



## RESEARCH ARTICLE

### Quinolone antibiotic residues in raw milk and chicken liver in Konya

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#### Özet

**Nizamlioğlu F, Aydın H.** Konya'daki çiğ süt ve tavuk karaciğerinde kinolon antibiyotik kalıntıları. *Eurasian J Vet Sci*, 2012, 28, 3, 154-158

**Amaç:** Bu çalışmanın amacı, Konya'daki çiğ süt ve tavuk karaciğeri örneklerinde kinolon antibiyotik kalıntılarının (ciprofloksacin, enrofloxacin, marbofloksacin, danofloxacin, difloksacin, flumequin, sarafloksacin and oxolinic acid) varlığını değerlendirmektir.

**Gereç ve Yöntem:** Elli adet çiğ süt ve 50 adet tavuk karaciğeri olmak üzere toplam 100 adet örnek çalışıldı. Örnekler enzyme-linked immunosorbent assay (ELISA) tekniği kullanılarak analiz edildi.

**Bulgular:** Kinolon kalıntıları için analiz edilen 50 adet tavuk karaciğeri örneğinin 17 (%34)'si pozitif bulundu ve bunlardan birinin değeri (147.88 µg/kg) maksimum rezidü limitlerinin (MRL) üzerindedir. Ortalama kontaminasyon düzeyi 47.5 µg/kg idi. Süt örneklerinin hiçbirinde tespit edilebilir düzeyde kinolon kalıntısına rastlanmadı.

**Öneri:** Analiz edilen örneklerin biri dışında tümü Avrupa Birliği ve Türkiye tarafından kabul edilen MRL'nin altında kinolon kalıntılarının varlığını gösterdi. Böylece süt ve tavuk karaciğeri örneklerinin analizinden elde edilen sonuçlar, gıda güvenliği açısından olumlu bir işaret olarak kabul edildi. Ayrıca bu analizler Türkiye Cumhuriyeti Ulusal Kalıntı İzleme Planına göre rutin olarak yapılmaktadır. Ülkede gıda güvenliğini sağlamak için süt, et ve yumurta gibi yenilebilir hayvansal ürünlerde rutin kalıntı gözetim ve izleme programlarına devam edilmelidir.

#### Abstract

**Nizamlioglu F, Aydın H.** Quinolone antibiotic residues in raw milk and chicken liver in Konya. *Eurasian J Vet Sci*, 2012, 28, 3, 154-158

**Aim:** The objective of this study was to evaluate the presence of quinolone antibiotic residues (ciprofloxacin, enrofloxacin, marbofloxacin, danofloxacin, difloxacin, flumequin, sarafloxacin and oxolinic acid) in raw milk and chicken liver samples in Konya.

**Materials and Methods:** A total of 100 samples, including 50 raw milk and 50 chicken liver samples, were examined for quinolone antibiotics. The samples were analyzed by an enzyme-linked immunosorbent assay (ELISA) screening method.

**Results:** Of the 50 chicken liver samples analyzed for residues of quinolone, 17 (34%) were positive and in one of them the value (147.88 µg/kg) was above the maximum residue limits (MRLs). The mean contamination level was 47.5 µg/kg. None of the milk samples were found to be positive for quinolone residues.

**Conclusion:** All of the analyzed samples except one showed the presence of quinolone residues below the MRLs established by European Union (EU) and Turkish Legislation. So that obtained results from analysis of milk and chicken liver samples were considered to be a positive sign in terms of food safety. Also these analyses are performed as routine according to the National Residue Monitoring Plan of the Republic of Turkey. Therefore, routine drug residues surveillance and monitoring programs in edible animal products like milk, meat and eggs should be continued to ensure food safety in the country.

