroquinolones and either the macrolides or tetracyclines (Neu, 1991) in general, fluoroquinolones are antagonistic with chloramphenicol.

PHARMACOKINETICS

Oral absorption of the fluoroquinolones depends on the specific agent administered with ofloxacin adsorbed better than ciprofloxacin, pefloxacin or enoxacin; all of these were more readily adsorbed than norfloxacin (Lode et al., 1987; Neu, 1988), with an absolute oral bioavailability of norfloxacin in dogs of approximately 35 % (Brown et al., 1990). Ciprofloxacin is absorbed primarily from the duodenum and jejunum when administered orally to monogastric (Wolfson and Hooper, 1991). Bioavailability is lower in ruminating

animals, although the mechanism for this anecdotal observation has not been determined. Bioavailability from parenteral injection sites is nearly 100 % for all fluoroquinolones. Food generally inhibits oral absorption of the fluoroquinolones, although there was no significant difference in enrofloxacin bioavailability in fed and fasted pigs (Gyrd-Hansen and Nielsen, 1994) or in ciprofloxacin bioavailability in humans on various high fat/high calcium diets (Frost et al., 1989a). Overall, oral bioavailability of fluoroquinolones ranges from 30-90 % in chickens (Chen et al., 1994; Martinez-Larranaga et al., 1994), turkeys (Gulkarov and Ziv, 1994) and pigs (Anadón et al., 1994; Gyrd-Hansen and Nielsen, 1994; Richez et al., 1994), although oral availability in donkeys was very low (Lavy et al., 1994).

The serum concentration peak is reached rapidly; the different fluoroquinolones display their maximum serum concentration peak between 1 and 2 hours after ingestion in man, and the times to the peak are similar in dogs, rodents and monkeys (Parpia et al., 1989). The time to serum peak concentrations after a single oral bolus administration of enrofloxacin is 2.5, 1.4, 0.9 and 0.5 hours, respectively, in the chicken, turkey, calf, dog and horse (VanCutsem et al., 1990). The concomitant administration of magnesium and aluminium containing anti-acids decreases the oral bioavailability of the fluoroquinolones. This action is attributed to the chelation of carboxylate groups by the bivalent cations (Gasser et al., 1987). The low serum concentration when administered with milk replacer may be due to the presence of minerals that could chelate the antimicrobial.

Parenteral availability of most quinolones is approximately complete in pre-ruminant and ruminant cattle (Giles et al., 1991a; Thomas et al., 1994a), although norfloxacin nicotinate availability from intramuscular injection sites was 70-90 % (Soback et al., 1994a). Supra-availability from extravascular routes has been seen in horses (Pyörälä et al., 1994), and may be a result of the enterohepatic recycling known to occur with some fluoroquinolones. The possibility of enterohepatic recycling of fluoroquinolones potentionally confounds many of the pharmacokinetic calculations that assume dose proportionally and no recycling (i.e. classical one- and two-compartment open pharmacokinetic models).

One of the most attractive pharmacokinetic characteristics of the fluoroquinolones is their large volume of distribution. Distribution of fluoroquinolones is very good to tissues, owing to their physicochemical properties. Plasma protein binding of the quinolones varies, with the newer quinolones less bound to plasma proteins than nalidixic acid.

The steady-state volume of distribution of the fluoroquinolones is large, being 2-3 L/kg for danofloxacin in cattle (Giles et al., 1991a; Grimshaw et al., 1990a), and 3.45 $\pm$

0.72 L/kg in horses (Dowling et al., 1995), 1.47 L/kg for norfloxacin in dogs (Brown et al., 1990) and 0.75 – 0.96 L/kg for flumequine in calves (Mevius et al., 1990). In most species, this distribution volume is over 3-fold greater than that for most β-lactam antibiotics and aminoglycosides, and probably represents intracellular sequestration of the drug in various tissues. Blister-fluid concentrations (indicative of interstitial fluid concentrations) equal serum concentrations within 2 h oral administration (Neu, 1988). Furthermore, tissue cage fluid concentrations of norfloxacin or ciprofloxacin were somewhat, but not substantially, higher than concurrent plasma concentrations after 6 h oral administration, and they were lower than concurrent plasma concentrations from 0-6 h dosing in normal dogs (Walker et al., 1989; Walker et al., 1990). Volume of distribution of enrofloxacin and ciprofloxacin increased in rabbits from 8 through 60 days of age, possibly due to changes in body composition (Abadía et al., 1994a).

High concentrations of fluoroquinolones are achieved in saliva, nasal secretions and nasal mucosa, and bronchial epithelium (Neu, 1988), although these are not substantially higher than concurrent plasma concentrations. In fact, nasopharyngeal concentrations of ciprofloxacin were much higher than the MIC_{90} for meningococci and *H. influenzae*, but they were below the MIC for methicillin-resistant *Staphilococcus aureus* in human patients (Darouiche et al., 1990). Enrofloxacin concentrations that were up to 3 times higher than serum concentrations were observed in tissue homogenates from calves taken 1 h after dosing, with 12 h concentrations in tissue homogenates exceeding concurrent serum concentrations (Sheer, 1987). Similarly, danofloxacin lung homogenate concentrations over time were 3.5-4.5 times the concurrent plasma concentrations (Giles et al., 1991a). These danofloxacin concentrations in lung homogenate appeared somewhat related to regional blood flow, although danofloxacin concentrations in consolidated lung homogenates were proportionally higher than in blood flow (Apley and Upson, 1993a). Furthermore, concentrations of danofloxacin in bronchial secretions reproduced concurrent plasma concentrations in swine in spite of higher concentrations in bronchial mucosa and whole lung homogenates (Friis, 1994); similar relationships between bronchial secretion and lung tissue homogenate concentrations may apply in other species, including cattle. In the dog, enrofloxacin concentrations in bile and urine exceeded serum concentrations 10-20 fold; tissue homogenate concentrations observed 1 h after drug administration in calves were in the following order: liver $\geq$ kidney > heart > lung $\geq$ spleen $\geq$ intestinal wall > serum = muscle = lymph nodes (Sheer, 1987). Concentrations of enoxacin in skin were

almost equal to concurrent plasma concentrations after multiple oral dosing (Malmborg and Rannikko, 1988).

Semen concentrations were half of those observed in the serum shortly after ciprofloxacin administration, but were 10 times higher than serum concentrations at 12 h and 24 h after dosing (Dalhoff and Weidner, 1984). Ciprofloxacin concentrations in expressed prostatic secretion after oral administration of 500 mg ciprofloxacin in human volunteers ranges from 0.9-15 µg/mL, indicating pronounced diffusion of ciprofloxacin into the prostatic fluid (Dalhoff and Weidner, 1984). Enrofloxacin showed similar penetration into prostatic fluid and tissue in dogs, such that both were higher than concurrent serum concentrations (Dorfman et al., 1995), and no differences were noted in the presence of chronic *Escherichia coli* prostatitis. Good penetration of enoxacin into myometrium, cervix, and Fallopian tubes has been shown in human beings (Bates and Elder, 1988). In dogs, uterine and prostatic fluid concentrations were 2.2 and 1.4 µg/mL 1 h after and oral dose of 2.5 mg, whereas 1 h serum concentrations were 1.2 µg/mL after an oral dose of 5 mg enrofloxacin/kg (Sheer, 1987). In cortical bone, enrofloxacin activity reached 29% of the concurrent serum activity (Duval and Budsberg, 1995), although it must be remembered that enrofloxacin and its dealkylated metabolite, ciprofloxacin; both contribute to *in vivo* activity. The ratio of concentrations of ciprofloxacin, pefloxacin, and ofloxacin in amniotic fluid compared to plasma ranged from 0.35 to 0.5 between 2-6 hours after dosing; comparable milk to plasma ratios were 0.75 to 1.84 (Giamarellou et al., 1989; Wolfson and Hooper, 1991). There is approximately 16 times more placental transfer of enrofloxacin than ciprofloxacin in rabbits (Aramayona et al., 1994), suggesting some very profound compound-specific transport processes through the placenta. In contrast, milk norfloxacin concentrations were up to 40 times higher than the corresponding serum concentrations after administration of norfloxacin nicotinate to ewes (Soback et al., 1994b). Enrofloxacin penetrates into milk to attain approximately twice the maximum concentration of ciprofloxacin at similar plasma concentrations, although the elimination of enrofloxacin from milk is approximately twice a fast as that for ciprofloxacin (Bregante et al., 1994). Penetration into the CNS is relatively good, and vitreous humor penetration is approximately 20 % (Barza, 1991). Apart from nasal secretions (Dobbs et al., 1988) and ejaculate, body fluid concentrations of fluoroquinolones rarely reach plasma concentrations (Sorgel et al., 1989). Thus, the high tissue concentrations are result of sequestration onto, or within, cells or cellular components of a tissue, although Carlier et al. (1990) found no specific subcellular structure affinity of pefloxacin. As an example, intracellular

concentrations of fluoroquinolones in polymorphonuclear leukocytes are 7-14 times those found in extracellular fluid (Zweerink and Edison, 1988).

The degree of metabolism of the fluoroquinolones varies widely. Biotransformation reactions involve predominantly the piperazinyl ring and its substituents. Most of the fluoroquinolone's primary metabolites are active against bacteria: however, these metabolites have a shorter elimination half-life than their parent compound.

In general, phase I metabolism occurs, primarily through hydroxylation and oxidation to oxoquinolones. Ofloxacin is not metabolized, whereas pefloxacin is nearly completely metabolized. Nalidixic acid is hydroxylated and then glucuronidated. Enrofloxacin and pefloxacin are N-dealkylated to form ciprofloxacin and norfloxacin, respectively, as is fleroxacin (Lode et al., 1987; Küng et al., 1993). Other more prominent metabolic pathways include oxidation at the piperazine ring to oxo-metabolites (Nix and Schentag, 1988), the major metabolites of ciprofloxacin, enoxacin, and norfloxacin (Anadón et al., 1995; Lode et al., 1990). Quite often, glucuronidation occurs, primarily on the carboxylic acid at position 3. Oxidized metabolites (like many of the N-desmethyl metabolites) have some antibacterial activity (Küng et al., 1993; Prescott and Yielding, 1990), whereas the glucuronide conjugates are devoid of activity (Nix and Schentag, 1988; Venezia et al., 1989). Other metabolic pathways include sulfoxidation and acetylation (Lode et al., 1990).

The excretion of the fluoroquinolones is primarily via the kidney and secondarily via the liver. High urinary concentrations are achieved due to glomerular filtration and to probenecid-sensitive tubular secretion.

Excretion is decreased in individuals suffering from renal failure and caution should be exercised with the use of fluoroquinolones in such patients. The percentage of elimination through the bile varies among species (Montay et al., 1984). For example, biliary excretion of the pefloxacin glucuronoconjugate is high in dogs and rats relative to all other species (Montay et al., 1984).

Nearly half of an intravenous dose of ciprofloxacin is eliminated in the feces, with slightly more than half of the dose being eliminated in the urine, after an oral dose more than 90 % is excreted in the feces (Nix and Schentag, 1988). The glucuronide conjugates of the fluoroquinolones may be excreted in the urine or bile, depending on the fluoroquinolone and the species to which it was administered (Nix and Schentag, 1988). There are indications that enterohepatic circulation of fluoroquinolones may occur, principally through the action of β-glucuronidases in the gastrointestinal tract that may liberate the parent agent or biologically active metabolites. Studies also suggest that

ciprofloxacin may be eliminated by active transepithelial elimination into the bowel lumen also (Ramon et al., 1994; Wolfson and Hooper, 1991).

Renal excretion of the fluoroquinolones is also variable, although glomerular filtration occurs for the unbound fraction of all fluoroquinolones. Active tubular secretion by the organic anion transport system also occurs to a more variable degree (Drusano et al., 1986). Probenecid blocks the renal tubular secretion of norfloxacin and ciprofloxacin but because of the other routes of excretion, drug accumulation does not occur to a great extent (Wolfson and Hooper, 1991). Renal excretion accounts for 100 % of cinoxacin (a non-fluoroquinolone) in 24 h (Drusano et al., 1986) 60 % of ciprofloxacin in 24 h in many species but only 30-40 % in dogs (Abadía et al., 1994b), and 30-40 % of norfloxacin and enrofloxacin in 24 h.

In normal animals, the biological half-life ($t_{1/2}$) of most fluoroquinolones ranges from 3 to 6 h: specifically, the $t_{1/2}$ of flumequine is 6-7 in calves (Mevius et al., 1990), 3.5-4.5 h for danofloxacin (IM.SC. or IV) in calves (Grimshaw et al., 1990a; Giles et al., 1991a), 5.4 $\pm$ 0.9 h for enrofloxacin in calves (Sheer, 1987), 2-4 h for ciprofloxacin in dogs (Abadía et al., 1994b) and horses (Dowling et al., 1995), 3.6 h for norfloxacin (IV) in dogs (Brown et al., 1990), and 3 hours for enrofloxacin in laboratory Beagles compared to 5.0$\pm$1.0 h in canine clinical patients. Interspecies differences are important: enrofloxacin has an elimination half-life of 7.3, 1.4, 1.2, 2.1 and 3.3 hours in the chicken, turkey, calf, dog and horse, respectively (VanCutsem et al., 1990). Fleroxacin has an elimination half-life of 1.6 hours in the rabbit, 9.4 hours in the dog (Sorgel et al., 1988) and 10.8 in man (Takayama et al., 1986).

Upon multiple dosing, ciprofloxacin, enoxacin, and other fluoroquinolones have shown anincrease in the $t_{1/2}$ and increased V_d from the first dose (Chang et al., 1988; Nix and Schentag, 1988); however, this phenomenon was not observed with norfloxacin in dogs using a dosage regimen of 5 mg/ kg every 12 h for 14 days (Brown et al., 1990) or for ciprofloxacin in other studies (Höffler et al., 1984; Drusano et al., 1986) or in dogs (Abadía et al., 1994b). The area under the concentration time curve normalized to a 1 mg/kg dose decreased as the dose of norfloxacin increased from 5 mg/kg to 20 mg/kg in healthy dogs (Brown et al., 1990). The multiple dose phenomenon described by Nix and Schentag (1988) and the non-linearity of the AUC with increasing doses in dogs observed by Brown et al. (1990) may reflect a decreased absorption of fluoroquinolones at higher doses, or may be a result of the complicated enterohepatic recycling that may occur after repeated doses. The pharmacokinetics seems to be independent of gender (Höffler et al., 1984)

although individual fluoroquinolones may vary depending on the metabolic pathways and routes of excretion.

PHARMACOKINETICS IN DISEASE

Oral absorption is not altered in human patients with diarrhea or in those with cutaneous infections. In cases of bacteriaemia, serum concentrations were still sufficient for effective treatment of gram-negative infections, although differences and increased variability were observed (Wolfson and Hooper, 1991). Human beings with hepatic cirrhosis exhibited reduced metabolism of ciprofloxacin to oxociprofloxacin but not desethylene ciprofloxacin or sulfociprofloxacin, with no change in parent ciprofloxacin pharmacokinetics from that observed in healthy humans (Frost et al., 1989b). Danofloxacin pharmacokinetics and lung disposition were not altered dramatically in pneumonic calves compared with healthy calves, although volumes of distribution were somewhat larger in pneumonic calves (Apley and Upson, 1993b). Pneumonic and macroscopically normal lung homogenates had similarly high danofloxacin concentrations and similar depletion profiles. In pre-ruminant calves, absorption from IM sites was not altered and elimination was not significantly slowed by experimental pneumonic pasteurellosis (Thomas et al., 1994b).

PHARMACOKINETIC PREDICTORS OF EFFICACY

The fluoroquinolones are classified as bactericidal compounds, and in fact have shown concentration-dependent bacterial killing within a couple of orders of magnitude of the MBC. Unlike β-lactam antibiotics, the efficacy of fluoroquinolones is related to both the maximum concentration and the time above the MIC (Blaser et al., 1987). *In vitro* pharmacokinetic systems have shown that peak concentrations exceeding 8 times the MIC were related with over 99 % reduction in bacterial counts and prevention of bacterial regrowth for 24 h. The study did not separate peak concentrations from time above the MIC by mimicking different pharmacokinetic profiles, precluding any definitive conclusions being made regarding the best pharmacokinetic predictor of efficacy. Similar results were observed in an *in vivo* model of *Streptococcus pneumonia* in mice with ciprofloxacin (Sullivan et al., 1993), indicating that the peak concentration/MIC ratio had to reach a value of 10.6 for optimum protection in that model. Drusano et al. (1993) provided some additional insight by administering lomefloxacin, to neutropenic rats with Ps. Aeruginosa sepsis, as a single daily dose which produced high peak concentration/MIC

ratios (approximately 20/1), or as the some total daily dose fractionated into four daily injections, the latter producing a longer time above the MIC. The single daily dose produced significantly better survival than the more fractionated regimen, indicating that peak concentration and/or intensity of exposure is linked more closely with efficacy than time above the MIC intensity of exposure has been quantified as the ratio of the area under the concentration-time curve to the MIC (AUC/MIC), otherwise known as the area under the inhibitory concentration curve (AUIC). Forrest et al. (1993) noted that, for ciprofloxacin, the probability of clinical and microbiological cures were above 80 % when the AUIC was greater than 125; when the AUIC was less than 125, the probabilities for clinical and microbiological cures were 42 % and 26 %, respectively. Time to eradication of the infection was similarly related to the AUIC, with 125 and 250 the cut off points for moderate and rapid eradication of the infection (Forrest et al., 1993). The observation that the AUIC is closely related to efficacy may also be related to increased coverage of more resistant strains whereas current expectations are that C_{max} will be more closely related to reducing resistance. Optimizing one or both of these ratios may ultimately reduce the likelihood that microbial flora will develop resistance. However, these are to date unproven hypotheses in the veterinary situation.

ADVERSE EFFECTS

With few exceptions, the adverse effects of the fluoroquinolones are not of severe consequence when compared to the beneficial features they exhibit. The target tissues are the juvenile cartilage, central nervous system, urinary tract and digestive tract. Some skin eruptions have also been seen in man (Ball, 1986). Embryonic losses in female monkeys exposed to very high doses have been described (Neer, 1988).

