

Study of enrofloxacin and flumequine residues depletion in eggs of laying hens after oral administration

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Two groups of laying hens (each $n = 12$) were administered 10 mg/kg enrofloxacin (ENRO) (group A) or 26.6 mg/kg flumequine (FLU) (group B) by gastric catheter daily for five consecutive days. A third group ($n = 6$) was untreated controls. Eggs were collected from day one of treatment and up to 30 days after withdrawal of the drug. Egg white and yolk from each egg were separated, and ENRO, its metabolite ciprofloxacin (CIP) and FLU residues were analysed by a high-performance liquid chromatography method with fluorescence detection. The sum of ENRO and CIP was detectable in egg white on the first day of treatment in high-level concentrations (2007.7 $\mu\text{g}/\text{kg}$) and remained steady during administration. In egg yolk, residues were detectable at day one in lower concentrations (324.4 $\mu\text{g}/\text{kg}$), increasing to the end of treatment. After treatment, these residues decreased and were detectable up to day 8 in egg white, and day 10 in yolk. FLU residues during drug administration in white were detectable in high concentrations from day one to five (6788.4–6525.9 $\mu\text{g}/\text{kg}$), and in yolk, concentrations were lower during administration (629.6–853.9 $\mu\text{g}/\text{kg}$). After drug withdrawal, FLU residues remained longer in egg white (30 days) than in yolk (26 days). For both drugs, differences of concentrations between matrices were significant.

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INTRODUCTION

Fluoroquinolones constitute an expanding group of synthetic antibiotics, widely used in the treatment of infections in both human and veterinary medicine. A number of these drugs have been licensed to be administered in broiler chickens for the prophylaxis and treatment of respiratory, renal and digestive infections in different regions of the world (Anderson & Macgowan, 2003; Martinez *et al.*, 2006). Fluoroquinolones are one of the few classes of antimicrobial agents with activity against the full range of pathogens involved in broiler chickens, such as *Campylobacter jejuni*, *Salmonella*, *Shigella* or *Escherichia coli*, being commonly used. In addition, fluoroquinolones have often been used for the treatment of *C. jejuni* and species of nontyphoidal *Salmonella* in humans (Luangtongkum *et al.*, 2009; Muhammad *et al.*, 2010).

Currently, the widespread use of these antimicrobials such as enrofloxacin (ENRO) and flumequin (FLU) in the poultry industry has become a matter of concern because it has led to

the emergence of resistance in *Salmonella* serovars (Esaki *et al.*, 2004; Zhao *et al.*, 2006; San Martín *et al.*, 2008), *Campylobacter* spp. (Griggs *et al.*, 2005; Humphrey *et al.*, 2005; Luangtongkum *et al.*, 2009) and *E. coli* (Khan *et al.*, 2005; Lee *et al.*, 2005; Lapierre *et al.*, 2008). This situation raises public health concerns regarding reduction in the clinical efficacy of FQs in human medicine (Norström *et al.*, 2006; Skjot-Rasmussen *et al.*, 2009).

In addition, the use of fluoroquinolones in food-producing animals may leave drug residues in foods. These residues represent a risk to public health, including stimulation of bacterial resistance, alterations on intestinal microflora and hypersensitivity reactions (Fàbrega *et al.*, 2008). To reduce this risk, maximum residue limits (MRL) – for ENRO and its metabolite ciprofloxacin (CIP), and for FLU – have been established in Europe and other countries for muscle, fat, liver and milk from several animal species, but not for eggs. Extra-label use of these drugs or unintentional contamination of feed for laying hens (cross-contamination during premix

manufacture or during feed transport) may be the source of violative drug residues in eggs for human consumption. Therefore, the depletion of these drugs in eggs should be assessed.

Considering the above-mentioned issues and the fact that published information regarding ENR depletion in eggs is scarce (Lolo *et al.*, 2005) and that no depletion studies have been reported for FLU, the present study was designed to assess the depletion time of ENR, its metabolite CIP, and FLU in egg white and yolk, after drug administration under therapeutic conditions. To this end, we studied the transfer and distribution of ENRO, CIP and FLU in eggs of treated laying hens. As no MRL has been established for these drugs in eggs, the depletion was observed up to the limit of detection (LOD) of the method.

