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OXYTETRACYCLINE RESIDUES IN EDIBLE TISSUES OF CATTLE SLAUGHTERED IN AKURE, NIGERIA

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SUMMARY

Meat and other edible tissues from slaughtered cattle from Akure metropolitan abattoir from January to June 2007 were analyzed with high performance liquid chromatography (HPLC) for oxytetracycline residue. The extraction was done using hydrochloric acid and acetonitrile for deproteinisation, while clean up was by liquid- liquid partitioning using dichloromethane and petroleum ether. Elution, detection and quantification were done on Lichrosorb RP–18 HPLC machine coupled with UV – detector. Out of a total of 180 beef samples analyzed during this study, 98 (54.44%) of the total samples had detectable levels of oxytetracycline residues from which 62(34.44%) had oxytetracycline residues at violative levels above the WHO/FAO maximum residue limits (MRLs). The mean residues for positive samples were 51.8µg/kg, 372.7µg/kg and1197.7µg/kg for muscle, kidney and liver respectively. The standard deviations (SD) of residue in samples tested positive were 718.9µg/kg, 366.8µg/kg, and 90.53µg/kg in liver, kidney and muscle respectively. These high level oxytetracycline residues in greater proportion of meat destined for human consumption at violative levels could be as a result of the indiscriminate use and misuse of veterinary drugs as commonly practiced among livestock producers and marketers without observing withdrawal period prior to slaughter. These results indicate that consumers may be predisposed to health hazards and hinder international meat trade from Nigeria. Regulatory authorities should therefore ensure compliance with good agricultural practices including withdrawal period of drugs used for treatment of food animals, while livestock producers should also be educated on responsible use of drugs in food animals. Routine drug residues surveillance and monitoring programs in meat and other edible livestock products should be established in the country to ensure food safety.

KEYWORDS: Oxtetracycline residue, Meat, HPLC, Food safety

INTRODUCTION

Antibiotics have been used in livestock farming for several decades in combating bacterial infections, but lack of proper application and handling can lead to occurrence of residues in foods of animal origin particularly meat, milk and eggs. Farm animals treated with antibiotics are required to be held for

specific withdrawal period until all residues are depleted to safe level before the animal tissue can be used as food for human consumption (KuKanich *et al.*, 2005). In Nigeria like most developing countries, antibiotics are used in animals indiscriminately for the prevention and treatment of bacterial infection (Dina and Arowolo, 1991; Kabir *et al., 2004*). A greater proportion of cattle in Nigeria are reared by the nomadic herdsmen who administer chemotherapeutic agents without veterinary prescription (Alhaji, 1976). When such laymen use these drugs, correct dosage are unlikely to be administered and the withdrawal periods are usually not observed. Tetracyclines are among various antibiotics widely used in livestock (Karimuribo *et al.*, 2005; Nonga *et al.*, 2009). Improper dosage of oxtetracycline especially at sub-therapeutic levels can result in acute or chronic public health problems, which could be toxicological, microbiological or immunological (Booth, 1978; FAO, 1999). Human health problems that could arise from the consumption of unacceptable levels of oxytetracycline residues in meat include gastrointestinal disturbances, hypersensitivity, bone and teeth problems in children and development of bacterial resistance (Woodward, 1991; Czeizel *et al.*, 1998 Larkin *et al.*, 2004). Excessive use of antimicrobials in agriculture, subject microorganisms to the same forces of evolution and resistance as occur when antimicrobials are used in humans (Van den Bogaard and Stobberingh, 2000). Humans may encounter bacteria from animals through food supply, direct contact with animals, or via contaminated water (Van den Bogaard and Stobberingh, 1999). Many of the antimicrobials used in animals are also used in human medicine; the use of antimicrobials in animals is part of the global problem of antimicrobial resistance (Aryal, 2001). Oxytetracycline is one of the most commonly used antibiotics in livestock production.