Toxicity of the fluoroquinolones is mild at therapeutic doses, and generally consists of gastrointestinal disturbances such as nausea, vomiting, and diarrhea (Norrby, 1991). At slightly higher doses, CNS signs of dizziness, restlessness, headache, depression, somnolence or insomnia may be seen (Neu, 1988). High serum concentrations may produce immediate toxic reactions, due possibly to overwhelming histamine release. These immediate reactions are believed to be principally CNS in nature, and consist of convulsions, defecation, urination, and emesis within 2-3 min of rapid IV injection of norfloxacin solution (Brown et al., 1990). These signs subsided within several minutes in the affected dogs, and slower infusion (for 2-3 minutes) did not produce such severe clinical signs. Others (Akahane et al., 1989) reported that the epileptogenic activity of

fluoroquinolones possibly relates to the γ-aminobutyric acid (GABA)-like structures of the substituents at the 7-position of some of the fluoroquinolones, which may allow them to act as GABA-receptor antagonists. Furthermore, enrofloxacin has increased the frequency and intensity of seizures in epileptic dogs (Van Cutsem et al., 1990). Other fluoroquinolones may not be as likely to produce these CNS effects. Crystalluria can occur in dogs and humans at high doses of norfloxacin, although the occurrence is rare in human beings treated with ciprofloxacin and has not been reported with either danofloxacin or enrofloxacin. Non-inflammatory, erosive arthropathies can be observed in growing animals treated with fluoroquinolones. Lesions of the weight-bearing cartilage of juvenile rats and beagle puppies have been observed after experimental exposure to nalidixic acid or fluoroquinolones (Kato and Onedara, 1988), causing lameness and pain severe enough to impose humanitarian euthanasia (Kato and Onedara, 1988, McQueen and Williams, 1987). Kato and Onedara (1988) observed the first histological changes as early as 5 hours after a very high dose of ofloxacin. It is apparently for this reason that the manufacturer of enrofloxacin does not advocate the administration of this product to dogs younger than eight months of age. The articular cartilage forms vesicles after a single very large dose, or after several moderately large doses, which can then progressively rupture and produce cartilaginous erosions. This observation is due to an early phase burst in oxidative metabolism in immature (but not mature) chondrocytes that may precipitate cell death (Hayem et al., 1994; Thuong-Guyot et al., 1994). These erosions are preferentially located at weight-bearing joints (Neu, 1988). For this reason, immature dogs, particularly those of large breeds, should not be treated with fluoroquinolones. In addition, most products labeled for human use state they should not be used in pregnancy, although this warning may be precipitated by lack of data. Furthermore, use of fluoroquinolones in horses has not been recommended for similar reasons (Berg, 1988). Although the basis for that recommendation has been made with very little published supporting information.

Photosensitization occurs with all marketed fluoroquinolones, especially pefloxacin, although it is rare for norfloxacin and ciprofloxacin (Neu, 1988; Norrby, 1991). Topical administration to the eye shows less toxicity to corneal epithelium than aminoglycosides (Cutarelli et al., 1991). However, ocular cataracts have been seen with prolonged use in humans (Neu, 1988).

Enrofloxacin has been shown not be mutagenic by the Ames test or by the Chinese hamster ovary-HGPRT forward mutation assay and unscheduled DNA synthesis test (Altreuther, 1987). In pregnant laboratory animals given very large doses of

fluoroquinolones, maternotoxicity has occurred and some embryonic deaths have been reported in laboratory animals; such observations have not been observed in the target species treated with fluoroquinolones at therapeutic doses.

Occasionally, laboratory tests may be altered in patients treated with fluoroquinolones, including increases in hepatocellular enzymes (alanine aminotransferase and aspartate aminotransferase), serum urea nitrogen and crystalluria, and decreases in haematocrit. These alterations may represent real perturbations of the organ systems of the animal or may be labortory artifact.

DRUG INTERACTIONS

The only possible drug interaction study that has been documented in animals is lack of effect of enrofloxacin on digoxin steady-state concentrations in dogs (Novotny and Shaw, 1991).

The following have been documented only in human studies. Oral absorption of the fluoroquinolones is drastically decreased by antacids containing magnesium and aluminium (Nix et al., 1989), and other agents such as sucralfate also decrease the absorption of the fluoroquinolones. Ranitidine did not alter the oral absorption of ciprofloxacin (Nix et al, 1989) but did decrease the oral bioavailability of enoxacin (Grasela et al., 1989), suggesting that gastric pH affects the oral absorption of some fluoroquinolones, perhaps through alterations in dissolution.

The fluoroquinolones, including enrofloxacin, after repeated administration have been shown to decrease the hepatic clearance and increase the elimination half-life of theophylline (Bowles et al., 1988; Rybak et al., 1987) and caffeine (Harder et al., 1988) reportedly by decreasing the demethylation of theophylline by the hepatic P450 enzymes, the 4-oxoquinolone metabolite. Ciprofloxacin administration over a period of 8-10 days prolonged the half-life of antipyrine from 9.45 to 14.9 h attributed to decreased clearance from 0.85 to 0.52 mL/min-kg in human patients (Ludwig et al., 1988). However, others have shown that oral doses of ofloxacin, enoxacin and norfloxacin showed no significant effect on the content of cytochrome P450, cytochrome b_5, NADPH- cytochrome P450 reductase, ethoxycoumarin O-deethylase, benzphetamine N-demethylase, or aniline hydroxylase in phenobarbital-responsive systems (Okazaki et al., 1988). Furthermore, clinically important drug-drug interactions between theophylline and ofloxacin have not been shown in several instances (Wolfson and Hooper, 1991). Enoxacin decreases the hepatic clearance of the R-enantiomer of warfarin but not the S-enantiomer, and the

anticoagulant effects of warfarin are increased by the concurrent administration of ofloxacin (Wolfson and Hooper, 1991).

Concurrent administration of the non-steroidal anti-inflammatory agent fenbufen with enoxacin has been associated with seizures in human beings, although patients given other fluoroquinolones concurrently with non-steroidal anti-inflammatory agents other than fenbufen have not developed seizures (Wolfson and Hooper, 1991). No drug-drug interaction studies have been published for danofloxacin.

THERAPEUTIC USES

The fluoroquinolones have shown efficacy against a variety of bacterial diseases and are indicated in the treatment of local and systemic diseases caused by a wide range of gram-positive and gram-negative bacteria, mycoplasma and chlamydia. Due to the wide array of spectrum the use of fluoroquinolones have been proposed in conditions such as deep-seated infections, prostatitis, CNS infections, bone and joint infections, and nosocomial infections resistant to other antibacterial agents.

In human beings, the fluoroquinolones are used for the treatment of a variety of severe infections that are either located in tissues inaccessible to other antibacterial agents or caused by bacterial pathogens resistant to other antimicrobial agents. These include (but are not limited to) purulent exacerbations of chronic respiratory infections (Maesen et al., 1987), complicated and uncomplicated urinary tract infections, Salmonella spp. infections, and other infections, such as otitis externa and ophthalmitis, that are resistant to agents (Barza, 1991). Norfloxacin and ciprofloxacin have received the most extensive clinical trials. Norfloxacin has been used most for treatment of urinary tract infections. In one study (Friis, 1991), 408 of 417 (98%) gram-negative isolates and 58 of 62 (94%) gram-positive isolates were susceptible to norfloxacin. Norfloxacin is active against pathogens that often require parenteral therapy, and therefore, an entire spectrum of urinary pathogens can be treated with a single oral drug. Because of this, many patients who once required long-term hospitalization for parenteral therapy of difficult urinary tract infections now can be discharged earlier and treated with these oral fluoroquinolones.

In animals, enrofloxacin, marbofloxacin, norfloxacin, norfloxacin nicotinate, difloxacin and danofloxacin are approved for use in animals. Enrofloxacin is used in dogs, for complicated and uncomplicated urinary tract infections (e.g., doses up to 11 mg/kg every 12 h) and for a variety of other infections, such as mycobacterial infections (Studdert and Hughes, 1992); prostatitis (Dorfman et al., 1995) and osteomyelitis (Duval and

Budsberg, 1995) caused by susceptible bacteria. Higher recommended doses have been calculated based on the assumption that concentrations of quinolones must exceeded the MIC_{90} for the entire dosing interval (Walker et al., 1992), this was later shown to be an incorrect assumption (Drusano et al., 1993; Forrest et al., 1993). In dogs, a therapeutically equivalent dose of ciprofloxacin has been suggested to be 4-5 times the dose (on a mg/kg basis) of enrofloxacin which is 2.5 mg/kg twice a day; however, the scientific justification for this recommendation is questionable. Studies have been published indicating that enrofloxacin was effective in the treatment of acute salmonella infections in calves, and produced negative fecal cultures both 5 and 12 days post-treatment in salmonella carrier calves (Berg, 1988). In swine, enrofloxacin is reported to eliminate the carrier state for Salmonella with an oral dose of 200 ppm in the feed for 10 days (Berg, 1988). Clinical field studies have been conducted with enrofloxacin and difloxacin in swine colibacillosis, poultry colibacillosis, and other poultry bacterial and mycobacterial diseases, with therapeutic success (Berg, 1988). Danofloxacin has undergone extensive field efficacy studies in bovine respiratory disease, indicating that a dose of 1.25 mg/kg every day for 3-5 days is effective under a variety of management systems (Jackson et al., 1990). Other efficacy studies with danofloxacin have shown promise for poultry mycoplasmosis (Jordan et al., 1993; Kempf et al., 1992). Parenteral enrofloxacin and oxytetracycline were both effective, and indistinguishable, in terms of clinical efficacy, from each other, against Actinobacillus pleuropneumoniae in swine as determined by rectal temperature and lung weight (Pijpers et al., 1994).

Efficacy rates of enrofloxacin for treating pneumonia and diarrhea in cattle and swine are from 76 to 100% (Lekeux and Art, 1988; Yamamoto et al., 1992), those of danofloxacin for cattle and swine pneumonia from 83 to 86% (Giles et al., 1991b; Grimshaw et al., 1990b). Enrofloxacin decreases mortality rates in poultry flocks with respiratory infections (Hinz and Rottmann, 1990), as do difloxacin, norfloxacin and danofloxacin. Danofloxacin may cause temporal sedentariness, and orbifloxacin may cause temporal walk failure.

The oral norfloxacin therapy of dogs suffering from acute enteritis removed the disease in 100% (Bhaumik, 1997), and in another study the urinary tract infection (Patil et al., 1995).

The pharmaceutical formulations of the veterinary new quinolones are solutions and powders.

Enrofloxacin, danofloxacin, difloxacin and norfloxacin nicotinate are available as solutions for injection in cattle, and only enrofloxacin is available as solution for oral use. For swine, all 4 drugs have been provided as solutions for injection. Danofloxacin, norfloxacin and norfloxacin nicotinate has been formulated as powder for feed and drinking water, and difloxacin, enrofloxacin, norfloxacin and danofloxacin as solutions for drinking water for swine. For poultry, danofloxacin, norfloxacin and norfloxacin nicotinate has been formulated as powder for adding to feed and drinking water, and difloxacin, enrofloxacin, norfloxacin and danofloxacin as solutions for adding to drinking water.

All drugs are administered for a maximum of 3 or 5 days. Injection sites should be changed when a large volume of drug is used, and the quinolones may cause indurations at the site of injection. Enrofloxacin should be used with caution because of its strong alkalinity.

INFLUENCE ON THE ENVIRONMENT

A further concern regarding the use of new quinolones in the veterinary field is the possible detrimental effect on the environment caused by the disposal of used drugs and from animal excreta. The first point to be considered is that veterinary new quinolones discarded into the environment are usually firmly adsorbed to soil and rarely pollute water. Furthermore, quinolones rapidly decompose on exposure to light. Finally, quinolones have virtually no effect on soil organisms such as protozoa and fungi or on insects and plants. It is therefore unlikely that controlled use of veterinary quinolones will give rise to unfavorable effects on the environment.

Although there are no definite data implicating veterinary use of anti-infectives in the development of drug resistance in human pathogens or in worsening environmental pollution, an urgent need exists for more appropriate selection and use of antimicrobial drugs. To this end, there are 3 important restrictions on the use of veterinary new quinolones. First, veterinary new quinolones are indicated only when the first-choice drugs are ineffective. Second, they are administered only by, or under the direction of, veterinarians. Third, professional and public education should be strengthened in the area of infectious diseases and antimicrobials to reduce inappropriate usage of these compounds.

The curriculum of health professional (medical, dental, nursing, and veterinary) schools and postgraduate educational programs should be strengthened in the areas of sterilization, disinfection, hazards of inappropriate antimicrobial drug use, appropriate

diagnosis and treatment of infectious diseases, and antimicrobial resistance. These efforts should result in reduction of spread of infectious agents and more prudent use of antimicrobials.

Better guidelines should be established and enforced to reduce the spread of infectious agents and antimicrobial resistance in the hospital environment, nursing homes, day care facilities, and food production industries.

Educational materials should be developed and widely distributed to patients and food producers. The need for partnerships in improving antimicrobial use for cost-effective treatment of infections and to preserve the effectiveness of antimicrobial drugs for the future should be emphasized.

More basic research is needed to determine the mechanisms of spread of pathogens, particularly in closed populations (i.e., hospitals, child care facilities, and food production facilities).

The laws of evolution dictate that microbes will eventually develop resistance to nearly every antimicrobial. Thus, more basic research is needed to facilitate development of effective vaccines and other prevention measures. Vaccines are the most cost-effective method of disease control and prevention for many diseases (American Society for Microbiology, 1995).

CONCLUSION

Fluoroquinolones are one of the most useful classes of antimicrobial agents used in human and animal medicine today, both because of their spectrum and their physicochemical properties. As such, their popularity in clinical situations is increasing.

Recently, however, concerns have been raised over the possible emergence of quinolone-resistant strains and the effects on the environment if such drugs are overused. At present it appears that, physicians and veterinarians can prolong their usefulness for many years if they use appropriate clinical judgment and proper dosing principles as they prescribe and administer these drugs to patients.

If used in a well-controlled manner, quinolones will contribute greatly to stock farming management, without adversely influencing human chemotherapy.

VIII. AIMS OF THE PRESENT STUDY

Norfloxacin is a third generation fluoroquinolone with a high antimicrobial activity against a wide range of Gram-negative and a number of Gram-positive aerobes as well as most pathogenic mycoplasmas.

The principal application of norfloxacin has been for gastrointestinal and respiratory infections. While pharmacokinetic evaluation of norfloxacin has been carried out in normal chickens, after extensive literature searches there is a lack of information on the clinical use of norfloxacin in chickens and turkeys in the treatment of fowl cholera and *Escherichia coli* infection and its pharmacokinetic and pharmacodynamic interrelationship during disease. We planned to develop an insight into the pharmacodynamics of outcome for the treatment of infections with the fluoroquinolones.

The basic questions that have been studied are:

- Implementation and development of a *Pasteurella multocida* and *Escherichia coli* infection model that is steadily functional for evaluating the efficacy of antimicrobial medication.

- Characterization of the efficacy of pulse- and continuous dosing oral norfloxacin treatment in the control of induced pasteurellosis and colibacillosis in broiler chickens and turkeys.

- Obtaining information about the general pharmacokinetic features of norfloxacin to determine the properties of pulse- and continuous oral administration in chickens and turkeys.

- Investigation of pharmacodynamics to establish optimal therapeutic dose and dose interval as well as determine the usefulness of pulse and continuous oral administration in chickens and turkeys. Optimizing one or both of these ratios may ultimately reduce the likelihood that microbial flora will develop resistance.

II

IX. TREATMENT OF EXPERIMENTALLY INDUCED *PASTEURELLA MULTOCIDA* INFECTIONS IN BROILERS AND TURKEYS: COMPARATIVE STUDIES ON DIFFERENT ORAL TREATMENT REGIMENS.[2]

SUMMARY

Experimental fowl cholera was induced in 60, healthy 10-week-old broiler chickens and 8 week old turkeys by intramuscular inoculation with approximately 80 CFU of *Pasteurella multocida* (X-73 strain) and with approximately 70 CFU of *Pasteurella multocida* (P-1059 strain), respectively. This method of infection proved to be useful for evaluating the efficacy of antimicrobial medication, by measuring mortality, weight gain, pathological responses and frequency of reisolation of *Pasteurella multocida*.

The efficacies of two different dosing methods, continuous and pulse dosing were compared.

By using the continuous dosing method, norfloxacin was administered to drinking water at 100 mg/L for 5 days in chickens. The efficacies were slightly improved compared to pulse dosing at 15 mg/b.w. for the same length of time. The opposite was observed in turkeys, to the degree of control of mortality and maintenance of weight gain.

Key words: norfloxacin, *Pasteurella multocida* infection, comparative study, oral treatment, continuous dosing, pulse dosing, broiler and turkey

INTRODUCTION

Avian cholera (fowl cholera) caused by the Gram-negative, nonmotile, coccobacillary bacterium *Pasteurella multocida*, has been known to occur in a variety of wild and domestic birds and cause major economic losses in the poultry industry. Death results primarily from peracute and acute septicemia and less frequently from the chronic and localized form of the disease (Rimler and Glisson, 1997). Most reported outbreaks of fowl cholera affected chickens, turkeys, ducks and geese but *Pasteurella multocida* has also been known to cause devastating outbreaks in free-living waterfowl. The wide range

[2] This study was published in the *Journal of Veterinary Medicine Series B* 2002, **49**: 130-134

of avian hosts in which fowl cholera has been reported suggests that all species of birds are susceptible. The most sensitive are young mature turkeys; but all ages are highly susceptible.

Mortality from fowl cholera in chicken usually occurs in laying flocks, this seems to be the most susceptible age (Rimler and Glisson, 1997). Although *Pasteurella multocida* has been successfully eliminated from most poultry flocks in many major areas of poultry production, those areas where infection remains endemic, measures to control the infection often depend on the widespread use of antimicrobials (Rhoades and Heddleston, 1980). Prevention is best achieved by a combination of good site security, adequate vaccination and vermin control (Horrox, 1987). The usual treatment regimen consists of sulfonamides and antibiotics; success is limited due to bacterial drug resistance (Walser and Davis, 1985), relapse (Hinz and Luders, 1991) and emergence of carrier birds.

A considerable debate reference the efficacy of vaccines for preventing fowl cholera is currently under discussion (Hinz and Luders, 1991).

Norfloxacin is a third generation fluoroquinolone that was first introduced for treating urinary tract infections in humans (Gootz, 1990). This drug is more potent than any earlier analogues, has a broad spectrum of activity and drug resistant bacteria is induced less frequently (Wolfson and Hooper, 1988).

The most attractive characteristics of norfloxacin are good absorption when given orally, and maintenance of effective serum and tissue levels against a broad range of pathogenic microorganisms causing systemic infections (Wise, 1984). After extensive literature searches, there appears to be no reports or publications on the use of norfloxacin in the treatment of fowl cholera.

Norfloxacin is not only well absorbed given orally (as cited above) but as other published benefits show, fluoroquinolone agents have a marked post-antimicrobial effect (PAE) on growth of either sensitive or resistant organisms for up to 8 hours (Neu et al., 1987). These facts support the discussion to use fluoroquinolones in a pulse-dosing manner by veterinarians. This activity, together with the importance of *Pasteurella multocida* as a poultry pathogen, led us to compare the efficacy of pulse-dosing oral norfloxacin treatment with that of an established medication of continuous-dosing, in the control of pasteurellosis induced in broiler chickens and turkeys by deep intramuscular injection of the bacteria.

MATERIALS AND METHOD

Pasteurella multocida. Two different *Pasteurella multocida* strains were used:

Pasteurella multocida X-73 (A:1 serovar). This strain is a reference strain ATCC No 11039 and is used officially as a challenge strain in the efficacy test of inactivated *Pasteurella multocida* vaccines in chickens by CEVA-Phyalxia Veterinary Biologicals Co. Ltd., Budapest, Hungary (CEVA-Phyalxia).

Pasteurella multocida P-1059 (A:3 serovar). This strain is a reference strain ATCC No 15742 and is used officially as a challenge strain in the efficacy test of inactivated *Pasteurella multocida* vaccines in turkeys by CEVA-Phyalxia.

The used *Pasteurella multocida* strains were propagated in Brain Heart Infusion (BHI, DIFCO Labs., USA) for 18 hours at 37 ^{o}C. Both strains are vaccine reference strains and were obtained from CEVA-Phylaxia. The minimum inhibitory concentration (MIC) of norfloxacin for both *Pasteurella multocida* isolates was determined using broth dilution method.

