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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Enrofloxacin Residue in Chicken Tissues from Tehran Slaughterhouses in Iran

Farahmand Salehzadeh¹, Aref Salehzadeh², Nordehr Rokni³, Rahim Madani⁴ and Fariba Golchinefar⁵

¹Iranian Veterinary Organization (IVO), Tehran, Iran

²School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

³Iran Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

^{4,5}Razi Vaccine and Serum Research Institute, Karaj, Iran

Abstract: Two hundred and seventy chicken muscle, liver and kidney samples from 90 broiler farm in Tehran province of Iran were collected over a period of one year starting from August 2001. All chicken had been slaughtered in Tehran slaughterhouse. High Performance Liquid Chromatography (HPLC) was used for separating, detecting and analyzing of Enrofloxacin residues in samples. All samples showed Enrofloxacin residues and samples from 22 (24% of farms showed residues of Enrofloxacin above MRLs (Maximum Residue Limits). 8 (8.88%), 12 (13.33%) and 22 (24.44%) of muscle, liver and kidney samples showed residues of Enrofloxacin above MRLs respectively. The mean concentrations of Enrofloxacin in muscle, liver and kidney samples were 18.32±32.29SD, 18.34±12.36 SD and 26.06±19.52 SD ng/g respectively. This study confirmed widespread misuses of Enrofloxacin in farms and lack of implementation of recommended withdrawal times. Also the results of this study emphasized on harder regulations for the use of antimicrobial drugs in poultry industry as well as the inspection of chickens for drug residues prior to marketing.

Key words: Antibiotics, Enrofloxacin, residues, poultry, Iran

Introduction

The antibacterial agents of the quinoline family are active against a wide range of Gram-negative organism but have activity against Gram-positive cocci (Barry and Fuchs, 1997). Enrofloxacin is a fluoroquinolone with a broad antibacterial spectrum and with bactericidal activity against major pathogenic bacteria found in diseased animal. Enrofloxacin is used in veterinary medicine in cattle, pigs, poultry, dog and cat (Droumev, 1983).

There is a worrisome world-wide trend of increased resistance to these agents among bacteria responsible for both hospital and community acquired infection including methicillin-resistant staphylococcus aureus, klebsiella pneumonia, pseudomonas aeruginosa, serratia marcesens, *Escherichia coli*, *salmonella* spp, *campylobacter* spp and neisseria gonorrhoeae (Johnston, 1998).

Cross-resistance between fluoroquinolones has been reported among a wide range of microorganisms including both Gram-negative and Gram positive bacilli (Linton, 1977).

The use of antimicrobial agents in food producing animals has recently become a very important public health issue. These agents are widely used for the prevention and therapy of infectious diseases in farm animals. An important measure when raising animal under intensive husbandry methods production (Maraschiello *et al.*, 2001; Manceau *et al.*, 1999; Mercer, 1975).

In addition, they are routinely used at sub-therapeutic levels as animal feed additives for their growth-

promoting properties (Okerman and Vanhoof, 1998).

European Union (EU) countries established a Maximum Residue Level (MRL) of 30 ng/g of muscle, liver and kidney for sum of enrofloxacin and ciprofloxacin (Prescott and Baggot, 1993; Posyniak *et al.*, 2001; Sogaard, 1973; Anonymus, 1969).

Enrofloxacin was introduced for veterinary use in Iran in 1990 and has been available since 1991 in pure powder and solution form for addition to poultry drinking water, prophylaxis or treatment of infectious due to Gram-negative microorganisms. In Iran about 7290317 kg antibiotics have been recently used in poultry.

During the past 7 years (from 1996). So that about 20% of this amount (1513794kg) was enrofloxacin. In this study enrofloxacin was determined in chicken tissues (liver, kidney and muscle) and compared with the permissible Maximum Residue Limits (MRL).

Chemicals: Enrofloxacin reference standard was purchased from Bayer. Hydrochloric acid, analytical grade, Na₂HPO₄, HPLC grade acetonitrile (CAN) and triethylamine (TEA) were purchased from Merck. Chicken kidney, liver and muscle were obtained from the Tehran slaughterhouse.

Materials and Methods

Over a period of one year starting from August 2001, a total of 270 muscle, liver and kidney samples from chicken were obtained from Tehran slaughterhouse, Iran. The chicken had been slaughtered in same slaughterhouse and were ready for marketing.