Oxytetracycline levels above the WHO/FAO recommended Maximum residues limits have been reported in edible tissues of slaughtered animal in Nairobi slaughterhouses in Kenya, (Muriuki *et al.*, 2001). In Nigeria various studies have been conducted on drug administration and residues deposition in meat and animal products, have demonstrated the presence of antibiotic residues in meat and animal products (Kabir *et al.*, 2004; Dipeolu and Alonge, 2002). Most of these studies were based on microbiological screening techniques that do not specifically classify and quantify the antibiotics. Hence the degree of risks to the consumers could not be ascertained due to low specificity and sensitivity of techniques. High performance liquid chromatography (HPLC) procedures are widely used to quantify various antibiotic residues in food products with good sensitivity and specificity (Moats, 1986; Dipeolu and Kanamaru, 1996; Senyuva *et al.*, 2000; Muriuki *et al.*, 2001). To our knowledge there has not been any documented report of oxytetracycline residue analysis in beef using HPLC from Nigeria. This study was therefore conducted to quantify the levels of oxytetracycline residue in edible tissues of cattle slaughtered in Akure municipal abattoir which is a major source of meat within Southwest Nigeria.

MATERIALS AND METHODS

Equipment:
High Performance Liquid chromatography (HPLC) machine (CECIL®, 1000 series) equipped with a constant flow pump, variable wavelength UV detector. Lichrosorb RP –18 (10µm, 250 x 4.6mm I.D) columns, a recorder operated at 10mV and chart speed of 5mm/min, meat scissors, high speed laboratory blender and different glass wares.

Chemicals:
Analytical grade oxytetracycline standard from Sigma chemical Co, St Louis, MO, USA., acetonitrile (HPLC grade), methanol (HPLC grade), oxalic acid, hydrochloric acid, methylene chloride, petroleum ether and distilled water. All chemicals were analytical grade and were properly degassed.

Location of study:
Akure is a city in Southwestern Nigeria and the capital of Ondo State. Akure is connected by road to other Nigerian cities such as Lagos and Ibadan and also has a domestic airport. In the city is a Federal University of Technology; it is also a tourist destination and departure point for visitors to the nearby Osse River and Idanre hill. Akure municipal abattoir is a major source of meat for the capital city and towns within the State. An average of 40 to 55 heads of cattle was slaughtered daily by individual private butchers who usually sourced the animals from nomadic herdsmen and cattle markets from northern Nigeria. Meat inspection was supervised by the Veterinary Department of the State Ministry of Agriculture (Ondo State Ministry of Agriculture, Fisheries, and Forest Resources, 2007).

Sample collection:
Approximately 50gms of liver, kidney and muscle (beef), 60 samples each were obtained by simple random sampling on a weekly basis from carcasses of cattle meant for public consumption at Akure municipal abattoir between January and June 2007. The samples were wrapped in polythene bags, transported in a cool box packed with ice to the Department of Veterinary Public Health and Preventive Medicine laboratory, University of Ibadan, for extraction and clean up processes.

Preparation of Standard Curve:
Oxytetracycline standard powder was accurately weighed and dissolved in methanol to make a stock solution and several serial dilutions of the stock solution were injected to the HPLC machine to obtain the standard curve by plotting the peak heights against the concentrations (Fig. 1). The detection limit for oxytetracycline was 0.01ppm while the retention time was 4 minutes.

Sample preparation:
The extraction and clean up procedures developed by Moats in 1986 was employed. This involves Liquid - Liquid partitioning extraction procedures to obtain the analyte. 25g of each sample was homogenized with 3 volumes of 1N hydrochloric acid. Eight millilters of the homogenate was thoroughly swirled with 32mls acetonitrile and allowed to stand for 5minutes after which the supernatant was decanted though a glass wool on the stem of a glass funnel. Twenty milliters of the filtrate was mixed with 20mls petroleum ether and 20mls methylene chloride in a separatory funnel and vigorously shaken

resulting in separation to two layers. About 4mls of the water layer containing the analyte was collected for HPLC analysis.