Poultry. Sixty clinically healthy 10 weeks old Ross breed broiler chickens (Prophyl Ltd, Mohács, Hungary), and sixty clinically healthy 8 weeks old Arbor breed turkeys (Prophyl Ltd, Mohács, Hungary) were allotted into three experimental groups: infected and untreated controls (n=20, Group 1); infected and continuous-dosing treated with norfloxacin 100 mg/L drinking water (n=20, Group 2); infected and pulse-dosing treated with norfloxacin 15 mg/b.w. (n=20, Group 3). During the experimental period, the groups were placed in separate deep bedding pens and were provided with broiler rations ad libitum and water according to the experimental design.

Inoculation of bacteria. A 7-day acclimatization period was taken. For infection the BHI culture was diluted with sterile physiological saline solution to the extent that each bird in all groups of chickens and turkeys were injected with 1 ml diluted broth culture containing approximately 80 CFU/ml X-73 (A:1) and 70 CFU/ml P-1059 (A:3), respectively. The injection was administered with a 23G needle as a deep intramuscular injection into the left, pectoral muscle. At the same time, the inoculum was cultivated on 5% bovine blood Trypto-Casein Soy Agar (TSA, Diagnostics Pasteur, France) for enumeration of viable germs and confirmation of identity of the bacteria.

Treatment regimen. Norfloxacin, as base (Vetriflox 200 Oral Solution A.U.V., Registration No.: 655/1996, CEVA-Phylaxia), was given to Group 2 and 3 in drinking water for 5 days, starting on the day of infection and one hour later of inoculation. Group 2 received continuous medication throughout the 5-day period; the drug was dissolved in drinking water and was available for birds ad libitum. In Group 3 drinking water was withheld for 2 hours; thereafter, the entire amount of drug was mixed with one-forth of the

daily water amount. Four hours later the birds received drinking water free from drug, ad libitum. The body weights of animals were measured each day and the dosage was adjusted. Table 1 shows the experimental design.

Table 1: Experimental design

Animals		
Group 1 (20 birds)	**Group 2 (20 birds)**	**Group 3 (20 birds)**
Infected, untreated (control)	Infected, continuous-dosing treated	Infected, pulse-dosing treated

Clinical follow-up. All animals were measured once daily, and examined twice daily (12 hours interval) before and during the onset of the experiment. Every day until day 14, the last day of the experiment, the animals were inspected individually for typical signs of fowl cholera, clinical signs were scored, and mortality was recorded. Five clinical signs characterizing fowl cholera were graded for their severity and expressed as the average of one bird showing lesions of each sign and was uttered for each examination time points. These included nasal discharge (1 point), diarrhea (1point), lameness (2 points), weakness (2 points), and moribund state (3 points).

Postmortem examination. All birds died during the experiments were necropsied. The survivers were euthanasied and necropsied at day 14 post infection. A detailed examination was performed on the presence or absence of lesions of pasteurellosis by conventional postmortem examination. Samples were taken from liver, lungs, heart, spleen, bone marrow, joint, brain and the pectoral muscle of inoculation. Postmortem signs characterizing fowl cholera were scored for their severity and expressed as a score for each individual bird. If a bird had more then one lesion, the scores were summed up. These included: inflammation of the spleen (4 points), subepicardial petechia (4 points), swollen liver (4 points), necrotized foci in liver (4 points), acute, hemorrhagic, catarrhal enteritis (4 points), necrotic, fibrous enteritis (2 points), fibroid exudates in peritoneum (2 points), fibrous pleuritis (2 points), croup pneumonia (2 points), fibrous arthritis (2 points) and leptomeningitis (2 points).

Reisolation and identification of Pasteurella. The samples taken from the postmortem examination (liver, lungs, heart, spleen, bone narrow, joint, brain and the pectoral muscle of inoculation) were cultivated on 5% bovine blood TSA and dextrose-starch (DSA, DIFCO Labs., USA) agar for 18 hours at 37 ^{o}C. Growth occurring or negative culture results were recorded. The minimum inhibitory concentration (MIC) for

norfloxacin for both *Pasteurella multocida* isolates was determined using broth dilution method.

Reisolation (confluent growth/no growth)	+/-
Colony morphology (capsular/non capsular)	+/-
Capsular type (by Staphylococcus hyaluronidase)	A/-
Serotype (by Heddleston method-AGDP)	Origin

Statistical analysis. Data were analyzed statistically using paired Student's t-test of pathological variables, clinical signs and weight gain into sources that were functions of the design and treatment structure of the study. Differences due to treatment were examined by Chi square and Fisher's exact test procedures. Statistical significance was defined as $P \leq 0.05$.

The critical **t** was determined by the degree of freedom and statistical significance ($P \leq 0.05$),

-if the calculated t (T_{calc}) < critical t (T_{cri}), meant not significant,

-if the calculated t (T_{calc}) > critical t (T_{cri}), meant significant different.

RESULTS

Weight gain. Following infection with *Pasteurella multocida*, chick-inoculation treatment produced significantly higher weight gain in surviving birds, compared with unmedicated birds (Group 2 T_{calc}: 2.17, Group 3 T_{calc}: 2.1 T_{cri}: 2.09). There were no significant differences in weight gain among treatment Group 2 and 3 in chickens (T_{calc}: 0.42 T_{cri}: 2.09). In the turkey-inoculation test there were no significant divergence between Group 1, Group 2 and 3 in turkeys.

Table 2 and table 3 respectively show the cumulated base data of chicken and turkey inoculation experiment.

Clinical signs. Severe pasteurellosis was induced first in the unmedicated birds after inoculation of the birds with either *Pasteurella multocida* X-73 (A:1) or *Pasteurella multocida* P-1059 (A:3) isolates, later some medicated birds showed signs as well. Clinical signs were first seen in both experiments in the control, Group 1 within 24 hours after inoculation. They included depression and listlessness, diarrhea and severe weakness. The most severe clinical signs were observed between 36 and 72 hours post infection. These included a drop in water and food consumption, nasal discharge, depression, diarrhea, weakness, recumbency and moribund status.

Table 2. Cumulated base data of chicken inoculation experiment. (10 weeks old broiler chickens).

	1. Group Control	2. Group Continuous dosing	3. Group Pulse dosing
No of animals	20	20	20
Inoculation with *P. multocida* (cfu)	80	80	80
Treatment (norfloxacin)	No	100 mg/l in drinking water	15 mg/b.w. in drinking water
Daily weight gain (kg) [1]	-0.13±0.24	0.10±0.07	0.09±0.04
Average daily clinical score [2]	1.71±1.34	0.12±0.06	0.37±0.20
Birds dying during experiment	20	0	3
Post mortem score [3]	20.95 ±1.81	0.20±0.62	2.40±4.71
No of bacteriology reisolate	117	0	20
Original *P. multocida* serotype	110	0	20
Average daily drug intake during treatment (mg/kg bodyweight)	0	18.23	15

1 Total daily mean weight gain/number of days.
2 Total daily mean clinical score/number of days.
3 Total post mortem score/number of birds.
cfu, colony-forming units.

Table 3. Cumulated base data of turkey inoculation experiment (8 weeks old turkeys).

	1. Group Control	2. Group Continuous dosing	3. Group Pulse dosing
No of animals	20	20	20
Inoculation with *P. multocida* (cfu)	70	70	70
Treatment (norfloxacin)	No	100 mg/l in drinking water	15 mg/b.w. in drinking water
Daily weight gain (kg) [1]	0.01±0.07	0.04±0.09	0.06±0.03
Average daily clinical score [2]	1.11±0.97	0.43±0.31	0.08±0.12
Birds dying during experiment	20	15	3
Post mortem score [3]	18.30±2.77	14.95±7.53	3.00±6.07
No of bacteriology reisolate	117	100	20
Original *P. multocida* serotype	117	100	20
Average daily drug intake during treatment (mg/kg bodyweight)	0	15.50	15

[1] Total daily mean weight gain/number of days.
[2] Total daily mean clinical score/number of days.
[3] Total post mortem score/number of birds.
cfu, colony-forming units.

In the chick-inoculation experiment, significant differences in the systemic signs were obtained among experimental groups from day 1 through day 14 post infection. Average daily clinical signs were significantly more severe in Group 1 and slightly in Group 3 (T_{calc}: 3.29 T_{cri}: 2.00) than in Group 2 (T_{calc}: 3.75 T_{cri}: 2.00). Group 2 and 3 also differed significantly (T_{calc}: 6.62 T_{cri}: 2.00).

In the turkey-inoculation experiment, significant difference was observed between Group 1 and Group 3 (T_{calc}: 2.89 T_{cri}: 2.00) and there was significant correlation between Group 1 and Group 2 (T_{calc}: 2.06 T_{cri}: 2.00). Group 2 and 3 also altered significantly (T_{calc}: 5.29 T_{cri}: 2.00).

Mortality. Mortality rates attributable to *Pasteurella multocida* during the chick-inoculation experiment in the unmedicated, control group (Group 1) were 100%, in Group 3 were 15% ($p<0.001$) and in Group 2 were 0% ($p<0.001$). In the turkey-inoculation experiment the mortality rate for Group 1 was 100%, Group 2 was 80% ($p=0.53$) and in Group 3 15% ($p<0.001$). In both experiments, norfloxacin significantly reduced mortality. However, during the chick-inoculation experiment the continuous-dosing seemed more valuable, in the turkey-inoculation experiment, the pulse-dose regimen was proved to be superior.

Postmortem examination. In all mortalities during the first 5 days after infection, inflammation of the spleen, subepicardial petechia, hemorrhages throughout the body, swollen liver, necrotized foci in liver, acute, hemorrhagic, catarrhal enteritis and severe necrotic pectoral muscle infection was observed. Mortalities after day 6, necrotic, fibrous enteritis, fibroid exudates in peritoneum, fibrous pleuritis, croup pneumonia, fibrous arthritis and leptomeningitis were witnessed. In surviving chickens in Group 2, only 2, in Group 3, only 3 animals showed mild signs of illness. In surviving turkeys in Group 2 almost all birds showed signs of infection, where in Group 3 only 2 animals demonstrated mild lesions. There was significant alteration between all three groups in the experiment with broiler chickens, where in the turkey-inoculation test there was no significant difference between Group 1 and Group 2 (T_{calc}: 1.84 T_{cri}: 2.02), but Group 1 and Group 3 (T_{calc}: 10.25 T_{cri}: 2.02) and Group 2 and Group 3 (T_{calc}: 5.51 T_{cri}: 2.02) altered significantly.

Recovery of Pasteurella multocida. Attempts to reisolate *Pasteurella multocida* were carried out. The original strains of *Pasteurella multocida* were reisolated from all dead birds. From surviving birds, reisolation was successful only in turkeys, where 1 animal had *Pasteurella multocida* in 1 organ from both Group 2 and 3. There was one case of insignificant data in the chicken-inoculation experiment because of Proteus overgrowth in Group 1 (Control). All strains reisolated were identified as the same strain of *Pasteurella multocida* as was used for infection.

Water consumption, drug intake. There was no significant difference in the average daily water consumption between Group 1, 2 and 3, however birds in Group 1

drank slightly less unmedicated water. There was also non-significant alteration in average daily drug intake among chickens and turkeys in Group 2 and 3.

MIC. Both *Pasteurella multocida* strains that were used for infection were susceptible for norfloxacin with isolates inhibited by modal MIC 0.16 μg/ml. All reisolated strains were also sensitive for norfloxacin with modal MIC 0.32 μg/ml. There was a marked decrease of susceptibility observed.

DISCUSSION

The advantage of induced disease models for evaluating the comparative efficacy of different dosing regimen has not been well documented for poultry. In the present study, intramuscular injection of chickens and turkeys with *Pasteurella multocida*, showed to be a reliable and reproducible method for inducing fowl cholera. The clinical signs that developed as a result of *Pasteurella multocida* infection were typical of fowl cholera (Rimler and Glisson, 1997), and by using them as indices; we were able to evaluate the efficacy of continuous- and pulse-dose treatment with norfloxacin. The preventive effect of norfloxacin therapy on intense reduction of mortality, morbidity, clinical- and postmortem scores was considered to be an index of efficacy. Although norfloxacin was first introduced to treat other diseases (Gootz, 1990) it was effective against fowl cholera in chickens and turkeys in the present study. Laczay and Semjén (Laczay et al., 1998) showed that norfloxacin was rapidly absorbed from the intestinal tract of chickens and turkeys, and its mean peak plasma concentrations exceeded the MIC for most avian pathogens. It is generally accepted that fluoroquinolones act in a concentration-dependent manner (Raemdonck et al., 1992, Schentag et al., 1993). Recent findings (Madaras-Kelly et al., 1996) indicate that the ratio of the area under the drug concentration-time curve (AUC) to the MIC (AUC/MIC), which quantifies the intensity of exposure of the infectious agent to the antimicrobial compound, is the most descriptive pharmacodynamic predictor of the antimicrobial activities of fluoroquinolone antimicrobial agents. In birds, both the magnitude of exposure (peak concentration) and the duration of exposure (time above the MIC) are important for an optimal antibacterial effect. Meinen et al. (1995) showed that the total dose of Enrofloxacin rather then the frequency of dosing was significant in determining drug efficacy.

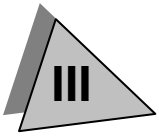
Administration of drugs in drinking water is by far the most flexible way to stop or rapidly change a therapy in commercial poultry production. Norfloxacin was offered to the chickens and turkeys in the drinking water at dose ranges based on earlier pharmacokinetic

baseline data (Laczay et al., 1998). The drug was applied according to the continuous- and pulse dosing method because of convenience for the poultry industry and because we intended to evaluate how continuous dosing and pulse dosing interfere with clinical efficacy against fowl cholera. The results from this study indicate that under practice conditions, fluoroquinolones can be applied in the drinking water in a flexible manner without compromising efficacy. This is important when considering the variety of husbandry conditions in the field and the consequent access to drinking water.

Norfloxacin administered in the water at 100 mg/L, continuous-dosing was as efficacious as norfloxacin administered in the water at 15 mg/b.w. pulse-dosing in chickens. There was no difference in daily weight gain, slight variation in mortality, but well characterized alteration in postmortem scores, daily clinical scores and recovery of bacteria. It should be considered that the inoculum was approximately 80 CFU X-73 (A:1), close to its LD_{50} (19.6 CFU) value. In turkeys, where 15 fold (approximately 70 CFU P-1059 (A:3)) of the LD_{50} (4.67 CFU) was used, significant alteration in all examined parameters was observed, except average daily weight gain, between the two different treatment schedule. We can proclaim that continuous dosing in chickens and pulse dosing in turkeys were significantly more valuable in treating *Pasteurella multocida* infection in the present study. These results were exactly the opposite as was expected by the pharmacokinetic properties described by Laczay and Semjén (Laczay et al., 1998).

Some previous studies indicated that even with an 8 hour lasting PAE (Neu et al., 1987) the fluoroquinolone treatment gives better result using as a continuous medication in drinking water, while others showed that severe bacterial infections are better treated with high dosages of antimicrobials (Lublin et al., 1993, Meinen et al., 1995). These results confirm our findings that low volume bacterial infection, 4 fold over the LD_{50}, improves with continuous-dosing, while severe bacterial infection, 15 fold over the LD_{50}, improves following a pulse-dosing schedule.

It can be suggested that norfloxacin therapy is an excellent choice in the treatment of fowl cholera, due to the broad and rapid action of the drug against the etiological agent, *Pasteurella multocida*. Although significant differences in clinical or pathological features of fowl cholera were found by comparing the two dosing regimen, we are not in a position to prove either administration method supremacy. Additional studies are required for elucidating the effect of dosing regimen in bacterial infections.



X. PULSE AND CONTINUOUS ORAL NORFLOXACIN TREATMENT OF EXPERIMENTALLY INDUCED ESCHERICHIA COLI INFECTION IN BROILER CHICKS AND TURKEY POULTS[3]

SUMMARY

Experimental colibacillosis was produced in 40, healthy, 7-day-old broiler chickens and turkeys by intratracheal injection of $1X10^8$ cfu/chick and $1.23X10^9$ cfu/poult bacteria of an O1:F11 strain of *Escherichia coli*, respectively. Two days before *E. coli* challenge all chicks were vaccinated with a live attenuated strain of bronchitis virus (H-52). This model of infection – at least in chicken - proved to be useful for evaluating the efficacy of antimicrobial medication, by recording mortality, weight gain, pathological alterations and frequency of reisolation of *E. coli*. Using this model, the efficacy of two different dosing methods of norfloxacin (continuous and pulse dosing) was evaluated. The once-per day pulse dosing of norfloxacin administered to drinking water at 15 mg/kg b.w. proved to be more efficacious than the continuous dosing method of 100 mg/L for 5 days in chickens, while there were no convincing differences between the two treatment regimens in turkeys. Results confirmed earlier observations on pharmacokinetic properties of norfloxacin in chicks and turkeys (Laczay et al., 1998).

Key words: norfloxacin, *E. coli*, oral treatment, continuous dosing, pulse dosing

INTRODUCTION

Colibacillosis, caused by *Escherichia coli* (*E. coli*), is a bacterial infection in chickens and turkeys may result in septicemia, respiratory tract infections, pericarditis, peritonitis and airsacculitis. *E. coli* may also be associated with other agents, such as infectious bronchitis virus (IBV), Newcastle disease virus (NDV) including vaccine strains, *Mycoplasma spp*., *Pasteurella spp*., causing the respiratory disease complex (Barnes and Gross, 1997). Although this disease is related with a number of pathogens, infection with *E. coli* is of particular concern because it commonly progresses to a more generalized condition associated with high mortality and condemnation losses at processing. Stress, exposure to poultry house dust, and ammonia provide additional

[3] This study was published in the *Acta Veterinaria Hungarica*, 2002, **153**: 199-210.

predisposing factors that may result in damage to the mucosal lining of the respiratory epithelium, and thus they contribute to the invasion of *E. coli* (Barnes and Gross, 1997). To help control the mortality associated with *E. coli* infections, certain fluoroquinolones – such as norfloxacin - have been approved for use in poultry.

Norfloxacin is a third generation fluoroquinolone that was first introduced for treating urinary tract infections in humans (Gootz, 1990). This drug is more potent than any earlier analogues, has a broad spectrum of activity and induces drug resistant bacteria less frequently (Wolfson and Hooper, 1988). Like other fluoroquinolones, norfloxacin acts by inhibiting bacterial DNA gyrase, which is responsible for the negative supercoiling of the DNA controlling replication, transcription and recombination (Reece and Maxwell, 1991, Hooper and Wolfson, 1993). The most attractive features of the drug are good absorption when given orally, and maintenance of effective serum and tissue levels against a broad range of pathogenic bacteria causing systemic infections (Wise, 1984).

The bactericidal effect of fluoroquinolones depends on rather the concentration of the drug than the time of exposure and they exert a marked post-antimicrobial effect (PAE) on growth of either sensitive or resistant organisms for up to 8 hours (Neu et al., 1987). Therefore, a pulse-dose regimen of fluoroquinolones has been suggested with the aim of achieving high peak concentrations and exploiting the PAE. This suggestion, together with the importance of *E. coli* as a poultry pathogen, led us to compare the efficacy of pulse-dosing oral norfloxacin treatment with that of an established medication of continuous-dosing, in the control of experimental colibacillosis induced in broiler chickens and turkeys by intratracheal injection.

