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PULSE AND CONTINUOUS ORAL NORFLOXACIN TREATMENT OF EXPERIMENTALLY INDUCED *ESCHERICHIA COLI* INFECTION IN BROILER CHICKS AND TURKEY POULTS

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Experimental colibacillosis was produced in 40 healthy, 7-day-old broiler chickens and turkeys by intratracheal injection of 1×10^8 CFU/chick and 1.23×10^9 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 infectious 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, body 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 via the drinking water at 15 mg/kg body weight 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. The results confirmed earlier observations on the pharmacokinetic properties of norfloxacin in chicks and turkeys (Laczay et al., 1998).

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

Colibacillosis, caused by *Escherichia coli* (*E. coli*), is a bacterial infection which may result in septicaemia, respiratory tract infections, pericarditis, peritonitis and airsacculitis in chickens and turkeys. *E. coli* may also be associated with other agents, such as infectious bronchitis virus (IBV), Newcastle disease virus (NDV) including vaccine strains, *Mycoplasma* spp. and *Pasteurella* spp., causing the respiratory disease complex (Barnes and Gross, 1997). Although this disease is associated with a number of pathogens, infection with *E. coli* is of particular

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concern because it commonly progresses to a more generalised condition associated with high mortality and condemnation losses at processing. Stress and exposure to poultry house dust and ammonia provide additional predisposing factors that may result in damage to the mucosal lining of the respiratory epithelium, and thus they contribute to invasion by *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 the concentration of the drug rather than on 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 had been 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). The strain 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 h at 37 °C in stationary culture. The minimum inhibitory concentration (MIC) of norfloxacin for the *E. coli* isolate was determined using the broth dilution method, and was found to be 0.02 μg/ml.

Poultry

Forty clinically healthy, 7-day-old Ross broiler chickens (Prophyl Ltd., Mohács, Hungary), and forty clinically healthy 7-day-old Arbor 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 acclimatisation period all birds were infected as follows. In order to make chickens more susceptible to the *E. coli* infection, 2 days prior to the infection all broiler chickens were vaccinated via the drinking water, according to the manufacturer's recommendation, with Bronchovac II (Ceva-Phylaxia Co. Ltd., Budapest) containing infectious bronchitis virus attenuated strain H-52. 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 1×10^8 CFU for chickens and 1.23×10^9 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 the 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 the drinking water (100 mg/L) and was available to the birds *ad libitum*. In Group 3 (receiving once-per-day pulse-dose) drinking water was withheld for 2 h; thereafter, the calculated daily dose (15 mg/kg body weight) of the drug was mixed with one-fourth 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.

Clinical follow-up

During the acclimatisation period (3 days) and the experimental phase (7 days from the infection) all animals were examined twice a day at 12-h intervals.

Clinical signs were scored, and mortality was recorded. During the experimental phase animals were inspected individually for signs considered to be characteristic of 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).

Table 1

Experimental design

	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

Postmortem examination

All birds that died during the experiments were necropsied and examined for gross lesions. All birds remained alive were euthanised 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 of colibacillosis were scored for their severity and expressed as the average of one bird for each sign. These included acute, haemorrhagic, 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 air sac) were cultivated on blood and dextrose-starch agar (DSA, Difco Labs., USA) for 18 h at 37 °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) of norfloxacin for *E. coli* isolates was determined using the 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 ($P \leq 0.05$):

– calculated t (T_{calc}) < critical t (T_{cri}) meant not significant,

– while 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 non-medicated control chicks pretreated with infectious bronchitis vaccine (Group 1). Clinical signs of depression and listlessness, diarrhoea and severe weakness developed within 24 h after inoculation. The most severe clinical signs were observed at 48 h post infection. These included a drop in water and feed 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 the turkey trial, infected and non-medicated control birds (Group 1) showed mild clinical signs including depression and diarrhoea between 24 and 72 h post infection. Significant difference was observed between Group 1 and Groups 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.

Table 2

Cumulated base data of the chicken inoculation experiment

	7-day-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)	1×10^8	1×10^8	1×10^8
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]	$12.40 \pm 6.32^*$	23.48 ± 9.50	22.52 ± 6.71
Average daily clinical score[2]	$1.77 \pm 1.27^*$	$0.25 \pm 0.11^{**}$	$0.18 \pm 0.08^{**}$
Birds died during experiment	4^*	0	0
Postmortem score[3]	$9.00 \pm 4.57^*$	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 average weight gain/number of days; [2]Total daily mean clinical score/number of days; [3]Total postmortem score/number of birds; *Significant difference between control (Group 1) versus treated groups (Groups 2 and 3); **Significant difference between Group 2 versus Group 3

Weight gain

Following infection with *E. coli*, both treatments produced significantly higher weight gain in surviving broiler chickens, compared with non-medicated 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 between treated Groups 2 and 3 in chickens (T_{calc}: 0.22 T_{cri}: 2.18).

There was no significant divergence between Group 2 and Group 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 non-medicated control group (Group 1) was 40%, while in the treated groups none of the birds died.