HPLC analysis for oxytetracycine:
The analysis and quantification of the oxytetracycline residues in the analyte was done at the Chemistry Laboratory, Department of Chemistry, University of Ibadan, using a high-performance liquid chromatography machine equipped with a constant flow pump and a variation wavelength UV detector set at 280nm and flow rate of 2mls/min. Elution of oxytetracycline from the analyte was done on a Lichrosorb RP- 18 (10µ, 250 x 4.0mm 1D) Column with Methanol-Acetonitrile-0.01m aqueous Oxalic acid solution, PH 2.0 (1: 1.5: 2.5) as the mobile phase as described by Muriuki *et al*., (2001). The analyte from each sample was injected in duplicate to obtain average peak height of positive samples corresponding to the retention time of 4 to 4.5 minutes as the reference standard. Quantification of oxytetracycline residues in the samples were obtained and calculated from the peak heights extrapolated from the calibration curves of the standard.

RESULTS
Out of the 180 beef samples analyzed during this study, 98(54.44%) comprising of 48 (80%) liver, 33 (55.0%) kidney and 17 (28.3%) muscle samples had detectable levels of oxytetracycline residues, while remaining 82 samples (45.6%) had no detectable residues. Out of the positive samples, 62 (63.2%) had oxytetracycline residue at violative levels while 36(36.8%) had residue levels below the WHO / FAO recommended maximum residue limits (MRLs) for oxytetracycline in meat, liver and kidney. The mean residue levels of oxtetracycline were 1197.0±718.9µg/kg, 372.7±366.8µg/kg, 51.80±90.53µg/kg in liver, kidney and muscle respectively (Fig. 2), while the ranges of oxtetracycline in the tissues were 424 to 2370µg/kg, 338 to 1016µg/kg, 0 to 220µg/kg in liver, kidney and muscle respectively (Table I). There were significant differences ($p<0.05$) in the mean residues of oxytetracyline in the different tissues using one-way ANOVA but there was no significant difference ($p>0.05$) in the mean residue levels obtained at different months of this study.

Table I: Result of meat/organ with OTC residue from Akure municipal abattoir

Meat/organ	Sample	Positive for OTC residue (%)	Samples with residue above MRL (%)
Liver	60	48 (80.0)	27 (45.0)
Kidney	60	33 (55.0)	21 (35.0)
Muscle	60	17 (28.3)	14 (23.3)
Total	180	98 (54.4)	62 (34.4)

TABLE II: Monthly result of oxytetracycline (OTC) residues levels (µg/kg) in carcasses from Akure municipal abattoir (January – June 2007)

Month	OTC residue	Liver	Kidney	Muscle
January	Positive	10/10	6/10	4/10
	Mean	1720	355.8	83.20
	Range	1016 to 2370	338 to 1016	220 to 240
February	Positive	8/10	6/10	5/10
	Mean	674.0	389.6	20.40
	Range	424 to 1354	1016	0 to 204
March	Positive	6/10	5/10	2/10
	Mean	1720	355.8	83.20
	Range	1016 to 2370	338 to 1016	204 to 220
April	Positive	8/10	5/10	2/10
	Mean	674.0	389.6	20.40
	Range	424 to 1354	424 to 1016	0 to 204
May	Positive	4/10	4/10	2/10
	Mean	1720	355.8	83.20
	Range	1016 to 2370	338 to 1016	204 to 220
June	Positive	10/10	7/10	2/10
	Mean	834.8	398.2	42.40
	Range	592 to 2032	424 to 1016	204 to 220
Total	Positive	**48/60**	**33/60**	**17/60**