MATERIALS AND METHOD

Escherichia coli. *E. coli* strain 260 (O1:F11) was used for infection of both chickens and turkeys. It was isolated from the bone marrow of a day old chick in Hungary and was provided by Prof. Dr. Béla Nagy (Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary), and it was sensitive to enrofloxacin in vitro. The bacteria of this *E. coli* strain were propagated in Brain Heart Infusion (BHI, DIFCO Labs., USA) for 18 hours at 37 ^{o}C in stationary culture. The minimum inhibitory concentration (MIC) of norfloxacin for *E. coli* isolate was determined using broth dilution method, and was found to be 0.02 μg/ml.

Poultry. Forty, clinically healthy, 7-day-old Ross breed broiler chickens (Prophyl Ltd, Mohács, Hungary), and forty, clinically healthy 7-day-old Arbor breed turkeys

(Prophyl Ltd, Mohács, Hungary) were allotted into three experimental groups: infected and untreated controls (n=10, Group 1); infected and continuous-dosing treated with norfloxacin (n=15, Group 2); infected and pulse-dosing treated with norfloxacin (n=15, Group 3). During the experimental period, the groups were placed in separate pens and were provided with broiler or turkey starter rations ad libitum and water according to the experimental design.

Infection procedure. After a 3-day acclimatization period all birds were infected as follows. In order to make chickens more susceptible to the *E. coli* infection, 2 days prior the infection, all broiler chickens were vaccinated through drinking water, according to the manufacturer's recommendation, with Bronchovac II (Ceva-Phylaxia) containing infectious bronchitis virus H-52 attenuated strain. Turkey poults did not receive any vaccine prior to infection. For infection, the overnight culture of *E. coli* strain 260 in BHI infusion was used. Each bird in all groups was injected with 0.2 ml broth containing $1X10^8$ cfu for chickens and $1{,}23X10^9$ cfu for turkeys, respectively. The infection was performed intratracheally with a sleeve of polyethylene tubing of a 23G cannula. At the same time, the inoculum was cultured on blood agar for enumeration and confirmation of identity and viability of the bacteria.

Treatment regimen. Norfloxacin, as base (Vetriflox 200 Oral Solution A.U.V., Registration No.: 655/1996, Ceva-Phylaxia), was given to Group 2 and 3 in drinking water for 5 days, starting on the day of infection. Group 2 received continuous medication throughout the 5-day period; drug was dissolved in drinking water (100 mg/L) and was available for birds ad libitum. In Group 3 (receiving once-per-day pulse-dose) drinking water was withheld for 2 hours; thereafter, the calculated daily dose (15 mg/kg b.w.) of the drug was mixed with one-forth of the daily water requirement of the group. Four hours later the birds received drug-free drinking water ad libitum. The body weight of animals was measured each day and the dosage was adjusted accordingly. Table 1 shows the experimental design.

Table 1: Experimental design in:

	Group 1 (10 birds)	**Group 2 (15 birds)**	**Group 3 (15 birds)**
Chickens	Vaccinated, infected, untreated (control)	Vaccinated, infected, continuous-dosing treated	Vaccinated, infected, pulse-dosing treated
Turkeys	Infected, untreated (control)	Infected, continuous-dosing treated	Infected, pulse-dosing treated

Clinical follow-up. During the acclimatization period (3 days) and experimental phase (7 days from the infection) all animals were examined twice a day with 12 hours intervals. Clinical signs were scored, and mortality was recorded. During the experimental phase animals were inspected individually for signs considered to be characteristic to colibacillosis. Clinical signs were scored for their severity and expressed as the average daily clinical score of one bird. These included nasal discharge (1 point), diarrhoea (1 point), lameness (2 points), weakness (2 points) and moribund state (3 points).

Postmortem examination. All birds that died during the experiments were necropsied and examined for gross lesions. All birds remained alive were euthanasied and necropsied at day 8 post infection. A detailed examination was performed for the presence or absence of lesions of colibacillosis by conventional postmortem examination. Samples were taken from liver, lungs, heart, spleen, bone marrow and the air sac for bacteriological investigation. Postmortem signs characteristic to colibacillosis were scored for their severity and expressed as the average of one bird for each sign. These included: acute, hemorrhagic, catarrhal enteritis (4 points), fibrous polyserositis (4 points), fibroid exudate on peritoneum (4 points), necrotic, fibrous enteritis (2 points), necrotic foci in the liver (2 points) and necrotic foci in the spleen (2 points).

Reisolation and identification of Escherichia coli. The samples taken during the postmortem examination (liver, lungs, heart, spleen, bone marrow and the air sac) were cultivated on blood and dextrose-starch agar (DSA, DIFCO Labs., USA) for 18 hours at 37 ^{o}C and the results were recorded. One discrete colony of the bacterial growth was transferred onto an agar slant for further identification. This included biochemical confirmation and detection of F11 antigen by slide agglutination. The minimum inhibitory concentration (MIC) for norfloxacin for *E. coli* isolates was determined using broth dilution method.

Statistical analysis. Data were analysed statistically using paired Student's t-test of pathological variables, clinical signs and weight gain into sources that were functions of the design and treatment structure of the study. Differences due to treatment were examined by Chi square and Fisher's exact test procedures. Statistical significance was defined as $P \leq 0.05$.

The critical t was determined by the degree of freedom and statistical significance ($P \leq 0.05$),

-if the calculated t (T_{calc}) < critical t (T_{cri}), meant not significant,

-if the calculated t (T_{calc}) > critical t (T_{cri}), meant significant difference.

RESULTS

Clinical signs. During the adaptation period the animals did not show any clinical symptoms. Inoculation of birds with the *E. coli* strain 260 (O1:F11) induced severe colibacillosis in infected and unmedicated control chicks pre-treated with infectious bronchitis vaccine (Group 1). Clinical signs of depression and listlessness, diarrhoea and severe weakness were developed within 24 hours after inoculation. The most severe clinical signs were observed at 48 hours post infection. These included a drop in water and food consumption, nasal discharge, depression, diarrhoea, weakness, recumbency and moribund status. Severe clinical signs were absent in all treated groups (Group 2, Group 3) in both experiments.

In the chick trial, significant differences were obtained among groups from day 1 through day 7, post infection. Average daily clinical signs were significantly more severe in Group 1 than in Group 2 (T_{calc}: 4.2 T_{cri}: 2.05) and Group 3 (T_{calc}: 4.02 T_{cri}: 2.05), respectively. Group 2 also differed significantly from Group 3 (T_{calc}: 2.1 T_{cri}: 2.05).

In turkeys, infected and unmedicated control (Group 1) showed mild clinical signs between 24 and 72 hours post infection. They included depression and diarrhoea. Significant difference was observed between Group 1 and Group 2 (T_{calc}: 2.12 T_{cri}: 2.04) and 3 (T_{calc}: 2.07 T_{cri}: 2.04) while there was a non-significant difference between Group 2 and Group 3 (T_{calc}:0. 41 T_{cri}: 2.04).

Tables 2 and 3 show the cumulated base data of chicken and turkey inoculation experiments, respectively.

Weight gain. Following infection with *E. coli*, both treatments produced significantly higher weight gain in surviving broiler chickens, compared with unmedicated birds (Group 2 T_{calc}: 2.9, Group 3 T_{calc}: 2.51; T_{cri}: 2.18). There was no significant difference in weight gain among treated Group 2 and 3 in chickens (T_{calc}: 0.22 T_{cri}: 2.18).

There was no significant divergence between Group 2 and 3 in turkeys (T_{calc}: 0.55 T_{cri}: 2.18) and there was also no significant difference between the control and the medicated turkey poults (Group 2 T_{calc}: 0.92, Group 3 T_{calc}: 1.23; T_{cri}: 2.18).

Mortality. Mortality rate attributable to *E. coli* during the chick-inoculation experiment in the unmedicated, control group (Group 1) was 40%, while in treated groups none of the birds died.

In the turkey experiment 10% of birds in Group 1 died due to the infection with *E. coli* O1:F11 but losses attributable to colibacillosis did not occur in treated groups. However, 3 animals (20%) died in Group 2 due to intercurrent disease (lesions could not

Table 2. Cumulated base data of chicken inoculation experiment (7 days old broiler chickens).

	Group 1 Control	Group 2 Continuous dosing	Group 3 Pulse dosing
No of animals	10	15	15
Inoculation with *E. coli* (cfu)	$1x10^8$	$1x10^8$	$1x10^8$
Vaccination with Bronchovac II	Yes	Yes	Yes
Treatment	No	100 mg/l in drinking water	15 mg/kg b.w. in drinking water
Daily weight gain (g) [1]	12.40±6.32*	23.48±9.50	22.52±6.71
Average daily clinical score [2]	1.77±1.27*	0.25±0.11**	0.18±0.08**
Birds died during experiment	4*	0	0
Post mortem score [3]	9.00±4.57*	1.67±2.32	0.87±1.55
No of samples with coliform isolate (total No of samples)	40 (60)*	11 (90)	16 (90)
Number of samples with F11 *E. coli* serotype	40*	0	0
Average daily drug uptake during treatment (mg/kg b.w.)	0	17.49	15

[1] Total daily mean weight gain/number of days
[2] Total daily mean clinical score/number of days
[3] Total post mortem score/number of birds
* Significant difference between control (Group 1) versus treated (Group 2 and 3) groups
** Significant difference between Group 2 versus Group 3

Table 3. Cumulated base data of turkey inoculation experiment (7 days old turkey poults).

	Group 1 Control	Group 2 Continuous dosing	Group 3 Pulse dosing
No of animals	10	15	15
Inoculation (cfu)	$1.23X10^9$	$1.23X10^9$	$1.23X10^9$
Vaccination	No	No	No
Treatment	No	100 mg/l in drinking water	15 mg/kg b.w. in drinking water
Daily weight gain (g) [1]	17.74±6.13	22.54±9.07	20.46±5.75
Average daily clinical score [2]	0.26±0.18*	0.14±0.15	0.16±0.07
Birds died during experiment	1	3[a]	0
Post mortem score [3]	2.70±3.27	1.13±1.64	0.47±1.06
No of samples with coliform isolate (total samples)	9 (60)	3 (90)	3 (90)
Number of samples with F11 *E. coli* serotype	9	3	3
Average daily drug uptake during treatment (mg/kg b.w.)	0	18.50	15

[a] These 3 birds died due to intercurrent disease.
[1] Total daily mean weight gain/number of days
[2] Total daily mean clinical score/number of days
[3] Total post mortem score/number of birds
* Significant difference between control (Group 1) versus treated (Group 2 and 3) groups

been found, though coliform colonies were isolated from the lung of one bird and from the air sac from another bird, these colonies did not give positive agglutination with F11+ antiserum; the third bird was bacteriologically negative).

Postmortem examination. Fibroid exudate on peritoneum, fibrinous polyserositis, necrotic-inflamed foci in liver, acute hemorrhagic-catarrhal enteritis were observed in all chickens and poults died in Group 1.

In surviving chickens in Group 2, only 5, in Group 3, only 2 animals showed mild lesions of diarrhoea and tracheitis. There was no significant difference between Group 2 and 3 (T_{calc}: 1.11 T_{cri}: 2.05).

In surviving turkeys in Group 2, only 5 birds showed signs of infection, where in Group 3 only three animals showed mild lesions, diarrhoea and catarrhal enteritis. Three birds in Group 2 died without showing any clinical signs, due to intercurrent disease. There was no significant alteration between Group1, 2 and 3 (T_{calc}: 1.36 T_{cri}: 2.05).

Recovery of *Escherichia coli.* Samples were taken from liver, lungs, heart, spleen, bone marrow and the air sac for inoculation from each bird. In the chick-inoculation experiment in the non-medicated, control group (Group 1), bacterial growth was obtained from 44 samples (total of 60 samples) out of which 40 colonies were classified as coliform. All 40 strains were identified as the F11+ strain of *E. coli*. In Group 2, bacteria were isolated from 18 samples, the yield was a few colonies of coliform bacterium in 11 samples (total of 90 samples). In Group 3, bacteria were isolated from 18 samples, coliform growth was found in 16 samples (total of 90 samples), but none of coliforms in Group 2 or 3 was identified as the F11+ strain as was used for infection. The remainder strains from Group 2 were haemolytic Gram-positive cocci, mixed cultures and in 3 chickens S. enteritidis growth was isolated from the spleen.

In the turkey-inoculation experiment, the F11+ strain of *E. coli* was reisolated from the bird that died in Group 1. The F11+ *E. coli* was also recovered from some birds in infected and treated groups as well. In exterminated birds of Group 1 we found 9 samples (total of 60 samples) where F11+ strain of *E. coli* growth occurred. In both Group 2 and 3, where bacterial growth was isolated from 10 and 8 samples, respectively, there were 3 samples (total of 90 samples) with F11+ *E. coli* growth.

Water consumption, drug intake. There was no significant difference in the average daily water consumption between Group 1, 2 and 3, however birds in Group 1 drank slightly less water. There was also non-significant alteration in average daily drug intake among chickens and turkeys in Group 2 and 3(Tables 2 and 3).

MIC. The *E. coli* used for infection was susceptible for norfloxacin (MIC 0.02 µg/ml). All reisolated strains were also sensitive for norfloxacin with modal MIC of 0.04 µg/ml.

DISCUSSION

Because a high rate of antibiotic resistance in avian isolates of *E. coli* has been reported in different studies and because there may be differences between in vitro and in vivo antibiotic susceptibility (Erganis et al., 1989, Premkumar et al., 1991, Allan et al., 1993), it is important to evaluate the therapeutic efficacy of new antimicrobial agents. Among these new compounds, norfloxacin is known as an effective antimicrobial agent against *E.* coli. Indeed it was effective against colibacillosis in chickens and turkeys in the present study. Laczay et al. (1998) showed that norfloxacin was rapidly absorbed from the intestinal tract of chickens and turkeys, and its mean peak plasma concentrations exceeded the MIC for most avian pathogens. It is generally accepted that fluoroquinolones act in a concentration-dependent manner (Raemdonck et al., 1992, Schentag et al., 1993). Recent findings (Madaras-Kelly et al., 1996) indicate that the ratio of the area under the drug concentration-time curve (AUC) to the MIC (AUC/MIC), which quantifies the intensity of exposure of the infectious agent to the antimicrobial compound, is the most descriptive pharmacodynamic predictor of the antimicrobial activities of fluoroquinolone antimicrobial agents. In birds both the magnitude of exposure (peak concentration) and the duration of exposure (time above the MIC) are important for an optimal antibacterial effect. Meinen et al. (1995) showed that the total dose of enrofloxacin rather then the frequency of dosing was significant in determining drug efficacy.

Another advantage of norfloxacin is its broad spectrum of antibacterial activity, which encompasses *E. coli*, *Salmonella*, *Klebsiella*, *Pasteurella*, *Yersinia*, *Haemophilus* and *Mycoplasma spp.* (Veere et al., 1996, Prasad et al., 1997).

Administration of drugs in drinking water is by far the most flexible way to stop or rapidly change a therapy in commercial poultry production. Norfloxacin was offered to the chickens and turkeys in the drinking water at dose ranges based on earlier pharmacokinetic baseline data (Lublin et al., 1993). In our experiments the drug was applied using the continuous- and pulse dosing methods. The results of this study confirmed that fluoroquinolones could be applied in the drinking water in a flexible manner without compromising efficacy. This is important when considering the variety of husbandry conditions in the field and the consequent access to drinking water.

The usefulness of *E. coli* induced disease models for evaluating the comparative efficacy of *different dosing* regimen has not been well documented for poultry. In the present study, intratracheal infection of IB-pre-treated chickens with the pathogenic strain of *E. coli* 260 (O1:F11), proved to be a reliable method for inducing *E. coli* infection. The clinical signs that developed as a result of *E. coli* infection were typical of colibacillosis (Barnes and Gross, 1997), and by using them as indices of colibacillosis; we were able to evaluate the efficacy of continuous- and pulse-dose treatment with norfloxacin in chickens. The effect of norfloxacin therapy on intense reduction of mortality, morbidity, clinical- and postmortem scores and recovery of challenge strains of *E. coli* was considered to be an index of efficacy.

The *E. coli* model used in the chick-inoculation experiment produced 40% mortality and 1.77±1.27 daily clinical score (60% morbidity) in infected, non-medicated birds. In an unpublished comparative study with norfloxacin we found that the same model as described above with $5X10^5$ CFU of *E. coli* (freshly isolated from chicken with colibacillosis, not-typed) created 20% mortality and 60% morbidity. In a recently published study with difloxacin and enrofloxacin, where the chickens received $2.5X10^9$ CFU of *E. coli* 260 (O1:F11+), the mortality was 83.3% and morbidity 100% (Sárközy and Laczay, 2001). These levels compare favourably to the 5% mortality and up to 50% morbidity suggested by Wray et al. (1996) as being typical for colibacillosis in the field. Dunnington et al. (1991) showed a mortality of 5% for chickens challenged with less then 10^4 CFU of *E. coli*, but this increased to 50% when the challenge dose was increased to 10^6 CFU. In the model of Charleston et al. (1998) the 43.5% mortality was related to the high, 10^6 CFU challenge dose. To enable comparisons of efficacy between dosing regimens with sensible numbers of birds, experimental models must produce pronounced disease otherwise large number of replicates must be used to detect differences between treatments. In the present study, both treatment procedures effectively reduced the experimental colibacillosis in chickens. Norfloxacin administered in the water at 15 mg/kg b.w. pulse dosing was more efficacious then norfloxacin administered in the water at 100 mg/L, continuous-dosing in chickens. There was no variation in daily weight gain and mortality, but well characterized alteration in daily clinical scores, postmortem scores for the benefit of pulse dosing. The results obtained in this study coincide with the conclusion from the pharmacokinetic properties described by Laczay et al. (1998).

Earlier studies indicated that even with an 8 hour lasting PAE (Neu et al., 1987) the fluoroquinolone treatment gives better result using as a continuous medication in drinking

water. Other studies showed that severe bacterial infections are better treated with high dosages of antimicrobials (Andon et al., 1993). These differences can be explained if we assume that low-level bacterial infection gives better recovery with continuous-dosing, while severe bacterial infection shows better improvement following pulse-dosing schedule.

The intratracheal injection of intact turkeys with *E. coli* was demonstrated to be a reasonable method for inducing colibacillosis (Barnes and Gross, 1997). The effect of norfloxacin therapy on reduction of clinical scores and recovery of the challenge strain of *E. coli* was assumed to be an indicator of efficacy. However, in our study the use of intact turkeys did not prove to be a good model for experimental colibacillosis. Consequently it could not be utilised for assessing efficacy of different norfloxacin treatment regimes. In order to improve this model it seems to be necessary to apply pneumovirus infection, as recently done by Van de Zande et al. (2001).

In conclusion, the results obtained from this study confirm that norfloxacin is an effective drug for the treatment of colibacillosis in chickens. The results also verified the recommended dose level of the compound. Although significant differences in clinical or pathological features of colibacillosis were found by comparing the two dosing regimen, yet we are not in the position to prove either administration method supremacy. Additional studies are required to improve our model for colibacillosis in turkey and to compare different norfloxacin treatment regimes.

IV

XI. PHARMACOKINETICS OF NORFLOXACIN IN BROILERS AND TURKEYS AFTER DIFFERENT METHODS OF ORAL ADMINISTRATION [4]

SUMMARY

Norfloxacin was administered to 2 groups of chickens and turkeys (6 birds/group) at a dose of 15 mg/kg b.w., as pulse dosing and 100 mg/L as continuous dosing for 5 consecutive days in drinking water. Blood samples were taken serially. Plasma norfloxacin concentrations were determined by using high-performance liquid chromatography and the obtained data were compared with published pharmacokinetic data.