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## ► Introduction

Quinolones (fluoroquinolones, FQs) are synthetic antimicrobials that inhibit the activity of bacterial DNA gyrase enzymes (Rodriguez et al 2008, Junza et al 2010, Jinqing et al 2011b). Several concerns regarding to the presence FQs have been approved for veterinary special use for the treatment of diseases, such as dermatological and respiratory diseases, urinary tract infections and gastroenteritis in food-producing animals (Sheng et al 2011).

Enrofloxacin (ENRO) is a FQ that was developed exclusively for veterinary use. After administration, ENRO is partly de-ethylated to ciprofloxacin (CIPRO) in vivo. This pharmacologically active metabolite which has been restricted to use in human medicine is excreted with milk after ENRO treatment (Suhren and Hammer 1998, San Martin et al 2009, Pena et al 2010).

Several concerns regarding to the presence of drug residues in foods are technological problems in fermented products, toxicity and potential allergic reactions in sensitized individuals (Brady and Katz 1988, Currie et al 1998, Van Coillie et al 2004). The most prominent human health risk associated with intensive animal farming and antibiotic use is antimicrobial resistance (Pena et al 2010). For these reasons, The EU has defined the MRLs for several of these compounds in the different food matrices of animal origin (EC 1990).

According to Commission Regulation (EU) 37/2010 (EC 2010), quinolones range between 100-1900 µg/kg in chicken liver, and 30-100 µg/kg in milk. Turkish Food Codex Regulation has also established the same levels of EU (Türk Gıda Kodeksi 2007).

Classical analytical methods, including high performance liquid chromatography (HPLC) (Roybal et al 1997, Zhao et al 2007), liquid chromatography-mass spectrometry (LC-MS) (Delepine et al 1998, Van Hoof et al 2005) and LC-MS/MS (Pikkemaat et al 2007, Hermo et al 2008), have been described for the detection of FQs in tissues and milk. Since these methods are labour intensive and require specific equipment, they cannot be used for the routine screening of large numbers of sample specimens (Wang et al 2007, Kato et al 2008). ELISA is very common as a biochemical and clinical analytical method, and available for the detection of veterinary medicine and pesticide residues in foods due to its high sensitivity, simplicity and ability to screen large number of small-volume samples (Nunes et al 1998, Watanabe et al 2001, Zhang et al 2007, Wang et al 2009, Jinqing et al 2011a).

The purpose of the present study is to determine the levels of quinolone in raw milk and chicken liver samples by ELISA and to compare the obtained results with antibiotic tolerance limits accepted by the European Commission.

## ► Materials and Methods

### • Samples

A total of fifty samples of raw milk were taken at milk-processing companies and from the collection tanks of milk routes in Konya. Samples were stored at 4-8 °C until analysis and then were examined within two days of the collection. For further investigations, milk samples were stored at -20 °C for 3 weeks. A total of fifty samples of chicken liver were purchased from large supermarkets and butcher shops and kept at -20 °C for further analysis.

### • Chemicals and equipment

A commercial ELISA kit (R-Biopharm, Darmstadt, Germany) was utilized. The kit contained the following quinolone standard solutions (1.3 mL each): 0, 0.5, 1.5, 3, 6 and 18 ppb ciprofloxacin in methanolic solution. The kit also contained the following reagents: antibody-coated microtiter strips, conjugate (peroxidase conjugated ciprofloxacin), anti-quinolone antibody, substrate/chromogen solution (Tetramethylbenzidine), stop solution (1N H<sub>2</sub>SO<sub>4</sub>) and washing buffer (PBS+Tween 20, pH: 7.4). Trichloroacetic acid (>99%) was provided by Merck (Darmstadt, Germany).

