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Oxytetracycline Residue in Chicken Tissues from Tehran Slaughterhouses in Iran

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Abstract: 270 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 Oxytetracycline residues in samples. All samples showed Oxytetracycline residues and samples from 86 (95.55%) of farms showed residues of Oxytetracycline above MRLs (Maximum Residue Limits). 25 (27.77%), 86 (95.55%) and 17 (18.88%) of muscle, liver and kidney samples showed residues of Oxytetracycline above MRLs respectively. The mean concentrations of Oxytetracycline in muscle, liver and kidney samples were 88.217 ± 44.503 SD, 576.657 ± 201.908 SD and 517.56 ± 186.64 SD ng/g respectively. This study confirmed widespread misuses of oxytetracycline 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, oxytetracycline, residues, poultry, Iran

Introduction

Tetracyclines are of great clinical importance because they possess a wide range of antimicrobial activity against aerobic and anaerobic Gram-positive and Gram-negative bacteria. They are also effective against some microorganisms that are resistant to cell-wall-inhibitor antimicrobial agents such as *Rickettsia*, *Mycoplasma pneumoniae*, *Chlamydia* spp., *Ureplasma* and some atypical *Mycobacteria* and *Plasmodium* spp. (Kapusnik-Uner *et al.*, 1996). Pharmacokinetic studies have demonstrated that about 60% of ingested oxytetracycline is absorbed through the gastrointestinal tract in human, compared to 4-9% in mice and swine. Following absorption by various routes of administration, oxytetracycline is widely distributed in the body, particularly in the liver, kidney, bone and teeth. Systemically available oxytetracycline is primarily excreted in the urine, as parent drug. The mutagenic potential of oxytetracycline has been investigated in a range of studies. Negative results have been recorded in bacterial test, a chromosomal aberration test, a sister chromatid exchange test (with and without metabolism activation) and a mouse lymphoma test without metabolic activation. A positive effect in the mouse lymphoma test with metabolic activation has been obtained using dose levels close to toxic concentration but the positive effect in the *in vivo* micronucleus assay in mice has not been dose related (JECFA, 1990). The use of antimicrobial agents in food-producing animal has recently become a very important public

health issue (AL-Ghamdi *et al.*, 2000). This is due to the fact that these agents are being increasingly used in animal farm production. Many antimicrobial agents are routinely added to animal feed at sub-therapeutic levels for their growth promoting properties (Droumev, 1983). In addition antimicrobials are widely used for disease prophylaxis and treatment as important measure when raising animals under intensive husbandry method of production (Aronsol, 1975; Droumev, 1983; Linton, 1977). Thus these agents are considered very valuable in preventing major economic losses to growers caused by diseases outbreaks. This practice, however carries many disadvantages, such as the stimulation of microbial resistance and presence of drug residues in animal products which may pose a major health risk to the public (Mercer, 1977). Internationally recognized organizations such as World Health Organization (WHO), Food and Agriculture Organization (FAO, 1988) and Veterinary Medicines Directorate (VMD) (VMD, 1997; VMD, 2000) of the European Union as well as Food and Drug Administration (FDA) of USA (Aronsol, 1975) have set tolerance or maximum residue limits (MRLs) and acceptable daily intakes (ADI) for humans and withholding times for pharmacologically active substances including antimicrobial agents prior to marketing.

Chemicals: Oxytetracycline reference standard was purchased from PFIZER, analytical-grade Na_2HPO_4 , EDTA-III And Citric acid obtained from Merck, Germany.

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

	Muscle	liver	kidney
Range mean concentration	6.6-255.3	210-1362	207-1498
Mean concentration \pm SD(ng/g)	88.75 \pm 44.503	576.65 \pm 201.906	517.56 \pm 186.64
%of positive oxytetracycline sample	100	100	100
%of oxytetracycline positive sample with concentration above MRL	27.77	95.55	18.88

Oxalic acid obtained from Reid-Dehaen, Germany. HPLC grade acetonitril (ACN) and methanol (MEOH) were purchased from Merck, Germany.

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. Separating, detecting and analyzing of oxytetracycline residues performed using high performance liquid chromatography.

