Comparison of the Efficacies of Three Fluoroquinolone Antimicrobial Agents, Given as Continuous or Pulsed-Water Medication, against *Escherichia coli* Infection in Chickens

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This study compared the efficacy of continuous or pulsed-water medication with enrofloxacin, danofloxacin, and sarafloxacin in eight groups of 90 chicks each by using an infectious bronchitis virus-*Escherichia coli* model of colisepticemia. The model produced lesions of typical those occurring in birds with severe colisepticemia; for the infected, nonmedicated birds the mortality was 43.5% and the morbidity was 89%, 17.8% of birds had severe lesions, and the birds had a mean air sac lesion score of 2.58. This experiment showed that continuous dosing and pulsed dosing are clinically equivalent. However, for all fluoroquinolones studied, there was a trend for the continuously medicated birds to have lower mortality and less severe disease than birds receiving pulsed doses. Compared with infected, nonmedicated controls, only birds continuously medicated with enrofloxacin had a significantly lower morbidity (32%), and only birds medicated with enrofloxacin and danofloxacin (continuous and pulsed treatments) had significantly lower mortality (6.7 and 11.0% and 16.8 and 19.2% for continuous and pulsed treatments with enrofloxacin and danofloxacin, respectively). A significantly lower proportion of birds only in the groups medicated with enrofloxacin had severe lesions (for birds receiving continuous and pulsed treatments, 2.2 and 6.7%, respectively). Birds medicated with any of the three fluoroquinolones (continuous and pulsed treatments) except pulsed-water treatment with sarafloxacin had significantly reduced mean air sac lesion scores compared with the scores for nonmedicated birds (air sac lesion scores, 0.60 and 0.83, 1.38 and 1.63, and 1.80 and 2.05 for birds receiving continuous and pulsed treatments with enrofloxacin, danofloxacin, and sarafloxacin, respectively). The performance of the birds that survived the challenge or that recovered after receiving medication was not compromised compared to the performance of noninfected birds. Enrofloxacin was more efficacious than either danofloxacin or sarafloxacin for the treatment of colisepticemia in chickens by medication in drinking water. Similarly, danofloxacin appeared to be more effective than sarafloxacin in treating colisepticemia.

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with the remaining daily water supply being antibiotic free. If enrofloxacin is given in a pulsed manner, the peak levels in serum and lungs are three- and fourfold higher, respectively, than the steady-state concentrations observed in birds dosed continuously throughout the day. It has been suggested (7), on the basis of the results obtained with a neutrophenic rat model and the fluoroquinolone lomefloxacin, that the once-daily administration of a drug dose which produced a high peak concentration in serum/MIC ratio is more efficacious than regimens with the same daily dose but given on a more fractionated schedule. However, for poultry this hypothesis has never been verified in a clinical setting with immunocompetent birds.

The objectives of the current experiment were to compare the efficacies of enrofloxacin, danofloxacin, and sarafloxacin for the treatment of disease due to experimental infection with *E. coli* 2- or 4-week-old chickens, and to assess whether pulsed dosing would improve the clinical outcome of therapy. The model used in this study was designed to produce a severe *E. coli* pathology so that statistically significant differences could be demonstrated between the treatment groups.

**MATERIALS AND METHODS**

**Birds and husbandry.** Seven hundred twenty unvaccinated Ross 1 broiler type chicks of mixed sex were obtained on the day of hatching from a commercial hatchery. The birds were shown to be free of infection with **Mycoplasma** spp. The birds were allocated to eight groups (groups 1 to 8) on a cavity with 6.5 log 10 50% ciliostatic doses (CD 50s) of IBV M41 (5). The embryo coccidiostats, and other chemical or biological growth promoters prior to the dom) which had been assayed to determine that it was free of antibiotics, that after 2 weeks the temperature was 25°C. Each room underwent 20 air changes per hour. A continuous lighting pattern was used (24 h of light each day), and feed and water (in bell drinkers) were provided ad libitum. The daily water intake of each replicate was recorded prior to and during medication. Birds were reared on a chick feed (HPG Feed; SDS Ltd., Cambridge, United Kingdom) which had been assayed to determine that it was free of antibiotics, coccidiostats, and other chemical or biological growth promoters prior to the start of the trial.