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Table 1: Summary of Enrofloxacin residues in 270 muscle, liver and kidney samples from 90 broiler farm of Tehran, Iran

	Muscle	Liver	Kidney
Range mean concentration-	2.8-233.2	4.3-66.2	4.2-133.0
Mean concentration \pm SD(ng/g)	18-32.295	18.348-12.632	26.06-19.526
%of sample positive Enrofloxacin	100	100	100
% of Enrofloxacin-positive sample with concentration above MRL	8.88	13.33	24.44

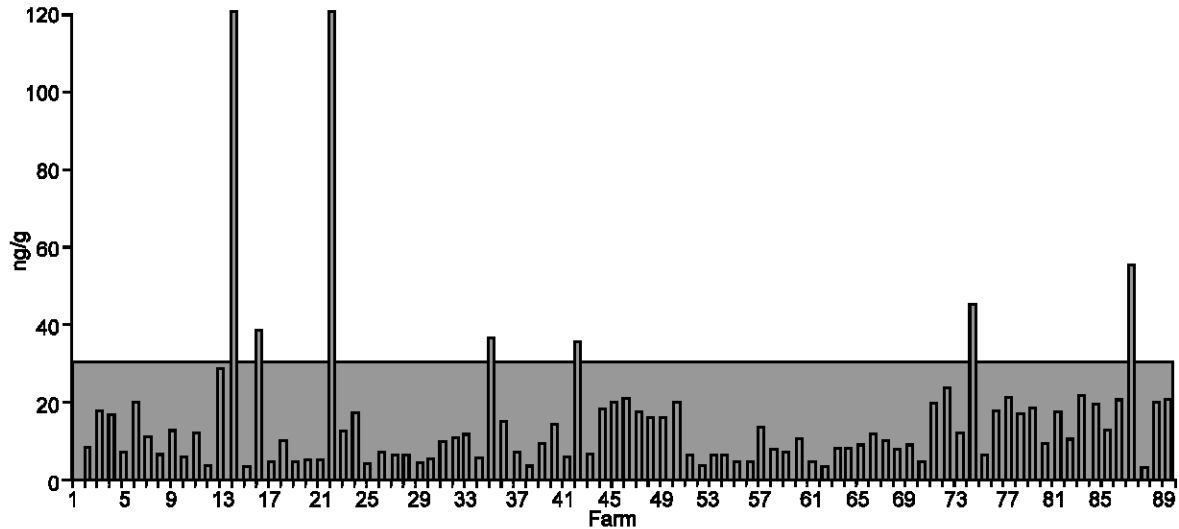


Fig. 1: Mean detectable concentrations of Enrofloxacin in muscle samples in comparison with the recommended maximum residue limit (MRL = 30 ng/g)

Separating, detecting and analyzing of Enrofloxacin residues performed using High Performance Liquidchromatography (HPLC).

Since the national Maximum Residue Limits (MRLs) for Enrofloxacin has not yet been fixed in Iran, the MRLs for Enrofloxacin that fixed by FDA and European Union (EU) countries has been used in this study.

Sample pretreatment: The samples were kept at-20 degrees centigrade until analysis. Analyzing of samples was carried out using 2.5 g of either kidney, liver or muscle tissues. In each case samples were allowed to defrost at room temperature. Then 300 mg kidney and liver and 400 mg muscle tissues was homogenized. The homogenized extract was then centrifuged at 4400 g for 10 min at 4°C. The exaction step was repeated twice and supernatants were pooled. In the case of the muscle tissue extract was left at 35 for 15 min and then centrifuged at 4400 g for 20 min at 4°C. and finally filtered by syringe filter.

Sample clean-up by solid-phase extraction: An SPE cartridge (SPE-pak vac 1cc (100mg) was conditioned with 2.5 mL of methanol and 2.5 mL of HPLC-grade water. The final extract (14 mL) was applied onto the cartridge. When the extract loading was completed, the cartridge was washed consecutively with 3 mL of HPLC-grade water, 3 mL of 0.2M Na₂HPO₄ (pH9) and 5 mL of

HPLC-grade water. The cartridge was subsequently dried by air aspiration. Enrofloxacin was eluted with 3.5 mL of MeOH, the eluted was evaporated to dry under nitrogen stream, The dry residue was redissolved in 200 mL of 0.2 m Na₂HPO₄ (PH9). The test tube was vortex-mixed for 30s and then centrifuged at 4400 g for 5 min at 4°C. The supernatant was trnsferd to an injection vial and 30 l was injected into the HPLC system. Enrofloxacin determination was performed by using a HPLC system consisting of a waters prep LC 4000 (USA) and a Spectroflow 783 UV-Vis detector (WATERS tm 486, tunablecabsorbance, USA) a 125 mm×4mm i.d. LiChrospher 100°C18 HPLC column (5 m) from waters was used. The detection wave length was set at 277 nm and a personal computer software (millennium v 12.15) was used for analyzing data.