Since the national maximum residue limits (MRLs) for Oxyteracycline has not yet been fixed in Iran, the MRLs for Oxytetracycline 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 tissues were homogenized and probe was rinsed twice with 2 ml Mcilvaine buffer-EDTA solution into centrifuge tube. 10 ml Mcilvaine buffer-EDTA solution was added to tube and was blended 30s with homogenizer and centrifuged 10 min at 2500 g. Then without transferring any intact tissue supernatant was poured into second 50 ml centrifuge tube. After adding 10 ml Mcilvaine buffer-EDTA solution, the first tube was capped and using vortex-mixer, tissue plug resuspended. The suspension was shaken for 10 min, centrifuged 10 min at 2500 g and then the supernatant was added to first supernatant in second tube. Tissue plug in first tube was suspended in 10 ml Mcilvaine buffer-EDTA solution. All steps were repeated until supernatants from 3 extraction were collected in the second tube. The suspension then was mixed and centrifuged for 20 min at 2500g. Then single GF/B filter paper was fixed in buchner funnel and moisturized with Mcilvaine buffer-EDTA. After that mixed supernates was filtered through funnel.

Sample cleanup by solid phase extraction: An SPE cartridge was conditioned with 10 ml methanol and 100 ml of HPLC grade water. The final extract was applied onto the cartridge, when the extract loading was completed. Oxytetracycline was eluted with 3 ml

methanolic oxalic acid solution and diluted to 5 ml with HPLC Grade water. Then tube vortex-mixed for 30s and 20 μ l was injected into the HPLC system. Oxytetracycline determination was performed by using a HPLC system (model 4000, USA) and a 783 uv-vis detector, Waters TM 486, tunable absorbance (USA), and Delta-Pack HPLC column. The detection wavelength was set at 350 nm and a personal computer software (Millennium v12.15) was used for analyzing data.

The mobile phase used was methanol-acetonitrile-methanolic oxalic acid (10:30:60). Flow rate was 1.5 ml/min. HPLC analysis of the samples was performed in 5 min.

Preparation of standards stock and working solution:

Stock solution of 100 μ g of oxytetracycline were prepared by diluting 100 mg of reference standard to 100 ml with methanol. The working solution of 100 μ g/ml was prepared by diluting of 10 ml of stock solution to 100 ml with methanol. The working solution of 25 μ g/ml was prepared by diluting of 2.5 ml of 100 μ g/ml solution to 10 ml with methanol.

Standard solutions were from .05 to 1 mg/ml (These solution were kept in -20°C) (Cooper *et al.*, 1998; Walsh *et al.*, 1992; Furusama, 1999)

Results

The results of this study indicated that Oxytetracycline was detectable in all samples by HPLC. The Oxytetracycline positive samples, which showed residues of Oxytetracyclin above MRLs, were 25 (27.77%), 86 (95.55%) and 17 (18.88%) in muscles, liver and kidney samples respectively.

The mean oxytetracycline concentrations in muscle, liver and kidney were 88.75 \pm 44.503 SD , 576.657 \pm 201.908 SD and 517.56 \pm 186.64 SD ng/g respectively (Table 1). Fig. 1 displays the mean detectable concentrations of oxytetracycline in muscle in comparison with the recommended maximum residue limit (MRL) for oxytetracycline (100ng/g). Fig. 2 illustrates the mean detectable concentration of oxytetracycline in kidney in comparison with the recommended maximum residue limit (MRL) for oxytetracycline (600 ng/g). Fig. 3 displays the mean detectable concentrations of oxytetracycline in liver in comparison with the recommended maximum residue limit (MRL) for oxytetracycline (300 ng/g).

These results indicated that 86(95.55%) of liver samples, 25 (27.77%) of muscle samples and 17