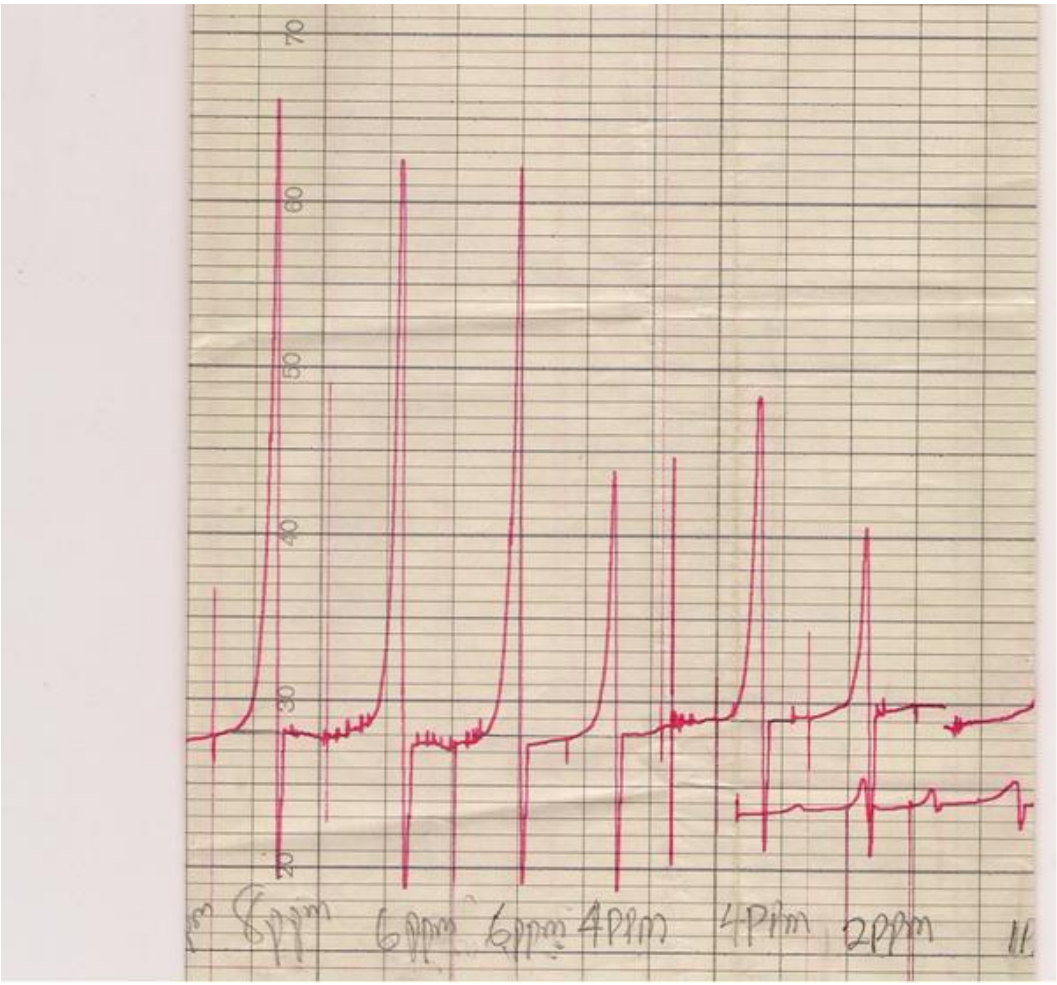
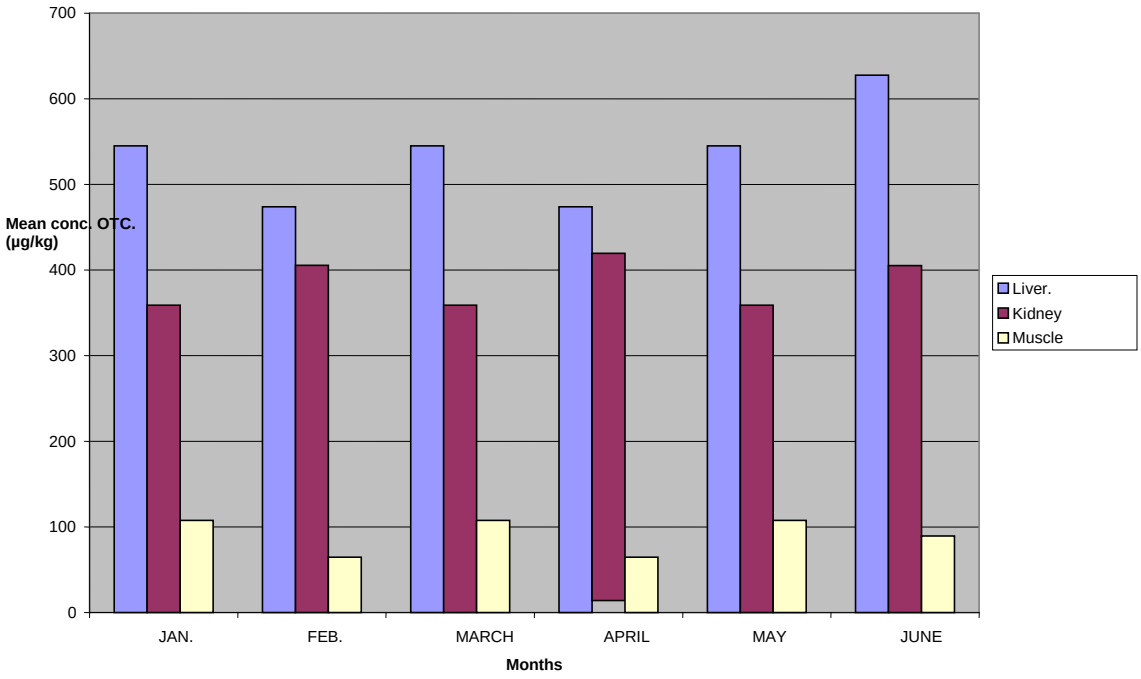