MATERIALS AND METHODS

This work was performed in the Laboratory of Veterinary Pharmacology of the Faculty of Veterinary Sciences, University of Chile, accredited under the ISO 17025 standard.

Animals and samples

Thirty White Leghorn laying hens were used for this study. Animals were maintained from 16 weeks of age in conventional cages with an automated system for temperature, humidity and ventilation control. The birds had *ad libitum* access to water and no medicated feed. The ration was formulated according to breed standard requirements and was prepared by an avian expert.

Experimental animals were kept in conditions in compliance with animal welfare guidelines approved by the Bioethics Committee of the Veterinary Sciences Faculty, Universidad de Chile, and the recommendations of the European Council Directive 2007/43 (2007). Throughout the experiment, an avian medicine veterinarian monitored the hens.

The animals were randomly allocated into three experimental groups: two groups of 12 chickens (A and B) and one group of six chickens (C). Group A was treated with an ENRO 10% solution, 10 mg/kg·bw⁻¹. Group B was treated with a FLU 20% solution, 26.6 mg/kg·bw⁻¹. Animals were treated individually once daily for five consecutive days. The drug was administered using a gastric catheter to ensure complete ingestion of the dose. Group C remained as untreated control chickens.

From day one of treatment and during 30 days after treatment, eggs from each group were collected and stored at 4 ± 1 °C until chromatographic analysis. Egg white and yolk were analysed separately.

Chemicals and reagents

The oral commercial formulations used in the experiment, ENRO 10% solution and FLU 20% solution, were purchased from the national market.

A standard of ENRO was supplied by USP Reference Standard (Rockville, Md.). Standards for CIP and FLU were supplied by Dr. Ehrenstorfer GmbH (Augsburg, Germany). The LC-grade

acetonitrile was supplied by Fisher Chemicals, (Fair Lawn, NJ, USA). The LC-Grade acetic acid, absolute ethanol and hexane were from Merck (Darmstadt, Germany). All other reagents were of analytical reagent grade and were purchased from Merck.

Standard solutions of ENRO, CIP and FLU were prepared in aqueous 0.03 M NaOH at 1000 µg/mL and stored at 4 ± 2 °C in the dark for no longer than 3 months. Working standard solutions were prepared from the standard solution immediately prior to extraction.

Analytical method

Egg white and yolk were separated and homogenized separately. The extraction protocol was based on the one developed by Zeng *et al.* (2005). Briefly, 1 g of homogenized sample was placed into a 50-mL polypropylene tube and 4 mL of an acetic acid/ethanol (1:99) solution was added. Samples were shaken for 5 min, and 500 µL of acetonitrile and 4 mL of the acetic/ethanol solution were added to yolk samples. Then, white and yolk samples were placed for 30 min in a shaker, and samples were centrifuged for 20 min at 5368 *g*. The upper layer was transferred into a 10-mL glass tube and evaporated under nitrogen stream at 40 ± 2 °C; the residue was dissolved with 500 µL of acetonitrile. Two millilitres of hexane was added to the samples and were shaken and sonicated, and the upper layer was discarded. The hexane defeating step was repeated in yolk samples. The lower layer was evaporated under a nitrogen stream and the residue was dissolved with 500 µL of mobile phase. Samples were transferred to an eppendorf tube and again centrifuged at 7729 *g* for 10 min. The upper layer was filtered by a Millipore filter to an HPLC vial. Residues were separated by liquid chromatography and identified by fluorescence detection (HPLC-FL).

For the matrix-matched calibration curve, 1.0 g of blank egg white or yolk homogenate was spiked with 20 or 40 µL of a series of diluted ENRO plus CIP or FLU working standard solutions. The calibration samples were extracted, cleaned and analysed as for the experimental samples. Matrix calibration curves were in a range of concentration of 0.5–10, 10–100 and 100–1000 µg/kg. The correlation coefficients of the calibration curves were >0.997. The concentrations of ENRO plus CIP and FLU in the experimental samples were interpolated from the matrix-based calibration curve which was constructed for each set of analyses.