### • Sample preparation

The milk sample preparation procedure was performed as reported by Sheng et al (2011). Fifty milliliter of milk was centrifuged at 4000 rpm for 10-15 min at 4 °C, and then fat was removed. A total of 1 ml of the rest milk was transferred into a centrifuge tube and 2 mL of 7.5% (w/v) trichloroacetic acid aquatic solution was then added. The sample was thoroughly vortexed for 1 min and centrifuged at 5000 rpm for 20 min to deproteinate. The supernatant was then diluted with washing buffer (1/2) and 50 µL portions were used for the test. The chicken liver samples were thawed at 4 °C overnight and homogenized using Ultra Turrax T25. Homogenized chicken liver were weighed (2.0 g) into 50 mL tubes, and 8 mL of 70% methanol solution was added, and the mixture was agitated on a shaker for 5 min. The samples were centrifuged at 4000 rpm for 10 min at 18-20 °C and the supernatants (1 mL) were added to an equal volume of dilution buffer and vortexed for 1 min and 50 µL portions were used for the test.

### • ELISA test procedure

The assay was performed as described in the package inserts provided by the manufacturers. As provided by the supplier, the ELISA kit showed cross-reactivity with ciprofloxacin (100%), norfloxacin, enrofloxacin, marbofloxacin, danofloxacin, difloxacin, flumequin, ofloxacin (>100%), sarafloxacin (43%) and oxolinic acid (24%). Absorbance was measured at 450 nm with a spectrophotometer for ELISA plates (ELX50, Bio-Tek Instruments, USA).

## ► Results

The mean values of the absorbances for the standards and the samples were evaluated according to the Rida Soft Win program (Ridavin.exe) prepared by R-Biopharm AG. According to the RIDASCREEN kit application data (Art. No. R3113), the detection limit of the kit for milk and liver were 10 µg/kg and the recovery rates in milk and liver samples were between 95-122% and 80-110% respectively. Fifty samples of raw milk and 50 of chicken liver were analysed. The quinolone residues were determined in 17 (34%) of the 50 chicken liver samples, with a range of 18.5-147.88 µg/kg. The mean contamination level was 47.5 µg/kg and only one sample was identified to contain quinolone residues above the MRLs. None of the milk samples were found to be positive for quinolone residues. As a result, all of the analyzed samples except one showed the presence of quinolone residues below the MRLs established by EU and Turkish Legislation.

## ► Discussion

Residues of antimicrobial agents have a potential hazard for the consumer and may cause allergic reactions, interference in the intestinal flora and to transfer of antibiotic resistance to human and animal bacterial pathogens, thereby rendering antibiotic treatment ineffective. For this reason, control of antibiotic residues is necessary to ensure food safety and to prevent exposure of the consumers to drug residues. Pena et al (2010) reported that comparison with FQ occurrence data at European and international levels is difficult because few studies are available in the scientific literature.

In a recent study (Temamoğulları and Kaya 2010), for the detection of ampicillin, amoxicillin, cloxacillin, danofloxacin, enrofloxacin, erythromycin and florfenicol residues, 240 milk samples collected from Ankara and only one pasteurized milk sample was found positive for ampicillin, and the other 239 samples were negative for antibiotic residues. As a result of the study performed by Zafra-Gomez et al (2008), for the determination of quinolones in bovine milk; 30 samples of whole milk, 30 of skimmed milk and 30 of powder milk were analysed and none of them gave a positive result.

A study undertaken by Chung et al (2009) in Korea, in 269 milk samples, using microbial screening assays and HPLC method with the goal of determining sulfonamide and quinolone antibiotic residues and among three samples which contained quinolone antibiotics in the microbial screening assay, only one sample was identified to contain ciprofloxacin (16.7 µg/kg) by HPLC. Another study conducted by Bilandzic et al (2011) in Croatia, a total of 1259 raw milk samples were examined over a three-year period for various antibiotics. The contamination levels were in general lower than the maximum levels proposed by the European Legislation. Junza et al (2010) devel-

oped a LC-MS/MS method for the determination of β-lactams and quinolones in tissues and milk samples. Forty-nine positive raw milk samples from animals medicated with different antibiotics were examined and only two samples were positive for ENRO.

The presence of quinolone antibiotics in this study were similar to that found by Temamoğulları and Kaya (2010) and Zafra Gomez et al (2008): No milk sample contained quinolone residues.