Table 3

Cumulated base data of the turkey inoculation experiment

	7-day-old turkey poults		
	Group 1 Control	Group 2 Continuous dosing	Group 3 Pulse dosing
No. of animals	10	15	15
Inoculation (CFU)	1.23×10^9	1.23×10^9	1.23×10^9
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
Postmortem 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 average weight gain/number of days; [2]Total daily mean clinical score/number of days; [3]Total postmortem score/number of birds; *Significant difference between control (Group 1) versus treated groups (Groups 2 and 3)

In the turkey experiment 10% of birds in Group 1 died due to 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 be found, though coliform colonies were isolated from the lung of one bird and from the air sac of 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, and acute haemorrhagic-catarrhal enteritis were observed in all chickens and poults that died in Group 1.

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

Only 5 surviving turkeys of Group 2 showed signs of infection, whereas in Group 3 only three animals showed mild lesions including diarrhoea and catarrhal enteritis. Three birds in Group 2 died without showing any clinical signs due to intercurrent disease. There was no significant difference among Groups 1, 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 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 bacteria 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 the coliforms in Group 2 or 3 was identified as the F11+ strain used for infection. The remaining strains from Group 2 were haemolytic Gram-positive cocci, mixed cultures and in 3 chickens *S.* Enteritidis 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 the infected and treated groups as well. In euthanised birds of Group 1 we found 9 samples (of a total of 60 samples) from which the F11+ strain of *E. coli* could be cultured. In both Groups 2 and 3, where bacteria were isolated from 10 and 8 samples, respectively, there were 3 samples (of a total of 90 samples) with F11+ *E. coli* growth.

Water consumption, drug intake

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

MIC

The *E. coli* strain used for infection was susceptible to norfloxacin (MIC 0.02 μg/ml). All reisolated strains were also sensitive to norfloxacin with model 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 than 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 regimens has not been well documented for poultry. In the present study, intratracheal infection of IB-pretreated 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 5×10^5 CFU of *E. coli* (freshly isolated from chicken with colibacillosis, not typed) gave rise to 20% mortality and 60% morbidity. In a recently published study with difloxacin and enrofloxacin, where the chickens received 2.5×10^9 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 of colibacillosis in the field. Dunnington et al. (1991) showed a mortality of 5% for chickens challenged with less than 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 numbers 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 than 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-characterised differences were observed in daily clinical scores and postmortem scores in favour 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 a PAE lasting 8 hours (Neu et al., 1987), fluoroquinolone treatment gives better results when used as continuous medication in the drinking water. Other studies showed that severe bacterial infections can be treated more successfully with high doses of antimicrobials (Ando 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 schedules.

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 the 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 regimens, yet we are not in the position to prove the superiority of either administration method. Additional studies are required to improve our model for colibacillosis in turkey and to compare different norfloxacin treatment regimes.

References

Allan, B. J., Van den Hurk, J. and Potter, A. A. (1993): Characterization of *E. coli* isolated from cases of avian colibacillosis, Can. J. Vet. Res. **57,** 146–151.

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.

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

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

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

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

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

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.

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. J. Vet. Pharmacol. Therap. **21,** 161–164.

Lublin, A., Mechani, S., Malkinson, M. and Weisman, Y. (1993): Efficacy of norfloxacin nicotinate treatment of broiler breeders against *Haemophilus paragallinarum*. Avian Dis. **37,** 673–679.

Madaras-Kelly, K. J., Ostergaard, B. E., Backer Horde, L. and Rotschafer, J. C. (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. Antimicrob. Agents Chemother. **40,** 627–632.

Meinen, J. B., McClure, J. T. and Rosin, E. (1995): Pharmacokinetics of enrofloxacin in clinically normal dogs and mice and drug pharmacodynamics in neutropenic mice with *E. coli* and staphylococcal infections. Am. J. Vet. Res. **56,** 1219–1224.

Neu, H. C., Kumada, T., Chin, N. X. and Mandell, W. (1987): The post-antimicrobial suppressive effect of quinolone agents. Drugs Exptl. Clin. Res. **XIII(2),** 63–67.

Prasad, V., Krishna Murthy, K. and Janardhana, R. (1997): *In vitro* antibiogram studies of *E. coli* in chickens. Indian Vet. J. **74,** 616–617.

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. Vet. Rec. **129,** 94–97.

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

Reece, R. J. and Maxwell, A. (1991): DNA gyrase: structure and function. Crit. Rev. Biochem. Mol. Biol. **26,** 335–375.

Sárközy, G. and Laczay, P. (2001): 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. 396.

Schentag, J. J., Nix, D. E. and Forrest, A. (1993): Pharmacodynamics of the fluoroquinolones. In: Hooper, D. C. and Wolfson, J. S. (eds) Quinolone Antimicrobial Agents, 2nd ed. American Society for Microbiology, Washington D.C. pp. 259–271.

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. Vet. Microbiol. **81,** 353–365.

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 Vet. J. **73,** 123–126.

Wise, R. (1984): Norfloxacin – a review of pharmacology and tissue penetration. J. Antimicrobiol. Chemother. **13,** 59–64. (Suppl. B).

Wolfson, J. S. and Hooper, D. C. (1988): Norfloxacin: a new targeted fluoroquinolone antimicrobial agent. Ann. Intern. Med. **108,** 238–251.

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