The LOD of the method was 0.5 µg/kg for FLU and 1 µg/kg for ENRO and CIP. To evaluate the repeatability and reproducibility of the method, fortified samples at three levels (1, 10 and 100 µg/kg) were prepared by spiking 1.0-g blank egg white or yolk homogenate with 40 µL of ENRO plus CIP or 20 µL FLU working solution. Six replicated samples at each level were prepared and analysed under the same experimental conditions during the same day and on different days. The mean recoveries were as follows: ENRO 85% for egg white and 80% for egg yolk; CIP 80% for egg white and 91% for egg yolk; and FLU 86% for white and 82% for yolk. The precision of the method expressed as CV (%) was as follows: ENRO 14.5% in egg white and 10.3% in yolk; CIP 15.4% in egg white and 10.3% in yolk; and FLU 16.5% in egg white and 15.3% for yolk.

Instrumentation

The Waters HPLC system consisted of a pump (510 series), an auto sampler (717 series) and a column oven. Chromatographic separation was achieved on a C-18 Symmetry column of 5 μm with 4.6 mm inside diameter, 250 mm in length (Waters, Milford, MA, USA). The HPLC system was coupled to a Series 2475 fluorescence Detector, (Waters). The mobile phase consisted of a mixture of an acetonitrile/aqueous solution (citric acid 50 mM, ammonium acetate 100 mM, pH adjusted to 4 with trimethylamine), in a 9:91 v/v.

Evaluation of drug concentrations in white and yolk during treatment and post-treatment

Eggs were collected from the first day of treatment to quantify ENRO plus CIP, and FLU concentrations in egg white and yolk during treatment. Ten egg samples from each day of treatment were collected from Group A and B and analysed. Two different statistical models were used to analyse the obtained data to assess any significant difference between drug concentrations in both egg compartments during treatment. The first analysis was performed with a model for completely randomized design, which was performed within each day to see the statistical differences between matrices (white and yolk) within 1 day and is expressed as:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij},$$

where Y is the observation of the treatment i (matrix) in day j ; μ is the general mean; τ_i is the treatment i (matrix) effect; ϵ_{ij} is the random error associated with the Y_{ij} observation.

A block design model was also used, to consider the time effect, beyond the treatment effect. This model is expressed as:

$$Y_{ij} = \mu + \tau_i + \beta_j + \epsilon_{ij},$$

where Y is the observation of the treatment i (matrix) in day j ; μ is the general mean; τ_i is the treatment i (matrix) effect; β_j is the effect of the j day (or the block); and ϵ_{ij} is the random error associated with the Y_{ij} observation.

The post-treatment concentration of drugs in white and yolk was also assessed. The statistical significance of the differences in drug concentrations between both compartments was also analysed using the above-mentioned models. Finally, the depletion of ENRO+CIP and FLU in both egg compartments was evaluated. For this purpose, the recommendations of the Committee for Veterinary Medicinal Products were adopted (EMA, 1995). The concentrations of the drugs in the final phase of depletion in each egg compartment were analysed and graphed as time vs. concentration.

RESULTS

ENRO plus CIP concentrations in egg white and yolk during the experiment are shown in Fig. 1. ENRO plus CIP residues were detected in egg white from the first day of treatment with mean