In Portugal, Pena et al (2010) reported that only one sample of 61 chicken muscle samples was contaminated with ENRO at levels higher than the MRLs. The study conducted by Liu et al (2009), danofloxacin was found at various concentrations in 5 of the 25 chicken liver samples. In a study performed by Salehzadeh et al (2007) in Iran, 8 (8.88%), 12 (13.33%), and 22 (24.44%) samples of muscle, liver, and kidney, respectively, contained residues of ENRO above the MRLs. In the study from Saudi Arabia (Al-Mustafa and Al-Ghamdi 2000), norfloxacin was detected in 35% and 56.7% of chicken muscles and livers, respectively. The mean concentration range was 80-1000 µg/kg and 110-1030 µg/kg for chicken muscle and liver, respectively. In this study, seventeen chicken liver samples were positive for residues of quinolone (ciprofloxacin, enrofloxacin, marbofloxacin, danofloxacin, difloxacin, flumequin, sarafloxacin and oxolinic acid), and in one of them the value (147.88 µg/kg) was above the MRLs. The prevalence of residues of quinolone was 34% and the mean contamination level was 47.5 µg/kg.

In Turkey, the contamination rates determined in tissue and milk samples of residue detection studies carried in recent years are much lower than those of the previous years. This was explained by an increased public awareness about food safety and healthy nutrition and efforts of producers to market high quality products after the media began emphasizing the issue (Ergin Kaya and Filazi 2010). The results of our study support this view.

In this study, the ELISA method was used. ELISA is fast and cheap, so it is suitable for the rapid generic screening of quinolones when large numbers of samples have to be analyzed to monitor legislative compliance. Jinqing et al (2011a) proved that the coupling of ELISA as screening method and LC-MS/MS as confirmatory method was an advantage for detecting FQ residues. Hernandez-Arteseros et al (2002) reported that ELISA and optical immunosensors methods have shown great potential as a screening tool, although most only allow semiquantitative analysis of the sum of ENRO and CIPRO.

## ► Conclusions

In this study, fifty samples of raw milk and 50 of chicken liver were analysed using ELISA method for the

presence of quinolone antibiotics. All of the analyzed samples except one showed the presence of quinolone residues below the MRLs established by EU and Turkish Legislation. However, positive screening results should also be confirmed using HPLC, LC-MS or LC-MS/MS. Analysis of milk and chicken liver samples showed satisfactory results for public health. Also these analyses are performed as routine according to the National Residue Monitoring Plan of the Republic of Turkey. Therefore, routine drug residues surveillance and monitoring programs in edible animal products like milk, meat and eggs should be continued to ensure food safety in the country. Nevertheless, veterinarians must be well aware of the importance of drug residues in food animals and their possible risk to the general public and they should educate to livestock producers on good agricultural practices and responsible use of antibiotics in food animals.