**Vaccine.** An 11-day-old embryonated egg was inoculated via the allantoic cavity with 6.5 log 10 50% ciliostatic doses (CD 50s) of IVB M41 (5). The embryo was killed 30 h after inoculation, and the allantoic fluid was harvested. Aliquots of the harvest fluid were stored at ultracold deep-freeze temperatures (approximated at −80°C), and the stock was thawed and diluted in phosphate-buffered saline before use. The titer of virus in the stored allantoic fluid was 7.5 log 10 CD 50s/ml.

**Bacteriology.** The *E. coli* challenge organism was originally isolated from the diseased air sacs of a chick with a field case of colisepticemia; its serotype was determined to be O2, and it was designated E518. The challenge model had been shown to be reproducible in pilot studies and consistently caused mortality or lesions on the pericardium or peritoneum (6); therefore, a group of 11-day-old chicks of mixed sex were obtained on the day of hatching from a commercial hatchery. The birds were shown to be free of infection with *Mycoplasma* spp. The birds were allocated to eight groups (groups 1 to 8) on a cavity with 6.5 log 10 50% ciliostatic doses (CD 50s) of IBV M41 (5). The embryo coccidiostats, and other chemical or biological growth promoters prior to the dom) which had been assayed to determine that it was free of antibiotics, that after 2 weeks the temperature was 25°C. Each room underwent 20 air changes per hour. A continuous lighting pattern was used (24 h of light each day), and feed and water (in bell drinkers) were provided ad libitum. The daily water intake of each replicate was recorded prior to and during medication. Birds were reared on a chick feed (HPG Feed; SDS Ltd., Cambridge, United Kingdom) which had been assayed to determine that it was free of antibiotics, coccidiostats, and other chemical or biological growth promoters prior to the start of the trial.

**Experimental model.** The experimental model used in this study was based on the model described by Bumstead et al. (3) and Dohoo et al. (6). IVB M41 was used to predispose the birds to colisepticemia and to mimic field cases of the disease. IVB M41 causes only transient cloudiness of the air sacs, with no mortality or lesions on the pericardium or peritonitis (6); therefore, a group of birds challenged with IVB alone was not included in this study. The disease model had been shown to be reproducible in pilot studies and consistently caused 40 to 50% mortality (data not shown). The experimental treatment for each group of birds is summarized in Table 1. At 14 days of age (day 0 of the experiment) each bird in groups 2 to 8 was given an intratracheal dose of 10^8 ciliostatic units of IVB M41 (5) in 0.1 ml of phosphate-buffered saline. Three days later each bird was given an intratracheal challenge of 10^8 CFU of *E. coli* in 0.2 ml of nutrient broth. The MICs of the different fluoroquinolones for the challenge strain were as follows: enrofloxacin, 0.015 μg/ml; danofloxacin, 0.03 μg/ml; and sarafloxacin, 0.03 μg/ml. At the completion of the study (day 21 of the experiment), all the birds were killed and the pericardium, peritoneum, and air sacs were examined. Postmortem examination was also performed within 48 h of death for chicks that were killed or that died during the study.

### Table 1. Summary of group allocation and treatment administration

<table>
<thead>
<tr>
<th>Group Challenge, medication</th>
<th>Dosage (mg/kg/day)</th>
<th>Treatment duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Noninfected, nonmedicated</td>
<td>0 0</td>
<td>Actual</td>
</tr>
<tr>
<td>2 Infected, nonmedicated</td>
<td>0 0</td>
<td>Actual</td>
</tr>
<tr>
<td>3 Infected, continuous enrofloxacin</td>
<td>10 11.3</td>
<td>3</td>
</tr>
<tr>
<td>4 Infected, pulsed enrofloxacin</td>
<td>10 9.5</td>
<td>3</td>
</tr>
<tr>
<td>5 Infected, continuous danofloxacin</td>
<td>5 5.7</td>
<td>3</td>
</tr>
<tr>
<td>6 Infected, pulsed danofloxacin</td>
<td>5 5.2</td>
<td>3</td>
</tr>
<tr>
<td>7 Infected, continuous sarafloxacin</td>
<td>8 8.8</td>
<td>5</td>
</tr>
<tr>
<td>8 Infected, pulsed sarafloxacin</td>
<td>8 8.2</td>
<td>5</td>
</tr>
</tbody>
</table>

| *a* Birds in groups 2 through 8 were infected with IBV on day 0 and *E. coli* on day 3.