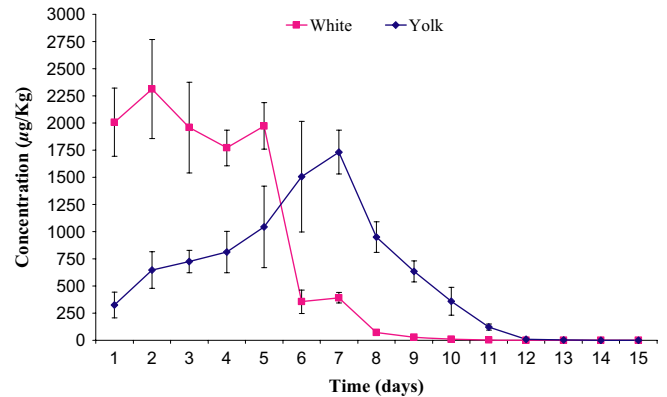


Fig. 1. Mean enrofloxacin (ENRO) + ciprofloxacin residues in egg white and yolk from laying hens treated with 10 mg/kg·bw⁻¹ of ENRO 10% solution.

drug concentrations of 2008 \pm 314 $\mu\text{g}/\text{kg}$ and remained steady during all the treatment administration period. In egg yolk, ENRO plus CIP mean concentration on the first day of treatment (324 \pm 119 $\mu\text{g}/\text{kg}$) was considerably lower than in white. In addition, ENRO plus CIP concentration in yolk constantly increased reaching 1044 \pm 376 $\mu\text{g}/\text{kg}$ on day 5 of treatment. According to the statistical analysis, the differences of the ENRO plus CIP residue concentrations during treatment between egg white and yolk were statistically significant ($P < 0.05$), when day and time effect was considered.

Figure 1 shows the concentrations of ENRO plus CIP in egg white and yolk during the experiment. It can be observed that residues in egg white dropped rapidly from an average concentration of 356 \pm 107 $\mu\text{g}/\text{kg}$ on the first day following withdrawal of the drug, to 29 \pm 6 $\mu\text{g}/\text{kg}$ on the fourth day following withdrawal of the drug, and were not detectable by the ninth day following treatment. After treatment, the concentration of ENRO-CIP in yolk continued to increase until day two, when it reached a maximum average concentration (1733 \pm 202 $\mu\text{g}/\text{kg}$). On the third day after withdrawal, the concentrations in egg yolk started to decrease, and residues were not detectable by the ninth day post-treatment in any eggs. After treatment, the differences in the concentrations of residues of both compartments were statistically significant.

Figure 2 shows the concentrations of FLU in egg white and yolk during the experiment. In white, the residues reached high concentrations during the 5 days of treatment, from 6788 \pm 575 $\mu\text{g}/\text{kg}$ on the first day of treatment to 6526 \pm 387 $\mu\text{g}/\text{kg}$ on the fifth day of treatment, with the higher residues level on day three (8631 \pm 816 $\mu\text{g}/\text{kg}$). In egg yolk, concentrations were much lower than in white and fluctuated from 630 \pm 34 $\mu\text{g}/\text{kg}$ on day one to 854 \pm 58 $\mu\text{g}/\text{kg}$ on day five. The difference between the concentrations of drug in egg white and yolk were considered significant after statistical analysis.

In Fig. 2 it can be observed that once the FLU was no longer administered to laying hens, the concentrations of drug in egg white decreased abruptly from 1228 \pm 297 $\mu\text{g}/\text{kg}$ on day one to 53 \pm 21 $\mu\text{g}/\text{kg}$ on the fifteenth day following the

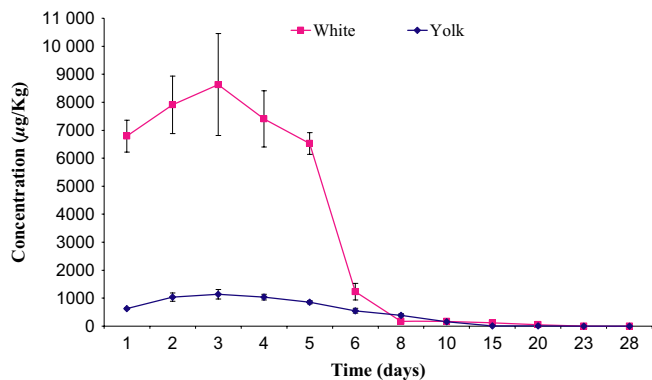


Fig. 2. Mean flumequine (FLU) residues in egg white and yolk from laying hens treated with $26.6 \text{ mg/kg}\cdot\text{bw}^{-1}$ of FLU 20% solution.

withdrawal of the drug. The residues of FLU in white were no longer detected by day 26 after treatment. In yolk, the levels of residues decreased from $548 \pm 86 \text{ µg/kg}$ on day one following the withdrawal of the drug to $9 \pm 3 \text{ µg/kg}$ on day fifteen after treatment, and the concentrations were below the LOD by day 20 after drug withdrawal. The difference of concentrations between both matrices was statistically significant ($P < 0.05$).