### ► References

- Al-Mustafa ZH, Al-Ghamdi MS, 2000. Use of norfloxacin in poultry production in the eastern province of Saudi Arabia and its possible impact on public health. *Int J Environ Health Res*, 10, 291-299.
- Bilandzic N, Kolanovic BS, Varenina I, Scortichini G, Annunziata L, Brstilo M, Rudan N, 2011. Veterinary drug residues determination in raw milk in Croatia. *Food Control*, 22, 1941-1948.
- Brady MS, Katz SE, 1988. Antibiotic-antimicrobial residues in milk. *J Food Prot*, 51, 8-11.
- Chung HH, Lee JB, Chung YH, Lee KG, 2009. Analysis of sulfonamide and quinolone antibiotic residues in Korean milk using microbial assays and high performance liquid chromatography. *Food Chem*, 113, 297-301.
- Currie D, Lynas L, Kennedy DG, McCaughey WJ, 1998. Evaluation of a modified EC Four Plate Method to detect antimicrobial drugs. *Food Addit Contam*, 15, 651-660.
- Delepine B, Hurtaud-Pessel D, Sanders P, 1998. Simultaneous determination of six quinolones in pig muscle by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. *Analyst*, 123, 2743-2747.
- EC 1990. Council Regulation 2377/90/EEC of 26 June 1990 on laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin. *Off J EC*, L224, 1-8.
- EC 2010. Commission Regulation (EU) No 37/2010 of 22 December 2009: On pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Off J EU*, L15, 1-72.
- Ergin Kaya S, Filazi A, 2010. Determination of antibiotic residues in milk samples. *Kafkas Univ Vet Fak*, 16 (Suppl-A), 31-35.
- Hermo MP, Nemutlu E, Kir S, Barron D, Barbosa J, 2008. Improved determination of quinolones in milk at their MRL levels using LC-UV, LC-FD, LC-MS and LC-MS/MS and validation in line with regulation 2002/657/EC. *Anal Chim Acta*, 613, 98-107.
- Hernandez-Arteseros JA, Barbosa J, Compano R, Prat MD, 2002. Analysis of quinolone residues in edible animal products. *J Chromatogr A*, 945, 1-24.
- Jinqing J, Haitang Z, Kun Z, Huaguo H, Ziliang W, 2011a. Comparison between direct competitive ELISA and LC-MS/MS method for detecting sarafloxacin residue in poultry. *Energy Procedia*, 11, 2717-2722.
- Jinqing J, Haitang Z, Junwei L, Junmin L, Ziliang W, 2011b. Development and optimization of an indirect competitive ELISA for detection of norfloxacin residue in chicken liver. *Procedia Environmental Sciences*, 8, 128-133.
- Junza A, Amatya R, Perez-Burgos R, Gökçe G, Grzelak E, Barron D, Barbosa J, 2010. Residues of  $\beta$ -lactams and quinolones in tissues and milk samples. Confirmatory analysis by liquid chromatography-mass spectrometry. *Ovidius University Annals of Chemistry*, 21, 109-122.
- Kato M, Ihara Y, Kasai D, Kodaira T, 2008. The development of release-competitive assay to detect residual quinolones in contaminated milk. *Food Agr Immunol*, 19, 241-246.
- Liu Z, Lu S, Zhao C, Ding K, Cao Z, Zhan J, Ma C, Liu J, Xi R, 2009. Preparation of anti-danofloxacin antibody and development of an indirect competitive enzyme-linked immunosorbent assay for detection of danofloxacin residue in chicken liver. *J Sci Food Agric*, 89, 1115-1121.
- Nunes GS, Toscano IA, Barcelo D, 1998. Analysis of pesticides in food and environmental samples by enzyme-linked immunosorbent assays. *Trac-Trend Anal Chem*, 17, 79-87.
- Pena A, Silva LJG, Pereira A, Meisel L, Lino CM, 2010. Determination of fluoroquinolones residues in poultry muscle in Portugal. *Anal Bioanal Chem*, 397, 2615-2621.
- Pikkemaat MG, Mulder PJP, Elferink JWA, De Cocq A, Nielsen MWF, Van Egmond HJ, 2007. Improved microbial screening assay for the detection of quinolone residues in poultry and eggs. *Food Addit Contam*, 24, 842-850.
- Rodriguez E, Moreno-Bondi MC, Marazuela MD, 2008. Development and validation of a solid-phase extraction method coupled to liquid chromatography with fluorescence detection for the determination of fluoroquinolone residues in powdered infant formulae Application to the analysis of samples from the Spanish and Latin American market. *J Chromatogr A*, 1209, 136-144.
- Roybal JE, Pfenning AP, Turnipseed SB, Walker CC, Huribut JA, 1997. Determination of four fluoroquinolones in milk by liquid chromatography. *J AOAC Int*, 80, 982-987.
- Salehzadeh F, Salehzadeh A, Rokni N, Madani R, Golchinfar F 2007. Enrofloxacin residue in chicken tissues from Tehran slaughterhouses in Iran. *Pak J Nutr*, 6, 409-413.
- San Martin B, Cornejo J, Lapierre L, Iragüen D, Perez F, Hidalgo H, Andre F, 2009. Withdrawal time of four pharmaceutical formulations of enrofloxacin in poultry according to different maximum residues limits. *J Vet Pharmacol Therap*, 33, 246-251.
- Sheng W, Li Y, Xu X, Yuan M, Wang S, 2011. Enzyme-linked immunosorbent assay and colloidal gold-based immunochromatographic assay for several (fluoro)quinolones in milk. *Microchim Acta*, 173, 307-316.
- Suhren G, Hammer P, 1998. Detection of residues of quinolones in milk. 3<sup>rd</sup> Karlsruhe Nutrition Symposium: European Research towards Safer and Better Food. Review and Transfer Congress, Congress Centre, Karlsruhe, Germany, October 18-20.
- Temamoğulları F and Kaya S, 2010. Ankara piyasasında satılan sütlerde bazı antibiyotik kalıntılarının ince tabaka kromatografisi ve biyootografik yöntemle saptanması. *Kafkas Univ Vet Fak*, 16, 187-191.
- Türk Gıda Kodeksi, 2007. Hayvansal Kökenli Gıdalarda Vet-