The plasma norfloxacin concentrations increased slowly during continuous dosing and reached the MIC_{90} (250 ng/ml) for the most Gram-negative pathogen bacteria by 12 h in chickens and 18 h in turkeys. The mean steady-state plasma concentration was also attained in 36 h. It remained approximately at the same plasma concentration level both in chickens and turkeys (776.67±33.23 ng/ml in chickens and 682.50±28.55 ng/ml in turkeys) during the whole treatment period. Pulse dosing produced half the steady-state concentration (365.32±39.31 ng/ml in chickens and 306.03±32.26 ng/ml in turkeys) as it was obtained after continuous-dosing.

After pulse dosing the plasma norfloxacin concentrations increased rapidly and significantly exceeded the MIC_{90} at 2 h in both chickens and turkeys and remained above for 8 h in chickens and 6 h in turkeys. Data of the daily pulse dosing suggested that every administration of the drug corresponds to a single, daily repeated bolus administration. However pulse dosing achieved higher plasma concentration more readily then continuous dosing.

Administration strategies such as continuous dosing at 100 mg/L or pulse dosing medication at 15 mg/kg b.w., indicated rational means of administering fluoroquinolones in herd situations, however, we recommend to treat bacterial infections of either high or

[4] This study will be published in the *Journal of Veterinary Pharmacology and Toxicology*, (presented for publication).

low pathogenicity starting with pulse dosing for 4 h and then maintaining continuous oral medication for 3-5 consecutive days with the fluoroquinolones.

INTRODUCTION

Fluoroquinolones, such as norfloxacin, have been used extensively in Hungary both as oral and injectable formulations for many domestic animals. This agent has been available to veterinarians for ten years. Norfloxacin is highly active against aerobic and facultative anaerobic Gram-negative bacilli. It has been shown to be concentration dependent in its rate of killing and also has a post antibiotic effect (PAE) against most Gram-negative pathogens (Neu et al., 1987). In an animal infection model (Drusano et al., 1993) once daily administration of a dose that produced high peak concentration/minimal inhibitory concentration (MIC) ratio of greater then 10-20:1 resulted in significantly better survival in neutropenic rats then did regimens in which the same dosage was used on a more fractionated schedule. Studies in patients (Forrest et al., 1993, Zhanel et al., 2001), demonstrated that the area under the inhibitory plasma concentration-time curve (AUIC = AUC/MIC) is also an important predictor of both clinical and microbiological cure.

It should be taken into account that antimicrobial therapy is an expression of the connection between pharmacokinetics and pharmacodynamics. The rational use of norfloxacin for the treatment of common infections in poultry requires detailed information on pharmacokinetic and pharmacodynamic properties of norfloxacin in birds to establish the orally administered dose necessary for maintaining bactericidal drug concentrations in the body. These properties, when combined with microbiological and pharmacokinetic data, provide the right tools needed for targeting the dosage of norfloxacin to birds on the basis of pharmacokinetics and the susceptibilities of the bacterial pathogens.

This information should be investigated to establish optimal therapeutic dose and dose interval as well as to compare the value of pulse and continuous oral administration. Optimizing one or both of these ratios may ultimately reduce the likelihood that microbial flora will develop resistance.

For this reason we studied the plasma disposition of norfloxacin after pulse and continuous oral administration in broiler chickens and turkeys.

MATERIALS AND METHODS

Poultry. 12 healthy, broiler chickens (Group 1 and 2) and 12 turkeys (Group 3 and 4), 7 weeks of age were used during the study. Arbor Acres broiler chickens and Big-6

turkeys were purchased from a poultry farm and housed in deep bedding in an experimental animal house. The body weights of chickens at the start of the experiment were 2085±206.7 g and turkeys 2676.6±200.1, respectively. Before the commencement of the experiment the animals were acclimatized for 1 week. The room temperature ranged between 20±2 ^{o}C and the relative humidity was maintained at 50-70 %. Commercial diets and water were provided ad libitum. The rations did not contain any drug or growth promoter.

Clinical follow-up. Before the onset of and throughout the examination the general health state of the animals were continuously monitored. All animals were weighted once per day, and examined every day during the onset of the experiment.

Experimental design. Norfloxacin, as base (Vetriflox 200 Oral Solution A.U.V., Registration No.: 655/1996, CEVA-Phylaxia), was given to Group 1, 2 3 and 4 in the drinking water for 5 days. Group 1 and 3 received continuous medication at a dose of 100 mg/L for 5 days; the drug was dissolved in drinking water and was available for birds ad libitum. In Group 2 and 4 drinking water was withheld for 2 h; thereafter, the entire amount of drug (15 mg/kg b.w.) was dissolved in one-forth volume of the daily water intake. Four hours later when the birds consumed the medicated water the animals received drinking water free from drug, ad libitum. The dosage was adjusted to the actual body weights of animals.

Sampling. In the case of the '0' schedule time point 5 ml, every other case 1,5 ml blood was drawn from each animal's wing vein into centrifugal (heparinised) tubes. During pulse dosing oral treatment, on the first day blood samples were drawn 6 times at 2, 4, 6, 8, 12 and 22 hours after the drug administration. Then, blood samples were taken 3 times a day, 2 hours prior the start of pulse-dosing treatment (22 hour) and 2 (6 hour) and 8 (12 hour) hours after the finishing of pulse dosing. In the course of continuous dosing plasma samples were taken every 12 hours after the start of the experiment. The withdrawn blood samples were centrifuged at room temperature for 10 minutes at 3000 rpm and the plasma samples were stored in deep freeze at -20 °C until analyzed. Plasma samples from both groups were assayed for norfloxacin.

Assay of Norfloxacin. Plasma norfloxacin concentrations were determined by reverse phase high-performance liquid chromatography (HPLC) with fluorescence detection. The applied method was based on those previously described by Anadón et al. (1992), Forchetti et al. (1984) and Nilsson-Ehle (1987). Briefly, the HPLC system was composed of a 510 pump (Waters, Milford, MA, USA), a Reodyne 7125 injector (Cotati,

Redwood, CA, USA), a 745B integrator (Waters) and a HP1046A fluorescence detector (Hewlett Packard, Waldbron, Germany). A Lichrosorb RP18 column (C_{18}, 5mn, 200x4.6mn; Hewlett Packard) was used for chromatographic separation. The mobile phase consisted of a mixture of acetonitril and bidistilled water containing 4.54g/l of KH_2PO_4, 5.94 g/l of Na_2HP_4 and 1.94g/l of (n-C_4H_9) $N^+HSO_4^-$ (10:90 v/v), ph 3.0, and flow rate was 1.0 ml/min. Fluorescence detection was performed at 25±2 ^oC by excitation at 280 nm and by monitoring the emission at a wavelength of 445 nm. The assay procedure was as follows: to 0.25ml of plasma 0.25ml of 0.5M sodium phosphate buffer (pH 7.5) and 10 ml methylene chloride were added. After shaking and centrifugation (2500 rpm, 10 min) the separated organic phase was evaporated under a nitrogen stream at 55 ^oC. The residue was redissolved in 0.5 ml mobile phase, centrifuged (2200 rpm, 10min) and 50ml was injected into the HPLC system. Norfloxacin plasma concentrations were quantified against calibration curves of plasma samples spiked with norfloxacin reference standard (Sigma, St. Louis, MO, USA).

The quantification limit was 0.002 mg/l and the standard curves were linear within the range of 0.002 and 2 mg/ml for plasma of chicken. The recovery rates were greater than 80 % for plasma of each animal tested. The intra- and inter-assay coefficients of variation at five different concentrations (0.002, 0.01, 0.1, 0.5 and 2 mg/ml) were less than 10 %. The method used was selective for the compound analyzed: endogenous interference was not observed on chromatograms.

Statistical analysis. Plasma concentration data were analyzed statistically using paired Student's t-test. Statistical significance was defined as $P \leq 0.05$.

The critical t was determined by the degree of freedom and statistical significance ($P \leq 0.05$),

-if the calculated t (T_{calc}) < critical t (T_{cri}), meant not significant,

-if the calculated t (T_{calc}) > critical t (T_{cri}), meant significant difference.

The plasma steady-state concentration was determined by AUC $(0,\infty)_{1st}$ /τ (tau-dosing interval) during pulse dosing using the equation of AUC $(0,\infty)_{1st}$ = AUC $(0,\tau)_{ss}$ and since AUC $(0,\tau)_{ss} = \tau \times C_{average}$, $C_{average}$ = AUC $(0,\infty)_{1st}/\tau$.

The other pharmacokinetic parameters, such as AUC $(0,\infty)_{1st}$, and AUC_{0-t} were calculated using Kinetica, Version 4.0.1. (InnaPhase Corporation) computer program.

RESULTS

The birds of both species readily consumed the medicated water each day during the treatment period.

The individual plasma norfloxacin concentrations during continuous dosing reached the steady-state concentration by 36 h after the start of treatment in both avian species and ranged between 710 and 820 ng/ml in chickens and 660 and 760 ng/ml in turkeys, respectively. The plasma concentrations rose relatively slowly in comparison with pulse dosing. Maximum, individual steady-state plasma concentrations varied between 790 and 870 ng/ml and 690 and 800 ng/ml in chickens and turkeys, with means of 835.0±28.81 and 750.0±37.42 ng/ml, respectively. Minimum steady-state plasma concentrations ranged between 650 and 790 ng/ml in chickens and 590 and 660 ng/ml in turkeys with means of 715.0±54.68 and 616.7±32.04, respectively. The average steady-state plasma concentration of norfloxacin was 776.7±33.23 ng/ml in chickens and 682.5±28.55 ng/ml in turkeys. Table 1 shows the corresponding data.

Table 1. Mean ± SEM plasma concentrations of norfloxacin obtained at various times after continuous dosing in chickens and turkeys.

Continuous treatment with 100 mg/L norfloxacin		
Time of sampling and parameters	Chickens	Turkeys
	Plasma norfloxacin concentrations (ng/ml)	
12 hours	245.8±18,55	180.8±17.44
24 hours	405.0±35,07	328.3±31.89
36 hours	773.3±45,02	700.0±40.49
48 hours	738.3±83,29	745.0±37.82
60 hours	800.0±65,12	703.3±52.41
72 hours	770.0±37,42	681.7±23.17
84 hours	771.7±30,61	668.3±42.15
96 hours	805.0±39,37	691.7±49.97
108 hours	791.7±26,39	646.7±42.27
120 hours	763.3±33,27	623.3±29.44
132 hours	300.8±18,00	237.5±15.41
144 hours	117.5±15,08	80.0±10.49
$Cpss_{max}$	835.0±28.81	750.0±37.42
$Cpss_{min}$	715.0±54.68	616.7±32.04
$Cpss_{average}$	776.7±33.23	682.5±28.55
Average daily norfloxacin intake	15.16 mg/kg b.w.	15.36 mg/kg b.w.

$Cpss_{min}$ = minimum steady state plasma concentration

$Cpss_{max}$ = maximum steady state plasma concentration

$Cpss_{average}$ = average steady state plasma concentration, mean of values of 36-120 hours

During pulse dosing the individual plasma norfloxacin concentrations at 2 h after the first administration ranged between 876 ng/ml and 1011 ng/ml in chickens and 741 ng/ml and 851 ng/ml in turkeys with means of 949.8±52.81 ng/ml and 810.8±40.99 ng/ml, respectively. The 4 h plasma concentrations ranged between 466 ng/ml and 676 ng/ml in chickens and 388 ng/ml and 563 ng/ml in turkeys with means of 538.0±75.00 ng/ml and 448.3±62.50 ng/ml, respectively. The 6 h plasma norfloxacin concentrations varied between 439 ng/ml and 250 ng/ml in chickens and 366 ng/ml and 208 ng/ml in turkeys, respectively, with means of 359.4±49.6 ng/ml and 299.5±41.3 ng/ml. 12 h plasma concentrations ranged between 304 and 139 ng/ml and 253 and 116 ng/ml in chickens and turkeys respectively, with an average of 187.5±39.5 ng/ml in chickens and 156.3±32.9 ng/ml in turkeys. 22 h plasma concentrations were changed between 156 and 104 ng/ml in chickens and 130 and 87 in turkeys, with means of 127.9±15.4 and 106.5±12.9 ng/ml in chickens and turkeys, respectively. The average steady-state plasma concentrations of norfloxacin were 365.32±39.31 ng/ml in chickens and 306.03±32.26 ng/ml in turkeys.

In the course of pulse dosing the AUC $(0,\infty)_{1st}$ was found to be 8767.7±943.52 ng/ml×h in chickens and 7344.7±774.24 ng/ml*h in turkeys. AUC_{0-t} was 5944.0±529.27 ng/ml×h in chickens and 4987.9±445.04 ng/ml*h in turkeys. Table 2 shows the related data.

Table 2. Mean ± SEM plasma concentrations of norfloxacin measured at defined time intervals after pulse dosing in chickens and turkeys.

Pulse-dosing treatment with 15 mg/kg b.w. norfloxacin		
Time of sampling and parameters	Chickens	Turkeys
	Plasma norfloxacin concentrations (ng/ml)	
2 hours	949.8±52.81	810.8±40.99
4 hours	538.0±75.00	448.3±62.50
6 hours	306.0±42.46	255.0±35.38
8 hours	227.6±26.03	189.7±21.69
12 hours	164.4±17.14	137.0±14.28
22 hours (22 hour)*	121.2±14.68	101.0±12.23
30 hours (6 hour)	379.0±31.49	315.8±26.24
36 hours (12 hour)	191.0±17.89	159.2±14.91
46 hours (22 hour)	126.2±15.95	105.2±13.29
52 hours (6 hour)	403.2±26.21	336.0±21.84
58 hours (12 hour)	252.4±28.81	210.3±24.01
68 hours (22 hour)	141.4±10.77	117.8±8.98
76 hours (6 hour)	379.8±40.81	316.5±34.00
82 hours (12 hour)	162.2±16.43	135.2±13.69
92 hours (22 hour)	122.6±14.08	102.2±11.74
100 hours (6 hour)	329.2±36.57	274.3±30.47

106 hours (12 hour)	167.6±19.57	139.7±16.31
Average at 6 hour	359.4±49.59	299.5±41.32
Average at 12 hour	187.5±39.50	156.3±32.92
Average at 22 hour	127.9±15.42	106.5±12.85
$Cpss_{average}$	365.3±39.31	306.0±32.26
AUC_{0-t} (ng/ml*hour)	5944.0±529.27	4987.9±445.04
$AUC_{(0,\infty)_{1st}}$ (ng/ml*hour)	8767.7±943.52	7344.7±774.24
Average daily norfloxacin intake	15 mg/kg b.w.	15 mg/kg b.w.

* Blood samples were taken 3 times a day from second day of treatment, 2 hours prior the start of pulse-dosing treatment (22 hour) and 2 (6 hour) and 8 (12 hour) hours after the finishing of pulse dosing.

$Cpss_{average}$= average steady state concentration, it was calculated as equivalent to $AUC_{(0,\infty)_{1st}}/\tau$ (dosing interval),

AUC_{0-t}=area under the concentration-time curve from 0-22 hours post dose,

$AUC_{(0,\infty)_{1st}}$ =area under the concentration-time curve to infinity during first dose

DISCUSSION

In the present study, the difference in the pharmacokinetic properties of pulse- and continuous dosing of norfloxacin in chickens and turkeys were evaluated.

The plasma norfloxacin concentrations increased rather slowly after starting of continuous dosing and reached the MIC_{90} (250 ng/ml) against the major poultry pathogen Gram-negative bacteria and mycoplasmas (Hannan et al., 1989) by 12 h in chickens and 18 h in turkeys. The mean steady-state plasma concentrations was attained in 36 h in chickens and turkeys and remained at 776.67±33.23 ng/ml and 682.50±28.55 ng/ml, respectively during the treatment period. These values were higher than those calculated after pulse dosing (365.32±39.31 ng/ml in chickens and 306.03±32.26 ng/ml in turkeys).

During pulse dosing the plasma norfloxacin concentrations increased rapidly and reached the MIC_{90}, 250 ng/ml in the first 20 minutes in both chickens and turkeys and remained above for 8 hours in chickens and 6 hours in turkeys. Therefore even with an 8 hour PAE the antimicrobial concentration is not sufficient to keep the microorganisms under continuous antibacterial pressure. Data of the daily pulse dosing demonstrated that every administration of the drug corresponds to a single, daily, repeated bolus administration. However pulse dosing achieved higher plasma concentrations more readily then continuous dosing.

Because of the fear about the development of resistance, there is great debate and political consideration on the use of fluoroquinolones in animals. Because the

fluoroquinolones are the drugs of choice for many bacterial infections not responding for any other antimicrobial in human beings, there has been an attempt to minimize the development of resistance to them by the medical profession (Beam, 1994).

In general the development of resistance is considered a 2-8-fold change in the MIC (Fernandes, 1988). However, decreased susceptibility caused by mutations was not considered clinically significant in the mid-1990s (Smith, 1984). Recently, resistance has been reported most often for *Pseudomonas aeruginosa, Serratia marcescens*, and *staphylococci* in chronic infections or bacterial exposure. Resistance has developed to some of the fluoroquinolones during clinical use in humans, as evidenced by an increased MIC observed in *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* isolates from human patients with chronic respiratory infections treated with enoxacin, pefloxacin (Maesen et al., 1987) or norfloxacin (Rowan et al., 1988). Therapy over prolonged periods of time (4-10 days) in human beings is also associated with the emergence of resistant strains of bacteria (Bayer et al., 1988).

The fluoroquinolones are classified as bactericidal compounds, and have shown concentration-dependent bacterial killing within a couple of orders of magnitude of the MBC. The efficacy of fluoroquinolones is related to both the maximum concentration and the time above the MIC (Blaser et al., 1987). *In vitro* pharmacokinetic systems have shown that peak concentrations exceeding 8 times the MIC were related with over 99% reduction in bacterial counts and prevention of bacterial regrowth for 24 h. Drusano et al. (1993) examined the impact of dose fractionating and altered MICs on survival by administering lomefloxacin, to neutropenic rats with *Pseudomonas aeruginosa* sepsis, as a single daily dose which produced high peak concentration/MIC ratios (approximately 20/1), or as the same total daily dose fractionated into four daily injections. The latter gave a longer time above the MIC. The single daily dose produced significantly better survival than the more fractionated regimen, indicating that peak concentration and intensity of exposure is linked more closely with efficacy than time above the MIC. Forrest et al. (1993) noted that, with ciprofloxacin, the probability of clinical and microbiological cures were 80% and 82%, respectively when the AUIC was above 125; when it went under 125, the probabilities were 42% and 26%, respectively. Time to eradication of the infection was similarly related to the AUIC, with 125 and 250 the cut off points for moderate and rapid eradication of the infection (Forrest et al., 1993). On the basis of these findings, it could be concluded that regimens of large doses (resulting in high AUC and AUIC) given relatively long intervals,

such as pulse-dosing (thus relying on PEA), might be more efficacious in terms of bacterial killing, eradication time and reducing the selection of resistant bacteria.