DISCUSSION

In this study, a 5-day treatment was adopted because it is the standard number of days used with fluoroquinolones administration to laying hens. The number of animals for each experimental group was established to comply with the European guidelines for the harmonization of withdrawal periods (EMA, 1995). Twelve laying hens were allocated to each experimental group, to obtain at least 10 eggs per day for the HPLC analysis during the decline period. Eggs of the untreated control group were used to validate the method and to construct the matrix spiked calibration curves used for the quantification of the experimental samples.

Fluoroquinolone depletion from egg white and yolk can be correlated with the egg formation process. The whole egg formation takes about 12 days. Lipoproteins synthesized in the liver are the main components of the egg yolk. The maturation process completes in about 10 days. The egg white is mainly formed by water-soluble proteins secreted by the hen oviduct (Donoghue & Myers, 2000; Kan, 2003). The distribution of the drugs between egg yolk and white is determined by the physiologic processes described above (Donoghue & Hairston, 2000; Kan & Petz, 2000). For this reason, ENRO plus CIP residues were detected from the first day after treatment in high-level average concentrations of over 2000 µg/kg .

In this study, ENR plus CIP residues in egg white remained relatively steady during the 5 days of treatment administration. For FLU residues in egg white, the pharmacokinetic behaviour was very similar to the one observed in ENR plus CIP residues. FLU residues reached high concentrations on the first day

postadministration in white, and they remained high during the 5 days of drug administration.

In egg yolk, both ENR+CIP and FLU residues were detectable after the first day of treatment but the average concentrations were lower than in white. For ENR plus CIP, the residue concentrations increased during the 5 days of drug administration (from 324 to 1043 µg/kg), while for FLU they started slightly higher than ENR+CIP initial concentrations (629 µg/kg), but they remained steady during treatment. Residues in yolk accumulate during growth of the follicle, and therefore, depending on the length and time of exposure to the drug relative to yolk deposition, drug levels can increase, be constant or decrease (Kan, 2003). According to Kan and Petz (2000), residues of drugs in yolk generally required exposure for about eight to 10 days to reach a constant level. However, Donoghue *et al.* (1996) state that a single exposure to a drug might be sufficient to detect the drug in either egg white or yolk, depending on the characteristics of the drug and sensitivity of the analytical method used.

During the depletion study, following drug withdrawal, ENRO+CIP residues showed different depletion profiles in egg white and yolk. ENRO+CIP residue concentrations in egg white dropped rapidly from day one to day four and were detectable for up to 8 days. FLU residue concentrations showed a similar profile in egg white decreasing rapidly from day one to day fifteen, once the drug was no longer administered. However, residues of FLU persist in egg white up to 26 days, a longer time than ENRO residues in the same matrix. In egg yolk, ENR+CIP and FLU concentrations were lower than in egg white, but they were detectable until day 10 and day 20, respectively.

On the basis of our results, we suggest that yolk is a better matrix than white for monitoring residues of both ENRO and its metabolite CIP in eggs intended for human consumption. The results obtained for ENR+CIP were similar to previous studies. Lolo *et al.* (2005) reported that ENR+CIP residues were detectable in both white and yolk for 15 days after drug withdrawal in concentrations of 6.4 and 3.5 µg/kg , respectively. Another study performed by Gorla *et al.* (1997) showed results for ENRO residues that were similar to those in the present study, with a 6-day depletion time for albumen and 9-day depletion time for yolk.