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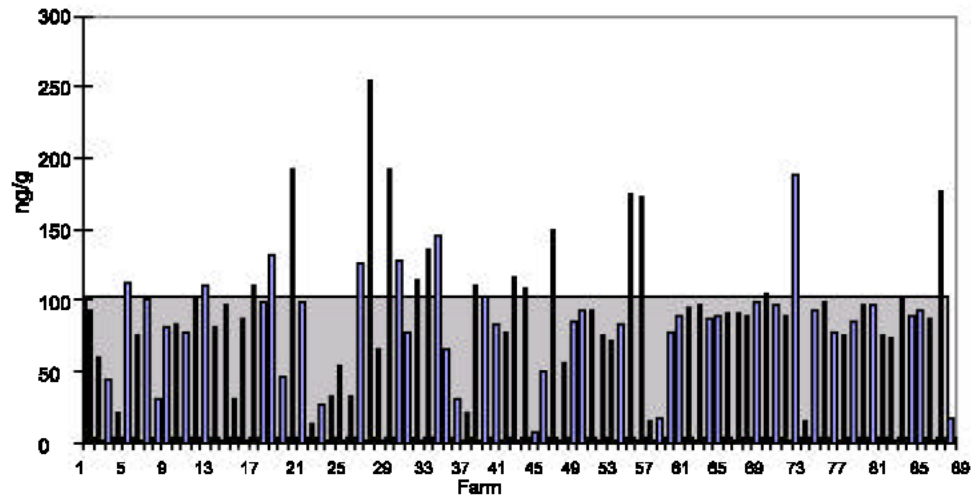


Fig. 1: Mean detectable concentrations of Oxytetracycline in muscle samples in comparison with the maximum residue limit (MRL=100 ng/g)

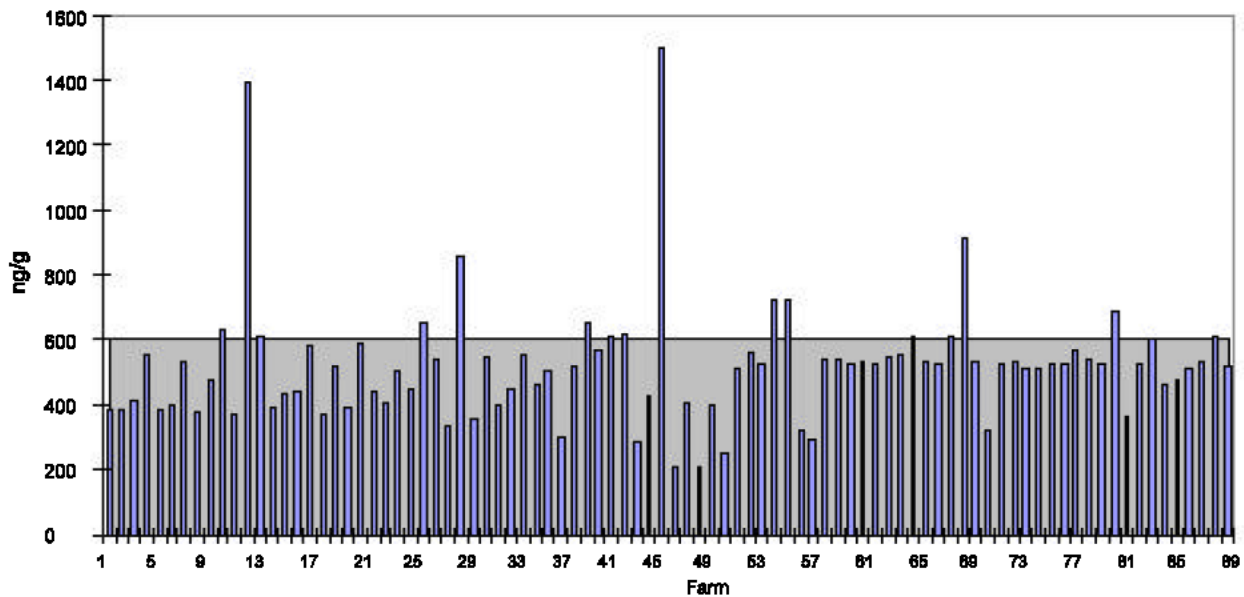


Fig. 2: Mean detectable concentrations of oxytetracycline in kidney samples the comparison with recommended maximum residues limit (MRL=600 ng/g)

(18.88%) of kidney samples were containing oxytetracycline in concentration above MRL.

Discussion

The results of present study showed that samples from edible tissues of 86 (95.55%) of samples of chickens of broiler farms in Tehran, had residues of Oxytetracycline above MRLs.

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 have indicated that microbial resistance to antibiotics may arise as result of animal exposure to these agents and the resistance may possibly be transferred to human pathogens (Soggard, 1973). In addition human exposure to animal products containing significant level of antibiotic residues may prove immunological response in susceptible individuals and cause disorder of intestinal flora (Linton, 1977). In the present study we examined samples of muscle, liver and kidney of chicken for presence of oxytetracycline residues. The results showed that all samples from the investigated broiler farms had detectable levels of oxytetracycline at the time of marketing.