- eriner İlaçları Maksimum Kalıntı Limitleri Tebliğinde Deđişiklik Yapılması Hakkında Tebliğ. TC Resmi Gazete: 09.03.2007-26457, Tebliğ No: 2007/17.
- Van Coillie E, De Block J, Reybroeck W, 2004. Development of an indirect competitive ELISA for flumequine residues in raw milk using chicken egg yolk antibodies. *J Agr Food Chem*, 52, 4975-4978.
- Van Hoof N, De Wasch K, Okerman L, Reybroeck W, Poelmans S, Noppe H and De Brabender H, 2005. Validation of a liquid chromatography-tandem mass spectrometric method for the quantification of eight quinolones in bovine muscle, milk and aquacultured products. *Anal Chim Acta*, 529, 265-272.
- Wang Z, Zhu Y, Ding S, He F, Beier RC, Li J, Jiang H, Feng C, Wan Y, Zhang S, Kai Z, Yang X, Shen J, 2007. Development of a monoclonal antibody-based broad-specificity ELISA for fluoroquinolone antibiotics in foods and molecular modeling studies of cross-reactive compounds. *Anal Chem*, 79, 4471-4483.
- Wang S, Xu B, Zhang Y, He JX, 2009. Development of enzyme-linked immunosorbent assay (ELISA) for the detection of neomycin residues in pig muscle, chicken muscle, egg, fish, milk and kidney. *Meat Sci*, 82, 53-58.
- Watanabe H, Satake A, Kido Y, Tsuji A, 2001. Monoclonal-based enzyme-linked immunosorbent assay and immuno-chromatographic rapid assay for salinomycin. *Anal Chim Acta*, 437, 31-38.
- Zafra-Gomez A, Garbalo A, Ballesteros O, Navalon A, Garcia-Ayuso LE, 2008. Simultaneous determination of quinolone antibacterials in bovine milk by liquid chromatography-mass spectrometry. *Biomed Chromatogr*, 22, 1186-1193.
- Zhang Y, Lu S, Liu W, Zhao C, Xi R, 2007. Preparation of anti-tetracycline antibodies and development of an indirect heterologous competitive enzyme-linked immunosorbent assay to detect residues of tetracycline in milk. *J Agr Food Chem*, 55, 211-218.
- Zhao SJ, Jiang HY, Li XL, Mi TJ, Li C, Shen JZ, 2007. Simultaneous determination of trace levels of 10 Quinolones in swine, chicken, and shrimp muscle tissues using HPLC with programmable fluorescence detection. *J Agr Food Chem*, 55, 3829-3834.