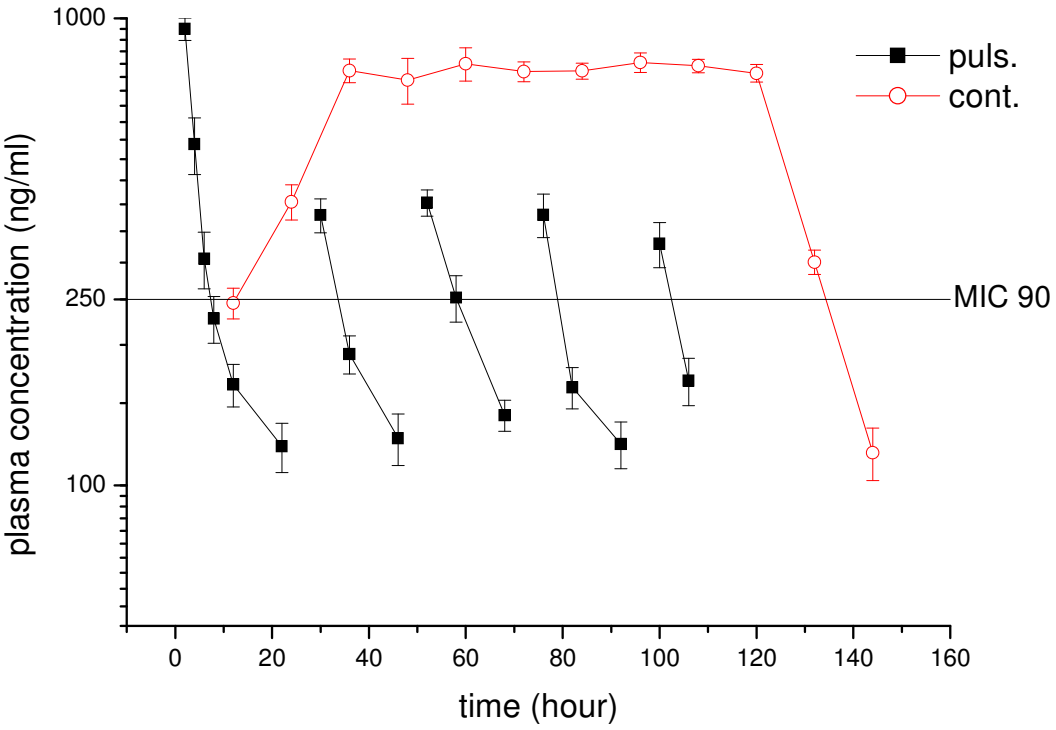
On the contrary, Zhanel et al. (2001) suggested that new respiratory fluoroquinolones with an AUC_{24}/MIC of 35-63 reduced the inoculum of multidrug-resistant *Streptococcus pneumoniae* below the level of detection without regrowth or development of resistance over 48 hours.

Considering the observation that the AUIC (AUC/MIC) is closely related to efficacy and high AUIC may also exerts an exposure of longer duration to less sensitive strains (Thomas et al., 1998). However, prevailing ideas are that C_{max} is more closely related to reducing resistance (Drusano et al., 1993). In our previously published artificial disease models of *Escherichia coli* and *Pasteurella multocida* infection in chickens and turkeys were performed for evaluating the comparative efficacy of continuous- and pulse dose treatment with norfloxacin (Sárközy et al., 2002a, Sárközy et al., 2002b). In both reports the C_{max} exceeded the MIC over 10 times for the strains used, however substantial differences were obtained between the dosing schedules. These reviews and our findings support the view that low pathogenicity bacterial infection gives better recovery with continuous-dosing, while severe bacterial infection shows improving effect following pulse-dosing schedule.

In conclusion, administration strategies such as continuous dosing at 100 mg/L or pulse dosing medication at 15 mg/kg b.w., may provide rational means of administering fluoroquinolones in herd situations, however, we recommend to treat bacterial infections of either high or low pathogenicity starting with pulse dosing for 4 hours and then maintaining continuous oral medication for 3-5 consecutive days with the fluoroquinolones. Nevertheless manufacturers recommend using fluoroquinolones in a pulse-dosing manner throughout the whole treatment period.

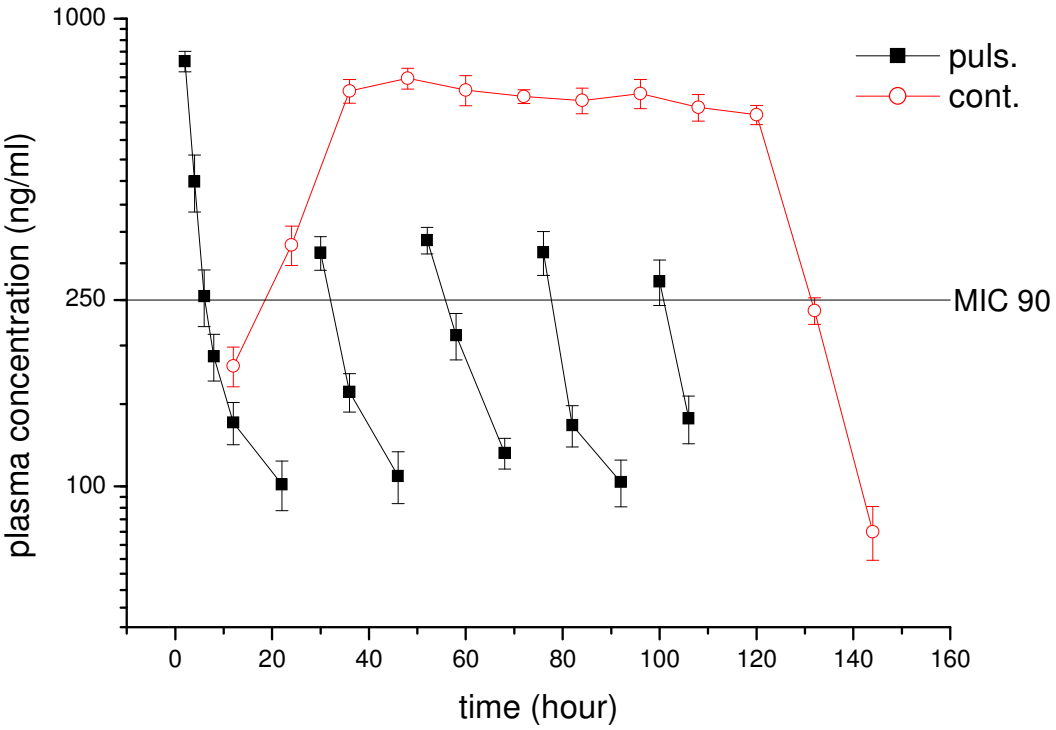
Our recommended method of administration should help in preventing both the severe and less serious bacterial infections and could prevent the emergence of resistance against the fluoroquinolones.

Figure 1.



Plasma concentrations of norfloxacin measured at defined time intervals after continuous- and pulse dosing in chickens in a semi logarithmic plot. MIC_{90} is the minimum inhibitory concentration for the 90% of Gram-negative bacilli.

Figure 2.



Plasma concentrations of norfloxacin measured at defined time intervals after continuous- and pulse dosing in turkeys in a semi logarithmic plot. MIC_{90} is the minimum inhibitory concentration for the 90% of Gram-negative bacilli.

XII. CONCLUDING REMARKS

Norfloxacin is a third generation fluoroquinolone that has high antimicrobial activity *in vitro* against a wide range of Gram-negative and Gram-positive bacteria.

As with many other agents, there is considerable confusion over the most appropriate way to administer this drug in order to obtain optimal outcome for both high and/or low pathogenicity infections in poultry.

A regimen of large doses (resulting in high AUC and C_{max}) given infrequent intervals, such as pulse dosing (thus relying on the PAE) has been suggested to be more efficacious in terms of bacterial killing, eradication time, and reducing the selection of resistant bacteria. This suggestion, together with the importance of *Pasteurella multocida* and *Escherichia coli* as a poultry pathogen, led us to compare the pharmacokinetic properties and efficacy of pulse dosing oral norfloxacin treatment with that of an established medication of continuous dosing in broiler chickens and turkeys.

The efficacy of artificial infection model relies on the virulence and the challenge dose of utilized bacteria. In our studies we were able to succesfully develop a reliable method for both *Pasteurella multocida* and *Escherichia coli* infection that made possible to answer the basic questions.

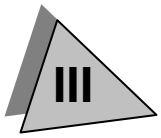
TREATMENT OF PASTEURELLA MULTOCIDA INFECTION

II

Norfloxacin administered at 100 mg/L, continuous dosing was as efficacious as norfloxacin administered at 15 mg/kg pulse dosing in chickens. There was no difference in daily weight gain, slight variation in mortality, but well characterized alteration in postmortem scores, daily clinical scores and recovery of bacteria. It should be considered that the inoculum was approximately 80 CFU X-73 (A:1), close to its LD_{50} (19.6 CFU) value. In turkeys, where 15 fold (approximately 70 CFU P-1059 (A:3)) of the LD_{50} (4.67 CFU) was used, significant alteration in all examined parameters was observed, except average daily weight gain, between the two different treatment schedule. We can proclaim that continuous dosing in chickens and pulse dosing in turkeys were significantly more valuable in treating *Pasteurella multocida* infection in the present study. These results were exactly the opposite as was expected by the pharmacokinetic properties described in the literature.

Some literature review indicated that even with an 8 hour lasting PAE the fluoroquinolone treatment gives better result using as pulse-dose medication in drinking water, while others showed that severe bacterial infections are better treated with high dosages of antimicrobials. These results confirm our findings that low volume bacterial infection, 4 fold over the LD_{50}, improves with continuous dosing, while severe bacterial infection, 15 fold over the LD_{50}, advances better following a pulse dosing schedule.

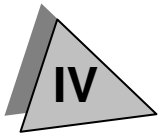
TREATMENT OF ESCHERICHIA COLI INFECTION



The *E. coli* model used in the chick-inoculation experiment produced 40% mortality and 1.77±1.27 daily clinical score (60% morbidity) in infected, non-medicated birds. These levels compare favourably to the 5% mortality and up to 50% morbidity suggested by different authors as being typical for colibacillosis in the field. In the present study, both treatment procedures effectively reduced the experimental colibacillosis in chickens. Norfloxacin administered at 15 mg/kg pulse dosing was more efficacious then norfloxacin administered at 100 mg/L, continuous dosing in chickens. There was no variation in daily weight gain and mortality, but well characterized alteration in daily clinical scores, postmortem scores for the benefit of pulse dosing. The results obtained in this study coincide with the conclusion from the pharmacokinetic properties described in the literature.

These differences can be explained if we assume that low-level bacterial infection gives better recovery with continuous dosing, while severe bacterial infection shows better improvement following pulse-dosing schedule.

PHARMACOKINETIC PROPERTIES



The plasma norfloxacin concentrations increased slowly during continuous dosing and reached the MIC for the most Gram-negative pathogen bacteria by 12 h in chickens and 18 h in turkeys. The mean steady-state plasma concentration was also attained in 36 h. It remained approximately at the same plasma concentration level both in chickens and turkeys (776.67±33.23 ng/ml in chickens and 682.50±28.55 ng/ml in turkeys) during the whole treatment period. Pulse dosing produced half the steady-state concentration (365.32±39.31 ng/ml in chickens and 306.03±32.26 ng/ml in turkeys) as it was obtained after continuous dosing.

After pulse dosing the plasma norfloxacin concentrations increased rapidly and significantly exceeded the MIC at 2 h in both chickens and turkeys and remained above for

8 h in chickens and 6 h in turkeys. Data of the daily pulse dosing suggested that every administration of the drug corresponds to a single, daily repeated bolus administration. However pulse dosing achieved higher plasma concentration more readily then continuous dosing.

Fluoroquinolones show concentration-dependent killing *in vitro*, and animal models have demonstrated that the principal predictors of *in vivo* killing are the 24-hour AUIC (AUC/MIC) and the C_{max}/MIC ratio. The AUIC appears to be important for killing, whereas the C_{max}/MIC ratio is important to prevent the selection of resistance mutants during treatment. During *in vivo* studies, bacteriostasis was achieved with an AUIC of around 35, while in a variety of animal models mortality is completely prevented once the ratio reached 100.

However, prevailing ideas are that C_{max}, over 2-3 times the MIC, is more closely related to reducing resistance. In our studies the C_{max} exceeded the MIC over 5-10 times for the strains used and the AUIC was over 100, however substantial differences were obtained between the dosing schedules. Our findings support the view that low pathogenicity bacterial infection gives better recovery with continuous dosing, while severe bacterial infection shows improving effect following pulse dosing schedule.

It can be concluded that regimens of continuous dosing at 100 mg/L or pulse dosing medication at 15 mg/kg may be used when administering norfloxacin in herd situations, however, we recommend to treat bacterial infections of either high or low pathogenicity starting with pulse dosing for 4 hours and then maintaining continuous oral medication for 3-5 consecutive days. Nevertheless manufacturers recommend using fluoroquinolones such as norfloxacin in a pulse-dosing manner throughout the whole treatment period.

Our recommended method of administration should help in preventing both the severe and less serious bacterial infections and could prevent the emergence of resistance against the fluoroquinolones.

Fluoroquinolones are one of the most useful classes of antimicrobial agents used in human and animal medicine today, both because of their

spectrum and their physicochemical properties. As such, their popularity in clinical situations is increasing.

Recently, however, concerns have been raised over the possible emergence of quinolone-resistant strains and the effects on the environment if such drugs are overused. At present it appears that, physicians and veterinarians can prolong their usefulness for many years if they use appropriate clinical judgment and proper dosing principles as they prescribe and administer these drugs to patients.

If used in a well-controlled manner, quinolones will contribute greatly to stock farming management, without adversely influencing human chemotherapy.

XIII. AKNOWLEDGEMENTS

I wish to express my gratitude to:

Professor **Gábor Semjén** (Department of Pharmacology and Toxicology, University of Veterinary Science, Budapest) who has been supervising my scientific work since I started my experiments in the field of pharmacology. Special thanks for his guidance and intensive efforts to provide a scientific background of international standards for my investigations. I truly admire his charisma in changing the world into something better. *Without his initiative and scientific advice, this study would not have been possible.*

Professor **Péter Laczay** (Department of Food Hygene, University of Veterinary Science, Budapest) who introduced me to the challanging world of science when I was an undergraduate student. I greatly appreciate his talents, which he shared with me both in research and in life. I am also deeply impressed by his efforts to create a productive research place emphasizing the importance of the intensive and high quality science and making a great deal in generating a friendly and collaborative atmosphere in the institute.

Professor **Béla Nagy** (Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest) for his support and scientific advice.

Dr. **Tibor Soós** and Dr. **Ernő Horváth** (State Control Institute for Biologicals, Drugs and Feed, Budapest) for all their support, and guidance in science.

My fellow researcher in CEVA-Phylaxia, Dr. **József Schmidt** with whom I spent many fruitful hours in the lab in splendid atmosphere.

Special thanks to Mr. **Lajos Svajcsik** and Ms. **Tímea Debreceni** who provided excellent laboratory and office background.

All the staff members of the Department of Pharmacology and Toxicology, University of Veterinary Science, Budapest and Department of Food Hygene, University of Veterinary Science, Budapest are acknowledged for the supporting millieu.

My **parents** for their love, encouragement, support and patience.

And finally my wife, **Julcsa**, for her love and also for providing a beautiful family background, which made me steady in these years.

XIV. REFERENCES

Abadía A.R., Aramayona J.J., Fraile L., Martinez C., Garcia M.A. and Bregante M.A. (1994a) Pharmacokinetics of enrofloxacin and ciprofloxacin during ontogeny in the rabbit. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 231.

Abadía A.R., Aramayona J.J., Muñoz M.J., Pla Delfina J.M., Saez M.P. and Bregante M.A. (1994b) Disposition of ciprofloxacin following intravenous administration in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, **17**: 384-388.

Akahane K., Segiguchi M., Une T. and Osoda Y. (1989) Structure-epileptogenicity relationship of quinolones with special reference their interaction with γ-amino-butiric acid receptor sites. *Antimicrobial Agents and Chemotherapy*, **33**: 1704-1708.

Allan, B.J., J. Van den Hurk and Potter, A.A. (1993) Characterization of *E. coli* isolated from cases of avian colibacillosis, *Canadian Journal of Veterinary Research*. **57**:146-151.

Althreuther P. (1987) Data on chemistry and toxicology of Baytril. *Veterinary Medicine Reviews*, **2**: 87-89.

Anadón A., Martínez-Larrañaga M.R., Velez C., Díaz M.J., Bringas P. (1992) Pharmacokinetics of norfloxacin and its N-desethyl- and oxo-metabolites in broiler chicken. *American Journal of Veterinary Research*, **53**: 2084-2089.

Anadón A., Martínez-Larrañaga M.R., Díaz M.J., Fernández R., Martínez M.A., Fernández-Cruz M.L. and Fernández M.C. (1994) Pharmacokinetic properties of norfloxacin in pigs. *Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 230-231.

Anadón A., Martínez-Larrañaga M.R., Díaz M.J., Bringas P., Martínez M.A., Fernández-Cruz M.L., Fernández M.C. and Fernández R. (1995) Pharmacokinetics and residues of enrofloxacin in chickens. *American Journal of Veterinary Research*, **56**: 501-506.

Ando, N., Ibayashi ,T. and Hidaka, S. (1993) Therapeutic effects of water medication of Neomycin on experimentally transmitted colibacillosis in chicks. *10th World Poultry Association Congress, Sydney, Australia*. P. 206.

Apley M.D. and Upson D.W. (1993a) Regional danafloxacin lung tissue concentrationsand their relationship to regional pulmonary blood flow in consolidated and nonconsolidated bovine lung. *American Journal of Veterinary Research*, **54**, 944-951.

Apley M.D. and Upson D.W. (1993b) Lung tissue concentrations and plasma pharmacokinetics of danofloxacin in calves with acute pneumonia. *American Journal of Veterinary Research*, **54**, 937-943.

Aramayona J.J., Garcia M.A., Fraile L.J., Abadfa A.R. and Bregante M.A. (1994) Placental transfer of enrofloxacin and ciprofloxacin in rabbits. *American Journal of Veterinary Research*, **55**, 1313-1318.

Ashby J., Piddock L.J.V. and Vise R. (1985) Correspondence: An investigation of the hydrophobicity of the quinolones. *Journal of Antimicrobial Chemotherapy*, **16**, 805-808.

Ball P. (1986) Ciprofloxacin: an overview of adverse experiments. *Journal of Antimicrobial Chemotherapy*, **18** (suppl. D): 187-193.

Barnes, H.J. and Gross, W.B. (1997) Colibacillosis. In Diseases of Poultry, Calnek, B.W. et al, 10th Edition. Pp. 131-141. (Ames, Iowa State University Press)

Barza M. (1991) Use of quinolones for treatment of ear and eye infections. *European Journal of Clinical Microbiology and Infectious Diseases*, **10**: 296-303.

Bates S.A. and Elder M.G. (1988) An evaluation of pelvic tissue concentrations after oral administration of enoxacin. *Journal of Antimicrobial Chemotherapy*, **21** (Suppl. B): 79-85.

Bayer A.S., Hirano L. and Yih J. (1988) Development of β-lactam resistance and increased quinolone MICs during therapy of experimental Pseudomonas aeruginosa endocarditis. *Antimicrobial Agents and Chemotherapy*, **32**: 231-235.

Beam T.R. jr. (1994) Fluoroquinolones in Animal Feeds. *American Society of Microbiology News*, **60**: 348-349.