**Medication.** At approximately 15 h postchallenge the drinking water was medicated with the appropriate antibiotic treatment (Table 1). Each medicated bird received the recommended duration and at the highest dose specified by the manufactures, i.e., enrofloxacin (Baytril 10% Oral Solution; Bayer) at 10 mg/kg of body weight for 3 days, danofloxacin (Advacicin, 16.7%; Pfizer) at 5 mg/kg for 3 days, and sarafloxacin (Saralox WSP, 10%; Abaxis) at 8 mg/kg for 5 days. Continguously dosed birds received their daily medication over a 24-h period. Pulse-dosed birds received their daily medication during a 4-h period, and their water was antibiotic free for the remaining 20 h of each day. The daily water intake of each replicate was recorded by measuring the water remaining in each bell drinker. Attendants were instructed to report accidental spillage, and losses due to evaporation were assumed to be constant for each replicate. The concentration of each antibiotic in the water to give the required dose per kilogram of body weight was calculated by determining the water consumption and body weight of each replicate of birds on the day of *E. coli* challenge. The actual antibiotic concentration in the water was assayed by high-pressure liquid chromatography to verify the correct dose. The daily consumption of medicated water was recorded for each replicate.

**Efficacy criteria and definitions.** Mortality was defined as the number of birds that were killed or that died before the end of the trial. Morbidity was defined as the number of birds with either air sac, pericardial, or peripheric lesions.

Lesions of colisepticemia were scored as follows. For air sacs, 0 indicates no lesions, 1 indicates cloudiness of air sacs, 2 indicates that air sac membranes are thickened, 3 indicates “meaty” appearance of membranes, with large accumulations of a cheesy exudate confined to one air sac, and 4 is the same as a score of 3 but with lesions in two or more air sacs. For the pericardial lesions, 0 indicates no visible lesions, 1 indicates excessive clear or cloudy fluid in the pericardium, and 2 indicates extensive fibrination in the pericardial cavity. For peripheric lesions, 0 indicates no visible lesions, 1 indicates definite fibration on the surface of the liver, and 2 indicates extensive fibrination, adhesions, liver swelling, and necrosis.

Birds with severe lesions were characterized as having an air sac lesion score of 4 and pericarditis and peripheritis scores of either 1 or 2. The mean body weight of the birds in each replicate was measured at 1 day of age and on days 2, 4, 7, and 21 of the experiment. A feed conversion ratio was calculated for each group of birds by taking the total amount of feed consumed by each replicate between days 4 and 21 and dividing it by the increase in mass of the birds over the same time period (data not corrected for dead birds). An index was constructed in order to estimate the overall clinical efficacy of each treatment.

The clinical efficacy index for group *n* is calculated as follows: morbidity (1) = 1 − ([1 − (group *n*/ group 2 − group 1)] × 100), mortality (b) = 1 − ([1 − (group *n*/ group 2 − group 1)] × 100), mean lesion score (c) = 1 − ([1 − (group *n*/ group 2 − group 1)] × 100), and clinical efficacy index = (a + b + c)/3.

**Statistical analyses.** The statistical significance of differences in the method of antibiotic administration on various factors was established by analysis of variance (ANOVA), with treatment method, antibiotic, and room used as variabes. Differences between antibiotic treatments were established by ANOVA, with antibiotic, treatment method, and room used as variables but with only data from two antibiotic groups being used. Differences between individual groups were established by Student’s *t* test. A significance level of 5% was used. No effect of room was detected in any of the analyses.

**RESULTS**

**Medication in water.** Table 1 presents the antibiotic intake for each group of birds. No incidents of water spillage were reported. The actual antibiotic intake for each group did not
The pathological signs and production parameters for each group of birds are presented in Table 2. There was no significant effect of the method of antibiotic medication (continuous or pulsed) on mortality, morbidity, mean air sac lesion score and clinical efficacy index (Fig. 1). However, fewer birds in the continuously dosed groups than in the pulsed-dosed groups had severe lesions (Table 3). The method of antibiotic medication had no effect on the final body weight or the feed conversion ratio between days 2 and 21 of the experiment.