The mobile phase used was water-CAN-TEA (83:14:0.45 v/v). ph was adjusted to 2.3 with 85% H₃PO₄ before adding CAN. The flow-rate was 1 ml/min. HPLC analysis of the samples was performed in 12 min.

preparation of standards stocks and working solutions: Stock solution of g/l of Enrofloxacin were prepared in 0.2M HCL. The working solution of 10 ng/l was prepared by diluting 10 l of the stock solution to 2 mL 0.2 NA₂HPO₄ (PH9). The working solution of 1ng/l was prepared by dilution 100 l of the 10 ng/l solution up to 1 mL with 0.2 M NA₂HPO₄ (PH9). Standards solution

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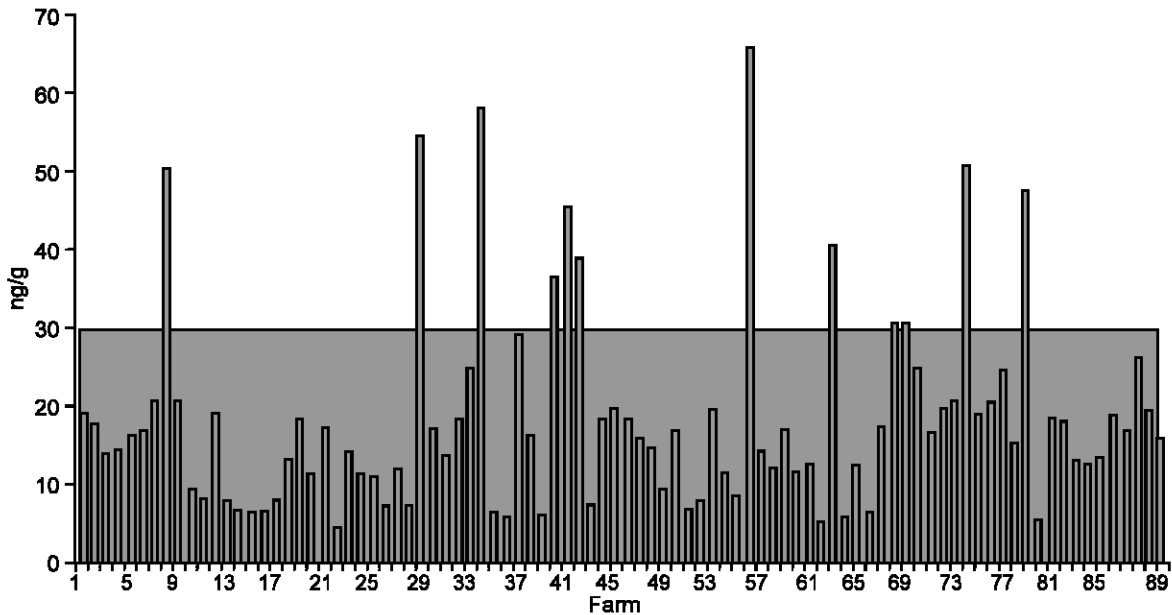


Fig. 2: Mean detectable concentrations of Enrofloxacin in liver samples in comparison with the recommended maximum residue limit (MRL = 30 ng/g)