Berg J. (1988) Clinical indications for enrofloxacin in domestic animals and poultry. In: Quinolones: A new class of antimicrobial agents for use in veterinary medicine. *Proc. West Vet. Conf, Las Vegas, Nevada: Mobay Corporation*

Bhaumik A. (1997) Effect of norfloxacin treatment in acute enteritis in dogs. *Indian Veterinary Journal*, **74**: 246-247.

Blaser J., Stone B.B., Groner M.C. and Zinner S.H. (1987) Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrobial Agents and Chemotherapy*, **31**: 1054-1060.

Blaser J. and Lüthy R. (1988) Comparative study on antagonistic effects of low pH and cation supplementation on *in-vitro* activity of quinolones and aminoglycosides against Pseudomonas aeruginosa. *Journal of Antimicrobial Chemotherapy*, **22**: 15-22.

Bowles S.K., Popovski Z., Rybak M.J., Beckman H.B. and Edwards D.J. (1988) Effects of norfloxacin on theophylline pharmacokinetics in steady-state. *Antimicrobial Agents and Chemotherapy*, **32**: 510-513.

Braunius W.W. (1987) Effect van Baytril™ (Bay Vp 2674) op jonge kalkoenen lijdende aan luchtweginfecties. *Tijdschr. Diergeneesksd*, **112**: 531-3.

Bregante M.A., Abadía A.R., Mora J., Aramayona J.J., Garcia M.A. and Fraile L. (1994) Milk transfer of enrofloxacin

and ciprofloxacin in the rabbit. *Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 231-232.

Brown S.A., Cooper J., Gauze J.J., Greco D.S., Weise D.W. and Buck J.M. (1990) Pharmacokinetics of norfloxacin in dogs after a single intravenous and single and multiple oral administrations of the drug. *American Journal of Veterinary Research*, **51**: 1065-1070.

Brumfitt W., Franklin I., Grady D., Hamilton-Miller J.M.T. and Iliffe A. (1984) Changes in the pharmacokinetics of ciprofloxacin and fecal flora during administration of a 7-day course to human volunteers. *Antimicrobial Agents and Chemotherapy*, **26**: 757-761.

Carlier M.B., Scorneaux B., Zenebergh A., Desnottes J.F. and Tulkens P.M. (1990) Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. *Journal of Antimicrobial Chemotherapy*, **26** (Suppl. B): 27-39.

Chamberland S., Bayer A.S., Schollaardt T., Wong S.A. and Bryan L.E. (1989) Characterization of mechanism of quinolone resistance in Pseudomonas aeruginosa strains isolated in vitro and in vivo during experimental endocarditis. *Antimicrobial Agents and Chemotherapy*, **33**: 624-634.

Chang T., Black A., Dunkey A., Wolf R., Sedman A., Latts J. and Welling P.G. (1988) Pharmacokinetics of intravenous and oral enoxacin in healthy volunteers. *Journal of Antimicrobial Chemotherapy*, **21** (Suppl. B.): 49-56.

Chapman J.S. and Georgopapadakou N.H. (1988) Routes of quinolone permeation in *E. coli*. *Antimicrobial Agents and Chemotherapy*, **32**: 438-442.

Charleston B., J.J. Gate, I.A. Aitken, B. Stephan, and R. Froyman. (1998) Comparison of the efficacies of three fluoroquinolone antimicrobial agents, given as continuous or pulsed water medication, against *E. coli* infection in chickens. *Antimicrobial Agents and Chemotherapy,* **42**: 83-87.

Chen Z., Fung K.F., Fang B. and Song Y. (1994) Antimicrobial and pharmacokinetic studies of fluoroquinolones in chickens. *Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 209-210.

Chu D., Fernandez P., Claiborne A., Shen L. and Pernet A. (1988) Structure-activity relationships in quinolone antimicrobials: design, synthesis and biological activities of novel isothiazoquinolones. *Drugs in Experimental and Clinical Research* **14**: 379-383.

Cooper A.D., Fuller J.R., Fuller M.K., Whitlestone P. and Wise, D.R. (1993) In vitro activity of danofloxacin, tylosin and oxytetracycline against mycoplasmas of veterinary importance. *Research in Veterinary Science*, **54**. 329-334.

Cutarelli P.E., Laso J.H., Lazarus H.M., Putnam S.P. and Jacobs M.R. (1991) Topical fluoroquinolones: antimicrobial activity and *in vitro* corneal epithelial toxicity. *Current Eye Research*, **10**: 557-563.

Dalhoff A. and Weidner W. (1984) Diffusion of ciprofloxacin into prostatic fluid. *European Journal of Clinical Microbiology*, **3**: 360-362.

Darouiche R., Perkins B., Musher D., Hamill R. and Tsai S. (1990) Levels of rifampin and ciprofloxacin in nasal secretions: correlation with MIC_{90} and eradication of nasopharyngeal carriage of bacteria. *Journal of Infectious Diseases*, **162**: 1124-1127.

Desgrandchamps D. (1989) Increasing rates of in vitro resistance to ciprofloxacin and norfloxacin in isolates from urine specimens. *Antimicrobial Agents and Chemotherapy* **33**: 595-6.

Desnottes J.F., Diallo N., Moret G. and Santonja R. (1987) Effects of subinhibitory concentrations of pefloxacin on the adherence of Staphylococcus aureus to human cells. *Drugs in Experimental and Clinical Research*, **13**: 69-73.

Dijkstra J.W., McConville M.L. and Nouws J.F.M. (1994) Effects of srafloxacin hydrochloride on human enteric bacteria under simulated human gut conditions. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland*, p. 75.

Diver J.M. and Wise R. (1986) Morphological and biochemical changes in *Escherichia coli* after exposure to ciprofloxacin. *Journal of Antimicrobial Chemotherapy*, **18** (suppl. D): 31-41.

Dobbs B.R., Gazeley L.R., Stewart I.A. and Edwards I.R. (1988) Pharmacokinetics and sputum concentrations of enoxacin after twice daily oral dosing for seven days. *Journal of Antimicrobial Chemotherapy*, **21** (Suppl. B), 61-66.

Dorfman M., Barsanti J. and Budsberg S. (1995) Enrofloxacin concentrations in dogs with the normal prostate and dogs with chronic bacterial prostatitis. *American Journal of Veterinary Research*, **56**: 386-390.

Dowling P.M., Wilson R.C., Tyler J.W. and Duran S.H. (1995) Pharmacokinetics of ciprofloxacin in ponies. *Journal of Veterinary Pharmacology and Therapeutics*, **18**: 7-12.

Drusano G.L., Johnson D.E., Rosen M. and Standiford H. C. (1993) Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of Pseudomonas spesis. *Antimicrobial Agents and Chemotherapy*, **37**: 483-490.

Drusano G.L., Plaisance K.I., Forrest A. and Standiford H.C. (1986) Dose ranging study and constant infusion evaluation of ciprofloxacin. *Antimicrobial Agents and Chemotherapy*, **30**: 440-443.

Dunnington, E.A., P.B. Slegel, and W.B. Gross. (1991) *E. coli* challenge in chickens selected for high or low antibody response and differing in haplotypes at the major histocompatibility complex. *Avian Diseases,* **35**: 937-940.

Duval J.M. and Budsberg S.C. (1995) Cortical bone concentrations of enrofloxacin in dogs. *American Journal of Veterinary Research*, **56**: 188-192.

Endtz H.P., Rujis G.J., Van Klingeren B., Jansen W.H., Van der Reyden T. and Mouton R. P. (1991) Quinolone resistance in campylobacter isolated from manand poultry following the introduction of fluoroquinolones in

veterinary medicine. *Journal of Antimicrobial Chemotherapy*, **27**: 199-208.

Erganis, O., Kaya, O., Corlu, M. and Istanbuluoglu E. (1989) Hemagglutination, hydrophobicity, enterotoxigenicity and drug resistance characteristics of avian *E. coli*. *Avian Diseases*, **33**:631-635.

Felmingham D., Foxal P., O`Hare M.D., Webb G., Ghosh G. and Grüneberg R.N. (1988) Resistance studies with ofloxacin. *Journal of Antimicrobial Chemotherapy*, **22** (Suppl. C): 27-34.

Fernandes P.B. (1988) Mode of action, and in vitro and in vivo activities of the fluoroquinolones. *Journal of Clinical Pharmacology*, **28**: 156-168.

Fitzgeorge R., Featherstone A., Baskerville A. (1988) The effect of ofloxacin on the intracellular growth of Legionella pneumophila in guinea pig alveolar phagocytes. *Journal of Antimicrobial Agents and Chemotherapy*, **22** (suppl. S): 53-57.

Foerster D. (1987) Visualization of the bactericidal action of Baytril by microphotography. *Veterinary Medicine Reviews*, **2**: 100-103.

Forchetti C, Flammini D, Carlucci G, Cavicchio G, Vaggi L, Bologna M. (1984) High performance liquid chromatography procedure for the quantitation of Norfloxacin in urine, serum and tissues. *Journal of Chromatography*, **309**: 177-182.

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

Friis C. (1994) Penetration of danofloxacin into respiratory tract tissues and secretions in pigs. *Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland* p. 208-209.

Friis C. (1991) Danofloxacin in calves: pharmacokinetics and penetration into the respiratory tract tissue, *Acta Vet Scand Suppl*, **87**: 104-6

Frost R.W., Carlson J.D., Dietz A.J.,jr., Heyd A. and Lettieri J.T. (1989a) Ciprofloxacin pharmacokinetics after a standard or high-fat/high-calcium breakfast. *Journal of Clinical Pharmacology*, **29**: 953-955.

Frost R.W., Lettieri J.T., Krol G., Shamblen E.C. and Lasseter K.C. (1989b) The effects of cirrhosis on the steady-state pharmacokinetics of oral ciprofloxacin. *Clinical Pharmacology and Therapeutics*, **45**: 608-616.

Furet Y.X. and Pechére J.C. (1991) Newly documented antimicrobial activity of quinolones. *European Journal of Clinical Microbiology and Infectious Diseases*, **10**: 249-254.

Gasser T., Graversen P. and Madsen P. (1987) Treatment of complicated urinary tract infections with ciprofloxacin. *American Journal of Medicine*, **82** (suppl. 4A): 278-281.

Gellert M, Mizuuchi K, O`Dea MH, et al. (1976) DNA gyrase: an enzyme that introduces superhelical turns to DNA. *Proc Natl Acad Sci*, **74**: 4772.

Giamarellou H., Kolokythas E., Petrikkos G., Gazis J., Aravantino D. and Sfikakis P. (1989) Pharmacokinetics of three newer quinolones in pregnant and lactating women. *American Journal of Medicine*, **87** (Suppl. 5A): 49-51.

Giles C.J., Magonigle R.A., Grimshaw W.T.R., Tanner A.C., Risk J.E., Lynch M.J. and Rice J.R. (1991a) Clinical pharmacokinetics of parenterally administered danofloxacin in cattle. *Journal of Veterinary Pharmacology and Therapeutics*, **14**: 400-410.

Giles C.J., Grimshaw W.T.R., Shanks D.J. et al. (1991b) The efficacy of danofloxacin in the therapy of acute bacterial pneumonia in housed beef cattle. *Veterinary Record*, **128**: 296-300.

Goodman L.S. and Gilman A. (1992) The Pharmacological Basis of Therapeutics. *8th ed. New York, Macmillan*, p. 1057.

Gootz, T.D. (1990) Discovery and development of new antimicrobial agents. *Clin. Microbiol. Rev*. **3**:13-31.

Gootz T.D., McGuirk P.R., Moynihon M.S. and Haskell S.L. (1994) Placement of alkyl substituents on the C7 piperazine ring of fluoroquinolones: Dramatic differential effects on mammalian topoisomerase II and DNA gyrase. *Antimicrobial Agents and Chemotherapy*, **38**: 130-133.

Gould I.M., Milne K. and Jason C. (1990) Concentration-dependent bacterial killing, adaptive resistance and post-antibiotic effect of ciprofloxacin alone and in combination with gentamycin. *Drugs in Experimental and Clinical Research*, **26**: 621-628.

Grasela T.J.jr., Schentag J.J., Sedman A.J., Wilton J.H., Thomas D.J., Schultz R.W., Lebsack M.E. and Kinkel A.W. (1989) Inhibition of enoxacin absorption by antacids or ranitidine. *Antimicrobial Agents and Chemotherapy*, **33**: 615-617.

Greenwood D., Baxter S., Cowlishaw A., Eley A. and Slater G.J. (1984) Antibacterial activity of ciprofloxacin in conventional tests and in a model of bacterial cystitis. *European Journal of Clinical Microbiology*, **3**: 351-354.

Grimshaw W.T.R., Magonigle R.A., Giles C.J., Tanner A.C., Risk J.E., Lynch M.J. and Rice J.R. (1990a) The pharmacokinetics of danofloxacin in cattle. *XVI World Buiatrics Congress, Salvador, Bahia, Brazil.*

Grimshaw W.T.R., Giles C.J., Cooper A.C., et al. (1990) The efficacy of danofloxacin in the therapy of pneumonia associated with *Pasteurella* species in housed calves. *DTW Deutsche Tierarztliche Wochenschrift*, **97**: 529-532.

Gulkarov A. and Ziv G. (1994) Some pharmacokinetic features of norfloxacin nicotinate in turkeys. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 235.

Gyrd-Hansen N. and Nielsen P. (1994)The influence of feed on the oral bioavailability of enrofloxacin, oxytetracycline, penicillin V and spiramycin in pigs. *Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, pp. 242-243.

Hannan P.C., O'Hanlon P.J. and Rogers N.H. (1989) In vitro evaluation of various quinolone antibacterial agents

against veterinary mycoplasmas and porcine respiratory bacterial pathogens. *Research in Veterinary Science*, **46** (2): 202-211.

Harder S., Staib A.H., Beer C., Papenburg A., Stille W. and Shah P.M. (1988) 4-Quinolones inhibit biotransformation of caffeine. *European Journal of Clinical Pharmacology*, **35**: 651-656.

Hayem G., Petit P.X., LeVacher M., Gaudin C., Kahr M.F. and Pocidalo J.J. (1994) Cytofluorometric analysis of chondrotoxicity of fluoroquinolone antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, **38**: 243-247.

Hinz K.H. and Rottmann S. (1990) Studies in vivo on the efficacy of enrofloxacin against *Mycoplasma gallisepticum*. *Avian Pathology*, **19**: 511-522.

Hinz, K.H. and Luders H. (1991) *Pasteurella multocida* as a cause of disease outbreaks in commercial poultry flocks. *Berliner-und-Munchener-Tierarztliche-Wochenschrift*, **104**: 298-303.

Hoiby N. (1986) Clinical use of nalidixin acid analogues: the fluoroquinolones. *European Journal of Clinical Microbiology*; **5**: 138-140.

Homles B, Brogden RN, Richards DM. (1985) Norfloxacin. A review of its antibacterial activity, pharmacokinetic properties, and therapeutic use. *Drugs*, **30**: 482-513.

Hoogkamp-Korstanje J.A.A. (1984) Comparative in vitro activity of five quinolone derivates and five other antimicrobial agents used in oral therapy. *European Journal of Clinical Microbiology*, **3**: 333-338.

Hooper D. and Wolfson J. (1985) The fluoroquinolones: Structures, mechanisms of action and resistance and spectra of activity in vitro. *Antimicrobial Agents and Chemotherapy* **28**: 581-586.

Hooper D.C. and Wolfson J.S. (1991) Mode of action of the new quinolones: new data. *European Journal of Clinical Microbiology and Infectious Diseases*, **10**: 223-231.

Hooper, D.C. and Wolfson, J.S. (1993) Quinolone antimicrobial agents, 2nd ed. *American Society for Microbiology, Washington D.C.* Pp. 53-76, 97-118.

Hoshino, K. et al. (1994): Comparison of inhibition of *Escherichia coli* topoisomerase IV by quinolones with DNA gyrase inhibition. *Antimicrobial Agents and Chemotherapy*, **38**. 2623-2627.

Horrox, N. (1987) Fowl cholera - the old enemy returns. *International Hatchery Practice*, **2**: 4-8.

Höffler D., Dalhoff A., Gau W., Beerman D. and Michael A. (1984) Dose- and sex independent disposition of ciprofloxacin. *European Journal of Clinical Microbiology*, **3**: 363-366.

Jackson J.A., Davidson J.N., Ter Hune T.N. and Magonigle R.A. (1990) A dose response study of the fluoroquinolone, danofloxacin, against induced bovine pneumonic pasteurellosis. *XVI World Buiatrics Congress, Salvador, Bahia, Brazil.*

Janin N., Meugnier H., Desnottes J.F., Woehrle R. and Fleurette J. (1987) Recovery of pefloxacin in saliva and feces and its action on oral and fecal floras of healthy volunteers. *Antimicrobial Agents and Chemotherapy*, **31**: 1665-1668.

Jenkins W. and Friedlander L. (1988) The pharmacology of the quinolone antibacterial agents. In: Quinolones: A new class of antimicrobial agents for use in veterinary medicine. *Proc. West Vet. Conf, Las Vegas, Nevada: Mobay Corporation Animals Health Division, Shawnee, Kansas,* p. 5-16.

Jordan F.T.W., Horrocks B.K., Jones S.K., Cooper A.C. and Giles C.J. (1993) A comparison of the efficacy of danofloxacin and tylosin in the control of Mycoplasma gallisepticum infection in broiler chicks. *Journal of Veterinary Pharmacology and Toxicology*, **16**: 79-86.

Kaatz G.W., Seo S.M. and Ruble C.A. (1991) Mechanisms of fluoroquinolone resistance of *Staphylococcus aureus*. *Journal of Infectious Diseases*, **163**: 1080-1086.

Kaatz, G.W. and Seo, S.M. (1998): Topoisomerase mutations in fluoroquinolone-resistant and methicillin-susceptible and –resistant clinical isolates of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, **42**: 197-198.

Kato M. and Onedara T. (1988) Effect of ofloxacin on the uptake of (3H) thymidine by articular cartilage cells in rat. *Toxicol. Lett.* **44**: 131-142.

Kempf I., Gesbert F., Guittet M. and Bennejean G. (1992) Efficacy of danofloxacin in the therapy of experimental mycoplasmosis in chicks. *Research in Veterinary Science*, **53**: 257-259.

Kotera Y., Watanabe M., Yoshida S., Inoue M. and Mitsuhashi S. (1991) Factors influencing the uptake of norfloxacin by *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*, **27**: 733-739.

Kresken M. and Wiedemann B. (1988) Development of resistance to nalidic acid and the fluoroquinolones after the introduction of norfloxacin and ofloxacin. *Antimicrobial Agents and Chemotherapy*, **32**: 1285-8.

Küng K., Riond J.L., Wolffram S. and Wanner M. (1993) Comparison of an HPLC and bioassay method to determine antimicrobial concentrations after intravenous and oral administration of enrofloxacin in four dogs. *Research in Veterinary Science*, **54**: 247-248.

Laczay, P., Semjén, G., Nagy, G. and Lehel, J. (1998) Comparative studies on the pharmacokinetics of norfloxacin in chickens, turkeys and geese after a single oral administration. *Journal of Veterinary Pharmacology and Therapeutics*, **21**:161-164.

Lavy E., Ziv G. and Glickman A. (1994) Pharmacokinetics of Norfloxacin in the donkey. *European Association of Veterinary Pharmacology and Toxicology*; **6**: 52-53.