Efficacies of fluoroquinolone treatments against \textit{E. coli} infection. The pathological signs and production parameters measured for all the medicated and control groups are summarized in Table 2, and the statistical significance of any differences between the treatments are summarized in Table 3. Generally, birds treated with fluoroquinolones had lower mortalities, morbidities, and mean air sac lesion scores and higher clinical efficacy indices than birds treated with danofloxacin and sarafloxacin, and a lower proportion of birds treated with enrofloxacin than birds treated with danofloxacin and sarafloxacin had severe lesions. In addition, the mortality for the enrofloxacin-treated birds was significantly lower than that for the sarafloxacin-treated birds. Due to the variation in mortality among replicate pens, no difference between enrofloxacin and danofloxacin could be shown for this specific criterion. Nevertheless from birds in 12 replicate cages treated with enrofloxacin, 4 had zero mortality, whereas only 1 of the 12 danofloxacin-treated replicates had zero mortality. Danofloxacin-treated birds had significantly lower mortalities and mean air sac lesion scores than sarafloxacin-treated birds.

**Bacteriology.** The bacteriological results are presented in Table 4. All of the isolates from birds in groups 2 to 8 were serovar O2, which was the same serovar as the \textit{E. coli} challenge strain. The numbers of birds positive for \textit{E. coli} was low, and visual inspection of the data did not suggest that there were significant differences between the groups, so statistical analysis of the data was not performed. Only birds continuously medicated with enrofloxacin were completely negative for \textit{E. coli}.

**DISCUSSION**

The \textit{E. coli} model used in this experiment produced 43.5% mortality and 89% morbidity in infected, nonmedicated birds. These levels are much higher than the 5% mortality and up to 50% morbidity suggested by Wray et al. (30) as being typical.

<table>
<thead>
<tr>
<th>% Mortality</th>
<th>% Morbidity</th>
<th>Mean air sac lesion score</th>
<th>% Birds with severe lesions</th>
<th>Clinical efficacy index</th>
<th>Mean total cage wt (g) of surviving birds (day 21)</th>
<th>Feed conversion ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>19.253 \pm 598</td>
<td>2.2 \pm 0.2</td>
</tr>
<tr>
<td>2</td>
<td>43.5 \pm 15.2</td>
<td>88.8 \pm 29.8</td>
<td>2.6 \pm 0.5</td>
<td>17.8 \pm 10.9</td>
<td>10.082 \pm 2.964</td>
<td>2.8 \pm 0.6</td>
</tr>
<tr>
<td>3</td>
<td>6.7 \pm 4.6</td>
<td>32.3 \pm 13.6</td>
<td>0.6 \pm 0.4</td>
<td>2.2 \pm 3.4</td>
<td>17.642 \pm 1.797</td>
<td>2.2 \pm 0.2</td>
</tr>
<tr>
<td>4</td>
<td>11.0 \pm 6.8</td>
<td>45.3 \pm 10.6</td>
<td>0.8 \pm 0.3</td>
<td>6.7 \pm 6.0</td>
<td>17.607 \pm 479</td>
<td>2.1 \pm 0.1</td>
</tr>
<tr>
<td>5</td>
<td>16.8 \pm 9.3</td>
<td>59.1 \pm 12.1</td>
<td>1.4 \pm 0.5</td>
<td>11.3 \pm 7.0</td>
<td>15.487 \pm 1.402</td>
<td>2.1 \pm 0.3</td>
</tr>
<tr>
<td>6</td>
<td>19.2 \pm 16.7</td>
<td>67.5 \pm 14.1</td>
<td>1.6 \pm 0.6</td>
<td>12.5 \pm 7.9</td>
<td>16.438 \pm 3.174</td>
<td>2.1 \pm 0.3</td>
</tr>
<tr>
<td>7</td>
<td>20.0 \pm 8.2</td>
<td>73.0 \pm 0.0</td>
<td>1.8 \pm 0.2</td>
<td>7.8 \pm 6.6</td>
<td>16.041 \pm 2.430</td>
<td>2.2 \pm 0.3</td>
</tr>
<tr>
<td>8</td>
<td>27.2 \pm 17.2</td>
<td>79.3 \pm 12.5</td>
<td>2.1 \pm 0.6</td>
<td>19.9 \pm 9.9</td>
<td>13.023 \pm 4.090</td>
<td>2.6 \pm 0.6</td>
</tr>
</tbody>
</table>

* Values are means \pm standard deviations.
* Air sac lesions were scored on a scale of from 0 to 4 (see text).
* The feed conversion ratio is calculated for each group of birds by taking the total feed consumed between days 2 and 21 and dividing it by the increase in the mass of the birds over the same time period (data not corrected for dead birds).
* Significantly different from group 1 \((P < 0.05)\).
* Significantly different from group 2 \((P < 0.05)\).
for colisepticemia in the field. The mortality observed in this study is consistent with that observed in a number of other colisepticemia models (3, 6, 8, 9, 31). For example, Dunnington et al. (8) 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 our model the high mortality observed among the infected, non-medicated birds may be related to the high challenge dose ($10^6$ CFU) given. To enable comparisons of efficacy between products and treatment 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.