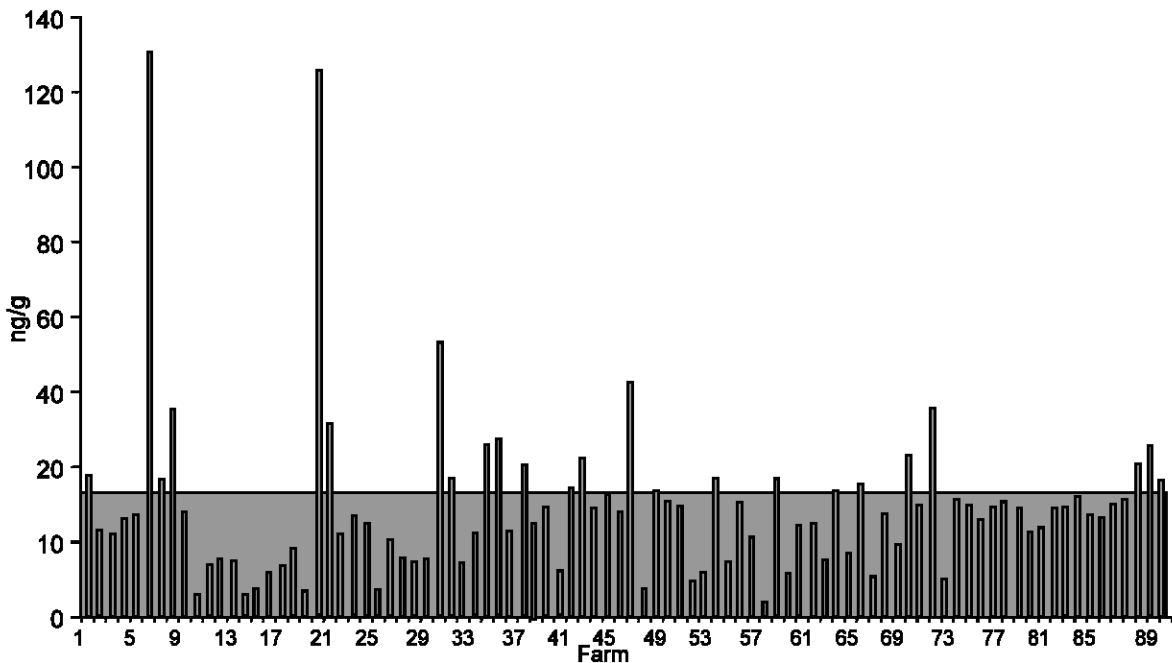


Fig. 3: Mean detectable concentrations of Enrofloxacin in kidney samples in comparison with the recommended maximum residues limit (MRL = 30 ng/g)

were from 30 to 500 ng/l (Barry and Fuchs, 1997; Droumev, 1983; Linton, 1977; Prescott and Baggot, 1993; Sogaard, 1973; Posyniak *et al.*, 2001).

Results

The results of this study indicated that Enrofloxacin was detectable in all samples by HPLC. The Enrofloxacin

positive samples, which showed residues of Enrofloxacin above MRLs, were 8 (8.88%), 12 (13.3%) and 22 (24.44%) in muscles, liver and kidney samples respectively. The mean Enrofloxacin concentrations in muscle, liver and kidney were 18.32 ± 32.29 SD, 18.34 ± 12.36 SD and 26.06 ± 19.52 SD ng/g respectively (Table 1). Fig. 1 displays the mean detectable

concentrations of Enrofloxacin in muscle in comparison with the recommended Maximum Residue Limit (MRL) for Enrofloxacin (30ng/g). Fig. 2 illustrates the mean detectable concentration of Enrofloxacin in liver in comparison with the recommended Maximum Residue Limit (MRL) for Enrofloxacin (30 ng/g) . Fig. 3 shows the mean detectable concentrations of Enrofloxacin in kidney in comparison with the recommended Maximum Residue Limit (MRL) for Enrofloxacin (30 ng/g). These results indicated that 12 (13.3%) of liver samples, 8 (8.88%) of muscle samples and 22 (24.44) of kidney samples were containing enrofloxacin in concentration above MRL (30 ng/g).

Discussion

The presence of antibiotic residues in food-producing animals has received enormous worldwide attention from local and international regulatory and public health Agencies. This is owing to the importance of the issue and its possible significant impact on public health. Many reports indicated that microbial resistance to antibiotics may arise as result of animal exposure to these agents and that the resistance may possibly be transferred to human pathogens (Yorke and Froc, 2000). In addition human exposure to animal products containing significant level of antibiotic residues may proven immunological response in susceptible individuals and cause disorder of intestinal flora (Zaki *et al.*, 2000). In the present study we examined chicken muscle, liver and kidney for the presence of Enrofloxacin. The results showed that all of the investigated broiler farms had detectable levels of Enrofloxacin at the time of marketing.

Enrofloxacin distributes widely into body tissues and found in high concentration in the excretory organs especially the liver and in the bile (Rao *et al.*, 2001).

Several organizations like food And agriculture organization (FAO), World Health Organization (WHO), Veterinary Medicine Directorate (VMD) of the European Union and Food and Drug Administration (FDA) of USA have set tolerance or Maximum Residue Limits (MRLs). Acceptable Daily Intakes (ADI) and withholding times for pharmacological active substance including antimicrobial agents. Different MRL have bee fixed for different quinoline compounds by the VMD of European Union. The fixed MRL for Enrofloxacin by VMD and European Union is 30 ng/g (Prescott and Baggot, 1993; Posyniak *et al.*, 2001; Anonymus, 1969). The result showed that 22 (24.44%) kidney samples, 12 (13.33%) liver samples and 8 (8.88%) muscle samples had Enrofloxacin residues higher than the MRL. These results confirm that enrofloxacin was heavily used in investigated poultry farms. They also suggest that the recommended withdrawal time was either not strictly applied or may be insufficient for this drug.

In comparison the residue violation for Enrofloxacin was detected only in 2 of 1388 broilers (0.14%) that had investigated by Okerman on 1998 in European Union (Okerman, 1998).

Conclusion: This study suggested widespread misuse of Enrofloxacin in farm and lack of implementation of recommended withdrawal times. This study therefore stresses the need for stricter regulation for the use of antimicrobial drugs in the poultry industry as well as the inspection of chicken for residues prior to marketing in IRAN.

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