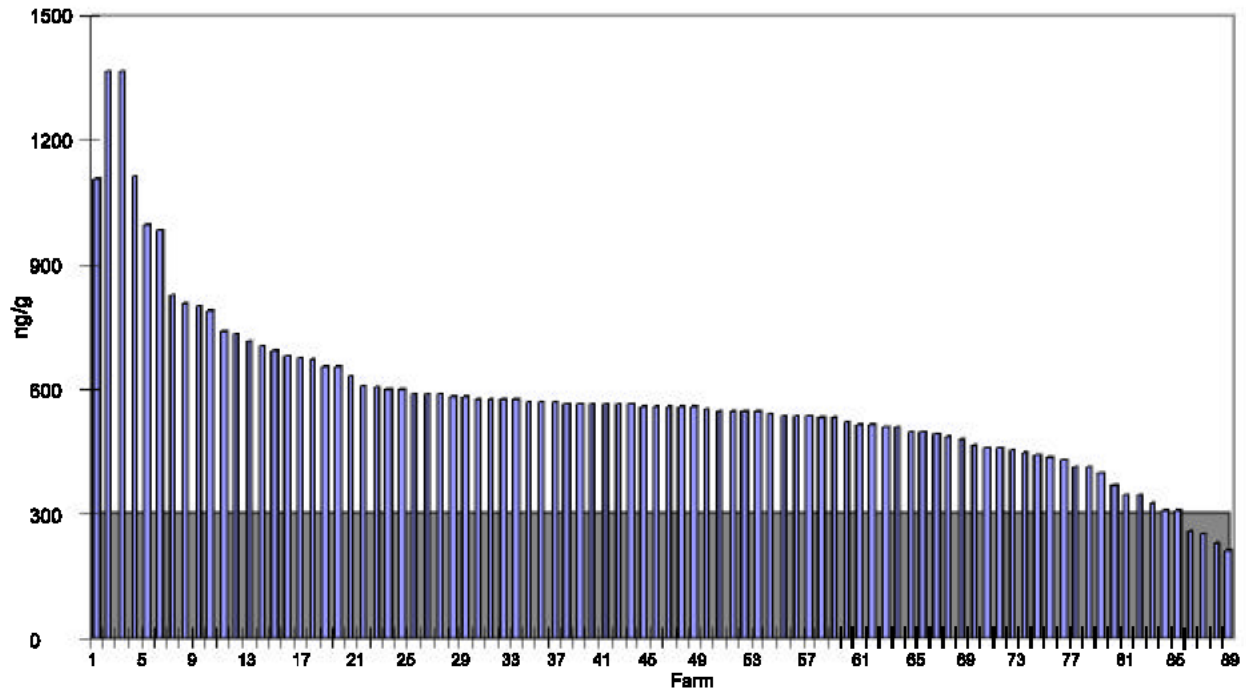


Fig. 3: Mean detectable concentrations of oxytetracycline in liver samples comparison with the recommended maximum residues limit (MRL=300 ng/g)

Oxytetracycline is distributed widely into body tissues and can be found in high concentrations in the excretory organs especially the liver and in the bile (Prescott and Baggot, 1993). Several organizations like the food and agriculture organization (FAO), World Health Organization (WHO), Veterinary Medicines Directorate (VMD) of the European and also food and Drug Administration (FDA) of USA have set tolerance or Maximum Residue Limits (MRLs). The recommended MRLs of this drug in all food-producing animals have been set at 100, 300 and 600 ng/g in muscle, liver and kidney respectively, while Accepted Daily Intake (ADI) for human is recommended not to exceed 3µg. The result of the present investigation indicated that 86 (95.55%), 17 (18.88%) and 25 (27.77%) of liver, kidney and muscle samples respectively had Oxytetracycline residues higher than the recommended MRLs. These results confirmed that Oxytetracycline was heavily used in poultry farms under investigation. 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 oxytetracycline detected in the antibiotic residues positive farms mean that concentration of at least one tetracycline compound had been in excess of the permissible maximum residues limits (MRL) in raw muscle and liver, respectively.

Conclusion: This study suggested widespread misuse of oxytetracycline 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 Tehran. IRAN.

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