Similar studies have been carried out with other fluoroquinolones. Yang *et al.* (2006) studied danofloxacin depletion in eggs. These authors reported that the residues were detectable up to day four in albumen and up to day 11 in yolk. This depletion profile was longer than the one described by Chu *et al.* (2002) for total radioactive residues of sarafloxacin, which were detectable for up to 1 day in egg white and for 6 days in egg yolk.

The literature that describes the depletion of flumequine (FLU) residues in eggs is sparse. In this work, the FLU depletion profile was assessed to establish the depletion time in eggs after extra-label use in laying hens. Unlike results obtained for ENR+CIP residues, FLU residues were detectable for a longer time in albumen (26 days) than in yolk (20 days). Similar results were obtained by Riberzani *et al.* (1993), in a simulated field trial. They found much higher concentrations of FLU in egg white

than in yolk, suggesting that it might be caused by the high solubility of the 'acid' drug FLU in the basic matrix egg albumen. Results differ from the ones reported for other fluoroquinolones (Gorla *et al.*, 1997; Chu *et al.*, 2002; Lolo *et al.*, 2005; Yang *et al.*, 2006), where residues remained for a longer time in egg yolk than in white. On the basis of the depletion profile for FLU in the present study, we suggest that when FLU use is suspected, egg white could be a better matrix for residue detection.

According to Kan (2003), the reason for the different distribution in egg white and yolk of the fluoroquinolones may be because of the differences in liposolubility and physicochemical characteristics of the drugs, such as their molecular weight, pKa value and binding capacity to plasma proteins. Although there is no general agreement regarding fluoroquinolone distribution, some authors described that these drugs accumulate mainly in the yolk, being deposited and incorporated to this matrix during the egg development (Donoghue *et al.*, 1996; Donoghue *et al.*, 1997a,b). In another study, Donoghue and Myers (2000) demonstrated that drugs can be incorporated in developing egg yolks, even after a single dose. However, other authors (Riberzani *et al.*, 1993; Gorla *et al.*, 1997; Roudaut, 1998; Kan & Petz, 2000; Kan, 2003) suggested that fluoroquinolone residue content is higher in egg white.

The high levels of ENRO and FLU residues in egg white and yolk can involve some risks to human health such as adverse effects on bacterial flora in the human gastrointestinal tract, and the emergence and dissemination of resistant bacteria (Anderson *et al.*, 2003; Liu *et al.*, 2005; Nelson *et al.*, 2007). Therefore, the egg withdrawal time following ENRO and FLU administration to laying hens should be established by regulatory authorities, with the aim of minimizing the risks associated with consumption of fluoroquinolones.

This work showed that it takes 8 days for ENRO residues in egg whites to deplete below the established LOD (0.5 µg/kg), and 10 days in egg yolks. For FLU residues, it takes longer to deplete from both matrices, 26 days in egg whites and 20 days in yolk, so the egg discard time should be longer. Therefore, considering that no MRL have been established for these drugs in eggs, we recommend egg withdrawal times of 10 and 26 days following 5-day administration of ENRO or FLU, respectively, based on the time needed for residue levels to decline below the LOD. In agreement with the obtained depletion profile, we suggest that when FLU use is suspected, egg white is a better matrix for residue detection whereas with ENRO, egg yolk is the preferred matrix for residue monitoring in eggs. This study showed that differences between the drug concentrations in egg compartments, white and yolk are significant ($P < 0.05$). So the selected matrix, egg white or egg yolk, can be used for the detection and confirmation of ENRO or FLU residues, respectively.