Lekeux P. and Art T. (1988) Effect of enrofloxacin therapy on shipping fever pneumonia in feedlot cattle. *Veterinary Record*, **123**: 205-207

Lode H., Höffken G., Prinzig C., Glatzel P., Wiley R., Olschewski P., Sievers B., Reimnitz D., Borner K. and

Koeppe P. (1987) Comparative pharmacokinetics of new quinolones. *Drugs*, **34** (Suppl. 1): 21-25.

Lode H., Höffken G., Boeckk M., Deppermann N., Borner K. and Koeppe P. (1990) Quinolone pharmacokinetics and metabolism. *Journal of Antimicrobial Chemotherapy*, **26** (Suppl. B4): 1-9.

Lublin, A., Mechani, Sara, Malkinson, M. and Weisman, Y. (1993) Efficacy of norfloxacin Nicotinate Treatment of Broiler Breeders against Haemophilus paragallinarum. *Avian Diseases*, **37**:673-679.

Ludwig E., Székely É., Csiba A. and Graber H. (1988) The effect of ciprofloxacin on antipyrine metabolism. *Journal of Antimicrobial Chemotherapy*, **22**: 61-67,

Madaras-Kelly, K.J., B.E. Ostergaard, L. Backer Horde, and J.C. Rotschafer. (1996) Twenty-four-hour area under the concentration-time curve/MIC ratio as a generic predictor of fluoroquinolone antimicrobial effect using three strains of Pseudomonas aeruginosa and in vitro pharmacodynamic model. *Antimicrobial Agents and Chemotherapy*, **40**: 627-632.

Maesen F.P.V., Davies B.I., Geraeds W.H. and Baur C. (1987) The use of quinolones in respiratory tract infections. *Drugs*, **34** (Suppl 1), 74-79.

Malmborg A.S. and Rannikko S. (1988) Enoxacin distribution in human tissues after multiple oral administration. *Journal of Antimicrobial Chemotherapy*, **21** (Suppl. B), 57-60.

Martínez-Larrañaga M.R., Díaz M.J., Bringas P., Fernández M.D., Fernández-Cruz M.L., Martínez M.A. and Anadón A. (1994) Bioavalability and residues of enrofloxacin and its metabolite ciprofloxacin in broiler chickens. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 238-239.

McQueen C. and Williams G. (1987) Effects of quinolone antibiotics in tests for genotoxicity. *American Journal of Medicine*, **82** (suppl. 4A): 94-6.

Meinen, J.B., J.T. McClure, and E. Rosin. (1995) Pharmacokinetics of Enrofloxacin in clinically normal dogs and mice and drug pharmacodynamics in neutropenic mice with *E. coli* and staphylococcal infections. *American Journal of Veterinary Research*, **56**: 1219-1224.

Mevius D.J., Breukink H.J., Guelen P.J.M., Jansen T. and De Gréve B. (1990) Pharmacokinetics, metabolism and renal clearance of flumequine in veal calves. *Journal of Veterinary Pharmacology and Therapeutics*, **13**: 159-169.

Montay G., Goueffon Y. and Roquet F. (1984) Absorption, distribution, metabolic fate and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys and humans. *Antimicrobial Agents and Chemotherapy*, **25**: 463-472.

Neer T.M. (1988) Clinical pharmacologic features of fluoroquinolone antimicrobial drugs. *Journal of the American Veterinary Medical Associacion*, **193**: 577-580.

Neu H.C. (1988) Quinolones: A new class of antimicrobial agents with wide potential uses. *Medical Clinics of North America*, **72**: 623-636.

Neu H.C., Kumada T., Chin N.X. and Mandell W. (1987) The post-antimicrobial suppressive effect of quinolone agents. *Drugs in Experimental and Clinical Research*, **13**: 63-67.

Neu H.C. (1991) Synergy and antagonism of combinations with quinolones. *European Journal of Clinical Microbiology and Infectious Diseases*, **10**: 255-261.

Nikaido H. and Thanassi D.G. (1993) Minireview: Penetration of lipophilic agents with multiple protonation sites into bacterial cells: Tetracyclines and fluoroquinolones as examples. *Antimicrobial Agents and Chemotherapy*, **37**: 1393-1399.

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

Nix D.E. and Schentag J.J. (1988) The quinolones: an overview and comparative appraisal of their pharmacokinetics and pharmacodynamics. *Journal of Clinical Pharmacology*, **28**: 169-178.

Nix D.E., Watson W.A., Lener M.E., Frost R..W., Krol G., Goldstein H., Lettieri J. and Schentag J.J. (1989) Effects of aluminium and magnesium antacids and ranitidine on the absorption of ciprofloxacin. *Clinical Pharmacology and Therapeutics*, **46**: 700-705.

Norrby S.R. (1991) Side effects of quinolones: Comparisons between quinolones and other antibiotics. *European Journal of Clinical Microbiology and Infectious Diseases*, **10**: 378-383.

Novotny M.J. and Shaw D.H. (1991) Effect of enrofloxacin on digoxin clearance and steady-state serum concentrations in dogs. *Canadian Joural of Veterinary Research*, **55**: 113-116.

Okazaki O., Kurata T. and Tachizawa H. (1988) Effect of new quinolones on drugmetabolizing enzyme system of rat hepatic microsomes. *Chemotherapy*, **34**: 149-154.

Oomori Y., Yasue T., Aoyma H., Hirai K., Suzue S. and Yokota T. (1988) Effects of fleroxacin on HeLa cell functions and topoisomerase II. *Journal of Antimicrobial Chemotherapy*, **22** (suppl. D): 91-7.

Parpia S., Nix D., Hejmanowski H., Wilton J. and Schentag J. (1989) Sucralfate reduces the gastrointetinal absorption of norfloxacin. *Antimicrobial Agents and Chemotherapy*, **33**: 99-102.

Patil V.K., Keskar D.V., Jagadish S., Bhalerao D.P. and Sharma L.K. (1995) Clinicopathology of urinary tract infections in dogs. *Indian Veterinary Journal*, **72**: 374-377.

Pérez-Trallero E., Urbieta M., Jimenez D., Garcia-Arenzana J.M. and Cilla G. (1993) Ten-year survey of quinolone resistance in *Escherichia coli* causing urinary tract infections. European *Journal of Clinical Microbiology and Infection Diseases*, **12**: 349-351.

Piddock L.J.V. and Wise R. (1989) Mechanism of resistance to quinolones and clinical perspectives. *Journal of*

Antimicrobial Chemotherapy, **23**: 475-483.

Piddock L.J. (1994) New quinolones and Gram-positive bacteria. *Antimicrobial Agents and Chemotherapy*, **38**: 163-169.

Pijpers A., Vernooy J.C.M., Cruijsen A.L.M., Van Leengoed L.A.M.G., Koeman J., Hessels A.H., Vandenhoek J. and Verheijden J.H.M. (1994) Efficacy of parenteral treatment with oxytetracycline and enrofloxacin against Actinobacillus pleuropneumoniae in swine. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 93.

Prasad, V., Krishna Murthy, K. and Janardhana Rao. (1997) In vitro antibiogram studies of *E. coli* in chickens. *Indian Veterinary Journal*, **74**: 616-617.

Preheim L., Cuevas T., Roccaforte J., Mellencamp M. and Bittner M. (1987) Oral ciprofloxacin in the treatment of elderly patients with complicated urinary tract infections due to trimethoprim/sulfamethoxazole-resistant bacteria. *American Journal of Medicine*, **82** (suppl. 4A): 295-297.

Premkumar, D., Purushothaman, V. and Venkatesan, R.A. (1991) Comparison of plasmid profile analysis, antibiogram testing,resistotyping and biotyping in the identification of *E. coli* isolates from poultry. *Veterinary Record*, **129**: 94-97.

Prescott J.F. and Yielding K.M. (1990) *In vitro* susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. *Canadian Journal of Veterinary Research*, **54**: 195-197.

Pyörälä S., Panu S. and Kaartinen L. (1994) Single-dose pharmacokinetics of enrofloxacin in horses. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland,* p. 45-46.

Raemdonck, D.L., A.C. Tanner, S.T. Tolling, and S.L. Michener. (1992) In vitro susceptibility of avian *E. coli* and *Pasteurella multocida* to Danofloxacin and five other antimicrobials. *Avian Diseases*, **36**: 964-967.

Ramadan A., Afifi N.A., Atef M. (1994) Pharmacokinetics of Norfloxacin in healthy and *E. coli*-infected chickens after single intravenous and single and multiple oral doses of the drugs. In Abstract *Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*; pp. 237-238.

Ramon J., Dautrey S., Farinoti R., Carbón C., Rubenstein E. (1994) Intestinal elimination of ciprofloxacin in rabbits. *Antimicrobial Agents and Chemotherapy*, **38**: 757-760.

Reece, R.J. and Maxwell A. (1991) DNA gyrase: structure and function. *Critical Review of Biochemichal and Molecular Biology*, **26**: 335-375.

Rhoades K.R. and Heddleston K.L. (1980) Pasteurellosis. In: Isolation and identification of avian pathogens, 2nd ed. Eds: Hitchner S.B., Domermuth C.H., Purchase H.G. and Williams J.E. *American Association of Avian Pathologists, Collage Station, Texas*, pp. 11-15.

Richez P., Dellac B. and Froyman R. (1994) Pharmacokinetics of enrofloxacin in pigs after single and repeated in-feed medication with Baytril 2.5%. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland*, p. 207-208.

Ridgway G.L., Mumtaz G., Gabriel F.G., Oriel J.D. (1984) The activity of ciprofloxacin and other 4-quinolones against *Chlamydia trachomatis* and Mycoplasmas in vitro. *European Journal of Clinical Microbiology*, **3**: 344-346.

Rimler R.B. and Glisson J.R. (1997) Fowl cholera. In: Diseases of poultry, 10th ed. Eds: Calnek B.W., Barnes H.J., Beard C.W., McDougald L.R. and Y.M. Saif. *Iowa State University Press, Ames, Iowa*. pp. 143-159.

Rowan R.C., Mullenix T.A., Arroyo J.C. and Voris J.C. (1988) Development of *Pseudomonas aeruginosa* resistance to norfloxacin during therapy. *Drug Intelligance and Clinical Pharmacy*, **22**: 773-776.

Rybak M.J., Bowles S.K., Chandrasekar P.H. and Edwards D.J. (1987) Increased theophylline concentration secondary to ciprofloxacin. *Drug Intelligence and Clinical Pharmacy*, **21**: 879-881.

Sárközy, G. and Laczay, P. (2002) Comparative study on the efficacy of difloxacin and enrofloxacin in an experimentally induced *Escherichia coli* infection in broilers. *Proceedings of the 12th World Veterinary Poultry Association Congress, Cairo, Egypt*. P. 226.

Sárközy G., Semjén G., Laczay P., Horváth E. and Schmidt J. (2002a) Pulse and continuous oral norfloxacin treatment of experimentally induced *Escherichia coli* infection in broiler chicks and turkey poults. *Acta Veterinaria Hungarica* **50** (2): 199-210.

Sárközy G., Semjén G., Laczay P. and Horváth E. (2002b) Treatment of experimentally induced *Pasteurella multocida* infections in broilers and turkeys - Comparative studies on different oral treatment regimens. *Journal of Veterinary Medicine Series B*, **49** (3): 199-210.

Schentag, J.J., D.E. Nix, and A. Forrest. (1993) Pharmacodynamics of the fluoroquinolones. In D.C. Hooper and J.S. Wolfson. Quinolone antimicrobial agents, 2nd ed. *American Society for Microbiology, Washington D.C.* pp. 259-271.

Semjén G. and Blaskó É. (1994) In vitro emergence of resistance of flumequine and enrofloxacin in strains of Staphylococcus aureus. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland*, p. 239-240.

Sheer M. (1987) Concentrations of active ingredient in the serum and in tissues after oral and parenteral administration of Baytril. *Veterinary Medicine Reviews*, **2**: 104-118.

Shungu D.L., Tutlane V.K., Weinberg E., et al. (1985) In vitro antibacterial activity of Norfloxacin and other agents against ocular pathogens. *Chemotherapy* (Bassel), **31**:112-118.

Smith J.T. (1984) Mutational Resistance to 4-quinolone antibacterial agents. *European Journal of Clinical Microbiology*, **3**: 347-350.

Smith J.T. (1986) The mode of action of 4-quinolones and possible mechanisms of resistance. *Journal of Antimicrobial Chemotherapy*, **18** (suppl. D): 21-9.

Soback S., Gips M. and Bialer M. (1994a) Norfloxacin nicotinate pharmacokinetics in unweaned and weaned calves. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland*, p. 63-64.

Soback S., Gips M., Bialer M. and Bor A. (1994b) Effect of lactation on single-dose pharmacokinetics of norfloxacin nicotinate in ewes. *Antimicrobial Agents and Chemotherapy*, **38**: 2336-2339.

Sorgel F., Seelman R., Naber K., Metz R. and Muth P. (1988) Metabolism of fleroxacin in man. *J. Antimicrobial Agents and Chemotherapy*, **22** (suppl. D): 169-78.

Sorgel F., Jaehde U., Naber K. and Stephan U. (1989) Pharmacokinetic disposition of fluoroquinolones in human body fluids and tissues. *Clinical Pharmacokinetics*, **16** (Suppl. 1), 5-24.

Stefan S., Marin G. and Bialer M. (1994) Norfloxacin nicotinate pharmacokinetics in unweaned and weaned calves. *European Association of Veterinary Pharmacology and Toxicology*; **6**: 62-63.

Studdert V.P. and Hughes K.L. (1992) Treatment of opportunistic mycobacterial infections with enrofloxacin in cats. *Journal of the American Veterinary Medical Association*, **201**: 1388-1390.

Sullivan M.C., Cooper B.W., Nightingale C.H., Quintiliani R. and Lawlor M.T. (1993) Evaluation of the efficacy of ciprofloxacin against *Streptococcus pneumoniae* by using a mouse protection model. *Antimicrobial Agents and Chemotherapy*, **37**: 234-239.

Swanson R.N., Hardy D.J., Chu D.T.W., Shipkowitz N.L. and Clement J.J. (1991) Activity of temafloxacin against respiratory pathogens. *Antimicrobial Agents and Chemotherapy*, **35**: 423-429.

Takayama S., Watanabe T., Akiyama Y. et al. (1986) Reproductive toxicity of ofloxacin. *Arzneimittelforschung*, **36**: 1244-8.

Takács-Novák K., Noszal B., Hermecz I., Keresztúri G., Podányi B. and Szász G. (1990) Protonation equilibria of quinolone antibacterials. *Journal of Pharmaceutical Sciences*, **79**: 1023-1028.

Thomas V., Deleforge J. and Bolsramé B. (1994a) Pharmacokinetics of marbofloxacin in preruminant and ruminant cattle. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland,* p. 60-61.

Thomas V., Deleforge J., Bolsramé B. and Espinasse J. (1994b) Pharmacokinetics of marboflaxicin in healthy and sick pre-ruminant calves. *Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland*, p. 61.

Thomas J.K., Forrest A., Bhavnani S.M., Hyatt J.M., Cheng A., Ballow C.H. et al. (1998) Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. *Antimicrobial Agents and Chemotherapy*, **42**: 521-527.

Thoung-Guyot M., Domarle O., Pacidalo J.J. and Hayem G. (1994) Effects of fluoroquinolones on cultured articular chondrocytes flow cytometric analysis of free radical production. *Journal of Pharmacology and Experimental Therapeutics*, **271**: 1544-1549.

VanCutsem P.M., Babish J.G. and Schwark W.S. (1990) The fluoroquinolone antimicrobials: Structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. *Cornell Veterinarian*, **80**: 173-186.

Veere, B.M., Krishna Murthy, G.V., Upadhye, A.S. and Raghavan, R. (1996) Serotypes of *E. coli* pathological conditions in poultry and their antibiogram. *Indian Veterinary Journal*, **73** (February): 123-126.

Venezia R.A., Prymas L.A., Shayegani A. and Yocum D.M. (1989) In vitro activities of amifloxacin and two of its metabolites. *Antimicrobial Agents and Chemotherapy*, **33**: 762-766.

Voight W.H. (1987) Electron microscopic studies on the effect of a quinolone carboxylic acid derivative on the ultrastructure of *Escherichia coli* and staphylococcal bacteria *in vitro. Veterinary Medicine reviews*, **2**: 119-121.

Walker R.D., Stein G.E., Hauptman J.G., MacDonald K.H., Budsberg S.C. and Rosser E.J.jr. (1990) Serum and tissue cage fluid concentrations of ciprofloxacin after oral administration of the drug to healthy dogs. *American Journal of Veterinary Research*, **51**: 896-900.

Walker R.D., Stein G.E., Budsberg S.C., Rosser E.J.jr and MacDonald K.H. (1989) Serum and tissue fluid norfloxacin concentrations after oral administration of the drug to healthy dogs. *American Journal of Veterinary Research*, **50**: 154-157.

Walker R.D., Stein G.E., Hauptman J.G. and McDonald K.H. (1992) Pharmacokinetic evaluation of enrofloxacin administered orally to healthy dogs. *American Journal of Veterinary Research*, **53**: 2315-2319.

Walser M.M. and Davis R.B. (1985) In vitro characterization of field isolants of *Pasteurella multocida* from Georgia turkeys. *Avian Diseases*, **29**: 1094-1107.

Watanabe M., Kotera Y., Yosue K., Inoue M. and Mitsuhashi S. (1990) In vitro emergence of quinolone-resistant mutants of *E. coli*, Enterobacter cloaceae and Serratia marcescens. *Antimicrobial Agents and Chemotherapy*, **34**: 173-175.

Wise, R. (1984) Norfloxacin-a review of pharmacology and tissue penetration. *Journal of Antimicrobial Chemotherapy*, **13**: 59-64 (Suppl. B).

Wolfson J.S. and Hooper D.C. (1985) The fluoroquinolones: structures, mechanism of action and resistance, and spectra of activity in vitro. *Antimicrobial Agents and Chemoterapy*; **28**: 581-586.

Wolfson, J.S. and Hooper, D.C. (1988) Norfloxacin: a new targeted fluoroquinolone antimicrobial agent. *Annual International Medicine*, **108**: 238-251.

Wolfson J.S. and Hooper D.C. (1991) Pharmacokinetics of quinolones: newer aspects. *European Journal of Clinical Microbiology and Infectious Diseases*, **10**: 267-274.

Wray, C., R.H. Davies, and J.D. Corkish. (1996) Enterobacteriaceae. In F.T.W. Jordan and M. Pattison (ed.), *Poultry Diseases. W.B. Saunders, London, UK.* pp. 9-43.

Yamamoto T., Watanabe K., Horikita T. et al. (1992) Therapeutic efficacy of enrofloxacin to swine Actinobacillus pleuropneumoniae infection. *Kachiku Shinryo*, **352**: 19-27

Van de Zande, S., Nauwynk, H. and Panseart, M. (2001) The clinical, pathological and microbiological outcome of an *Escherichia coli* O2:K1 infection in avian pneumovirus infected turkeys. *Veterinary Microbiology*, **81**: 353-365.

Zhanel G.G., Walters M., Laing N. and Hoban D.J. (2001) *In vitro* pharmacodynamic modeling simulating free serum concentrations of fluoroquinolones against multidrug-resistant *Streptococcus pneumoniae*. *Journal of Antimicrobial Chemotherapy*, **47**: 435-440.

Zweerink M.M. and Edison A.M. (1988) The uptake of 3H-norfloxacin by human polymorphonuclear leukocytes. *Journal of Antimicrobial Chemotherapy*, **21**: 266-267.