Figure 1: Chromatograph of oxytetracyline reference standard showing peak heights of the corresponding concentrations

FIG. 2: Monthly mean distribution of OTC residue in the tissues (Jan-June 2007)



DISCUSSION

Tetracyclines have served for decades as an important class of antibiotics in food animal health and production. As such, they have also been a source of concern for residue monitoring authorities around the world. In response to this concern the World Health Organization and the Food and Agriculture Organization Joint Committee on residues of some veterinary drugs in animals and foods recommended maximum residue limits for various drugs in edible tissues of food animals. The recommended maximum residue limits (MRLs) for oxytetracycline are 200µg/kg, 600µg/kg and 1200µg/kg in meat (beef), liver and kidney respectively. About 63.2% (comprising 44 liver, 4 kidney and 14 muscle samples contained residue levels above (FAO, 1999) recommended standards. Liver and kidney samples yielded more positive result of the residue than muscle. This agreed with findings of most workers (Moat, 1986; Dipeolu and Alonge, 2002; Muriuki *et al.*, 2001). These organs of metabolism and excretion of the drugs are delicacies by some meat consumers, and portends greater risk of exposure to residues in this group.

The total prevalence obtained in this study is higher than 16.11% in cattle (Dipeolu and Alonge, 2002) and 33.1% obtained in broilers (Kabir *et al*., 2004) in Nigeria and higher than 45.6% oxytetracycline residues reported from slaughtered cattle in Kenya by Muriuki *et al.,* (2001). This could also be a result of the higher sensitivity and specificity of HPLC in quantitation of drug residues than the microbial inhibition techniques used by the previous authors in Nigeria. The high prevalence (54.4%) of

oxytetracycline residues obtained from this study may be an indication of widespread misuse of veterinary drugs by food animal producers across Nigeria, since these animals were sourced from different parts of the country. This may be due to the fact that greater proportion of cattle rearing in Nigeria is mostly by nomadic herdsman using veterinary drugs indiscriminately. Also in Nigeria, these drugs are easily obtained over the counter without veterinary prescription and supervision. This is also a consequence of lack of adequate Veterinary and Public Health regulatory control in the country (Dina and Arowolo, 1991). This portends great risks and hazards to human health that could result in allergy, cancer, embryo toxicity and antibiotic resistance effects on the consumers. These authors (Kabir *et al.*, 1999; Aliu *et al.*, 2001) reported the socio-economic implications of drugs and chemical residues in carcasses as resulting physico-chemical changes in meat leading to condemnation and severe economic losses by the stakeholders. This is also a critical factor in international meat trade that can deprive the country earnings from meat and meat products in the international market.

CONCLUSION

This study has shown that oxyetracycline residues of 54.44% prevalence are present in edible portions of bovine carcasses meant for human consumption in Akure metropolis. Oxytetracyline is routinely misused in Nigerian livestock and are deposited within their tissues at significant levels rendering most of the meat unsafe and unwholesome for human consumption. Livestock producers in the country should be given extension education on good agricultural practices and responsible use of antibiotics in food animals; including correct diseases diagnosis, adequate dosage and observance of withdrawal periods of drugs used for treatment of food animals. Regulatory authorities should also ensure proper meat inspection and drug residues surveillance program should be established in the country to ensure food safety.

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