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REFERENCES

- Anderson, M. & Macgowan, A. (2003) Development of the quinolones. *Journal of Antimicrobial Chemotherapy*, **51**, 1–11.
- Anderson, A.D., Nelson, J.M., Rossiter, S. & Angulo, F.J. (2003) Public health consequences of use of antimicrobial agents in food animals in the United States. *Microbial Drug Resistance*, **9**, 373–379.
- Chu, P., Wang, R. & Chu, H. (2002) Liquid chromatographic determination of fluoroquinolones in egg albumen and egg yolk of laying hens using fluorometric detection. *Journal of Agricultural and Food Chemistry*, **50**, 4452–4455.
- Donoghue, D.J. & Hairston, H. (2000) Food safety implication: certain antibiotics may rapidly contaminate egg albumen during the process of its formation. *British Poultry Science*, **41**, 174–177.
- Donoghue, D. & Myers, K. (2000) Imaging residue transfers into egg yolks. *Journal of Agricultural and Food Chemistry*, **48**, 6428–6430.
- Donoghue, D.J., Hairston, H., Gaines, S., Bartholmew, M.J. & Donoghue, A.M. (1996) Modeling residue uptake in eggs: similar drug residue patterns in developing yolks following injection with ampicillin or oxytetracycline. *Poultry Science*, **75**, 321–328.
- Donoghue, D.J., Hairston, H., Henderson, M., McDonald, M., Gaines, S. & Donoghue, A.M. (1997a) Modeling residue uptake in eggs: yolks contain ampicillin residues even after drug withdrawal and non-detectability in the plasma. *Poultry Science*, **76**, 458–462.
- Donoghue, D.J., Schenck, F.J., Hairston, H. & Podhorniak, L.V. (1997b) Modeling drug residue uptake by eggs: evidence of a consistent daily pattern of contaminant transfer into developing preovulatory yolks. *Journal of Food Protection*, **60**, 1251–1255.
- EMA. The European Agency for the Evaluation of Medicinal Products. (1995) Note for Guidance: Approach towards harmonization of withdrawal periods. EMEA/CVMP/036/95, 1–37
- Esaki, H., Morioka, A., Ishihara, K., Kojima, A., Shiroki, A., Tamura, Y. & Takahashi, T. (2004) Antimicrobial susceptibility of *Salmonella* isolated from cattle, swine and poultry (2001–2002): report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Journal of Antimicrobial Chemotherapy*, **53**, 266–270.
- European Council Directive. (2007) Laying down minimum rules for the protection of chickens kept for meat production. *Official Journal of the European Communities*, L182/19, 2007/43/EC, 1–10.
- Fábrega, A., Sánchez-Céspedes, J., Soto, S. & Vila, J. (2008) Quinolone resistance in the food chain. *International Journal of Antimicrobial Agents*, **31**, 307–315.
- Gorla, N., Chiostri, E., Ugnia, L., Weyers, A., Giacomelli, N., Davicino, R. & Ovando, H.G. (1997) HPLC residues of enrofloxacin and ciprofloxacin in eggs of laying hens. *International Journal of Antimicrobial Agents*, **9**, 253–256.
- Griggs, D., Johnson, M., Frost, J., Humphrey, T., Jørgensen, F. & Piddock, L. (2005) Incidence and mechanism of ciprofloxacin resistance in *Campylobacter* spp. isolated from commercial poultry flocks in the United Kingdom before, during, and after fluoroquinolone Treatment. *Antimicrobial Agents and Chemotherapy*, **49**, 699–707.
- Humphrey, T.J., Jørgensen, F., Frost, J., Wadda, H., Domingue, G. & Elviss, N. (2005) Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrobial Agents and Chemotherapy*, **49**, 690–698.
- Kan, C. (2003) *Residues of Veterinary Drugs in Eggs and their Distribution Between Yolk and White*. Wuppertal University, Alemania.
- Kan, C. & Petz, M. (2000) Residues of veterinary drugs in eggs and their distribution between yolk and white. *Journal of Agricultural and Food Chemistry*, **48**, 6397–6403.