The birds that survived the *E. coli* challenge or that recovered after medication grew at a rate similar to that for the noninfected birds between days 14 and 21 of the study (data not shown). It appeared that birds with mild and severe lesions grew at the same rate, but birds with severe lesions tended to be in the groups with high mortality rates. The reduction in competition for cage and trough space may allow the growth rate of birds with severe lesions to be higher than expected.

This experiment showed that compared to continuous dosing, pulsed dosing of fluoroquinolones does not significantly reduce pathological signs and mortality; indeed, there was a trend for lower mortality and fewer pathological signs in the continuously dosed birds than in the pulse-dosed birds. The comparisons between the pulse-dosed and continuously dosed birds were consistent for all three fluoroquinolones studied.

It is generally accepted that fluoroquinolones act in a concentration-dependent manner (22, 24). Recent findings (18) 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 antimicrobial compound to the infectious agent, is the most descriptive pharmacodynamic predictor of the antibacterial activities of fluoroquinolone antibiotics. The similar efficacies of continuous and pulsed dosing obtained in our in vivo study with immunocompetent birds supports the pharmacodynamic findings that both the magnitude of exposure (peak concentration) and the duration of exposure (time above the MIC) are important for an optimal antimicrobial effect. The results of this study also confirm the findings of Meinen et al. (21), who attempted to correlate the pharmacokinetics of enrofloxacin in healthy dogs and mice to the pharmacodynamics in neutropenic mice infected with *E. coli* or staphylococci. The latter study showed that the total dose of enrofloxacin rather than the frequency of dosing was significant in determining drug efficacy. The assumption that the AUC/MIC ratio is the best predictor of clinical efficacy and the comparable clinical outcomes from pulsed and continuous dosing in our study are corroborated by a kinetic study of Stegemann (27). In that work it was shown that the serum AUC and lung AUC for broilers given 10 mg of enrofloxacin/kg of body weight continuously (24 h) or as a pulsed medication (3 h) in drinking water were close to identical, even though the maximum concentration in serum by the pulsed-

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**TABLE 4. Bacteriology results**

<table>
<thead>
<tr>
<th>Group no.</th>
<th>No. of birds positive for <em>E. coli</em> no. of birds sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/15</td>
</tr>
<tr>
<td>2</td>
<td>2/15</td>
</tr>
<tr>
<td>3</td>
<td>0/15</td>
</tr>
<tr>
<td>4</td>
<td>2/15</td>
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<tr>
<td>5</td>
<td>3/15</td>
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<tr>
<td>7</td>
<td>1/15</td>
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<tr>
<td>8</td>
<td>4/15</td>
</tr>
</tbody>
</table>

* The airsacs of 15 birds per group were sampled postmortem. The numbers of swabs positive for *E. coli* are presented for each group.

* The treatment for each group is described in Table 1.

* The serotype of isolate was not the same as that of the challenge strain.
dosing regimen (1.1 μg/ml) was almost three times higher than the mean steady-state concentration (0.38 μg/ml) by the continuous medication program.

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, e.g., broilers on a permanent light program versus layer replacements which receive restricted lighting. Nevertheless, from observations of the differences between continuous and pulsed dosage, it could be recommended that the time of the pulsed-dose administration be not shorter than 4 h.

The treatments with all the fluoroquinolone antibiotics reduced colibacillosis in this experimental study. However, in general the performance of birds that survived the challenge or that recovered after receiving medication was not compromised compared to the performance of noninfected birds. Even though all the fluoroquinolones could be shown to be efficacious, marked differences among the three distinct drugs were obvious, as exemplified by the clinical efficacy index (Fig. 1), which collates all the clinical criteria.

On the basis of the results obtained with this experimental challenge model, enrofloxacin is more efficacious than either danofloxacin or sarafloxacin for the treatment of colisepticemia in chickens by water medication. Similarly, danofloxacin was more effective than sarafloxacin in treating colisepticemia.

REFERENCES