- Khan, A.A., Nawaz, M.S., Summige West, C., Khan, S.A. & Lin, J. (2005) Isolation and molecular characterization of fluoroquinolone-resistant *Escherichia coli* from poultry litter. *Poultry Science*, **84**, 61–66.
- Lapierre, L., Cornejo, J., Borie, C., Toro, C. & San Martín, B. (2008) Genetic characterization of antibiotic resistance genes linked to class 1 and class 2 integrons in commensal strains of *Escherichia coli* isolated from poultry and swine. *Microbial Drug Resistance*, **14**, 265–272.
- Lee, Y.J., Cho, J.K., Kim, K.S., Tak, R.B., Kim, A.R., Kim, J.W., Im, S.K. & Kim, B.H. (2005) Fluoroquinolone resistance and *gyrA* and *parC* mutations of *Escherichia coli* isolated from Chicken. *Journal of Microbiology*, **43**, 391–397.
- Liu, J.H., Chen, Z.L., Li, Y. & Liu, Y.W. (2005) Effects of low concentration enrofloxacin on SPF mice intestinal microflora. *Scientia Agricultura Sinica*, **38**, 1905–1910.
- Lolo, M., Pedreira, S., Fente, C., Vaázquez, B., Franco, C. & Cepeda, A. (2005) Study of enrofloxacin depletion in the eggs of laying hens using diphasic dialysis extraction/purification and determinative HPLC-MS analysis. *Journal of Agricultural and Food Chemistry*, **53**, 2849–2852.
- Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C.M. & Zhang, Q. (2009) Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. *Future Microbiology*, **2**, 189–200.
- Martinez, M., McDermott, P. & Walker, R. (2006) Pharmacology of the Fluoroquinolones: a perspective for the use in domestic animals. *Veterinary Journal*, **172**, 10–28.
- Muhammad, M., Muhammad, L.U., Ambali, A.G., Mani, A.U., Azard, S. & Barco, L. (2010) Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents. *Veterinary Microbiology*, **140**, 131–135.
- Nelson, J.M., Chiller, T.M., Powers, J.H. & Angulo, F.J. (2007) Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clinical Infectious Diseases*, **44**, 977–980.
- Norström, M., Hofshagen, M., Stavnes, T., Schau, J., Lassen, J. & Kruse, H. (2006) Antimicrobial resistance in *Campylobacter jejuni* from humans and broilers in Norway. *Epidemiology and Infection*, **134**, 127–130.
- Riberzani, A., Fedrizzi, G. & Espositi, S. (1993) Presence of flumequine in eggs: experimental results of a simulated field trial. *Euroresidue*, **2**, 576–580.
- Roudaut, B. (1998) Elimination of oxolinic acid in eggs after oral treatment of laying hens. *British Poultry Science*, **39**, 47–52.
- San Martín, B., Lapierre, L., Cornejo, J. & Bucarey, S. (2008) Characterization of antibiotic resistance genes linked to class 1 and 2 integrons in strains of *Salmonella* spp. isolated from swine. *Canadian Journal of Microbiology*, **54**, 569–576.
- Skjöt-Rasmussen, L., Ethelberg, S., Emborg, H.D., Agersø, Y., Larsen, L.S., Nordentoft, S., Olsen, S.S., Ejlertsen, T., Holt, H., Nielsen, E.M. & Hammerum, A.M. (2009) Trends in occurrence of antimicrobial resistance in *Campylobacter jejuni* isolates from broiler chickens, broiler chicken meat, and human domestically acquired cases and travel associated cases in Denmark. *International Journal of Food Microbiology*, **131**, 277–279.
- Yang, G., Dong, A., Zeng, Z., Huang, X. & Chen, Z. (2006) Study of danofloxacin depletion in eggs of laying hens after oral administration. *International Journal of Antimicrobial Agents*, **28**, 128–131.
- Zeng, Z., Dong, A., Yang, G., Chen, Z. & Huang, X. (2005) Simultaneous determination of nine fluoroquinolones in egg white and egg yolk by liquid chromatography with fluorescence detection. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences*, **821**, 202–209.
- Zhao, S., McDermott, P.F., Friedman, S., Abbott, J., Ayers, S., Glenn, A., Hall-Robinson, E., Hubert, S.K., Harbottle, H., Walker, R.D., Chiller, T.M. & White, D.G. (2006) Antimicrobial resistance and genetic relatedness among *Salmonella* from retail foods of animal origin: NARMS retail meat surveillance. *Foodborne Pathogens and Disease*, **3**, 106–117.