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Two hundred and fifty beef samples were collected from five slaughterhouses in and around the city of Nairobi. The beef animals were sourced from various parts of the country. Samples of 50-100 grams were collected randomly from the liver, kidney and muscle of different beef carcasses. The samples collected were processed using multiresidue analytical methods that included liquid-gas partitioning and set-pat C18 cartridges chromatographic clean up. Chlortetracycline and oxytetracycline detection was done using Knauer Model 128 HPLC with an electron capture detector. Out of the 250 samples that were analyses for tetracycline residues 114 (45.6%) had detectable tetracycline residues. Of the 114 samples with detectable tetracycline residues, 60 (24%) were liver samples, 35 (14%), were kidney samples and 19 (7.6%) were muscle samples. The mean (p>0.05) residue levels of tetracycline for the five slaughterhouses studied were as follows: Athi River 1,046 μg/kg, Dandora 594 μg/kg, Ngong 701 μg/kg, Kiserian 524 μg/kg and Dagoretti 640 μg/kg. Of the 250 samples analysed 110 (44%) had oxytetracyclines while 4 (1.6%) had chlortetracyclines. The mean residue levels of the detected tetracyclines were higher than the recommended maximum levels in edible tissues. This study indicates the presence of tetracycline residues in the various edible tissues. Regulatory authorities should ensure proper withdrawal periods before slaughter. This study indicates the presence of tetracycline residues in the various edible tissues. Regulatory authorities should ensure proper withdrawal period before slaughter of the animals.

Key words: Tetracycline residue, Nairobi, Kenya

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Introduction

Antibiotics are widely used in animal health practice. In Kenya, as in many other countries, antibiotics may be used indiscriminately for the treatment of bacterial diseases of domestic animals [10]. When such drugs are administered by laymen correct dosages are unlikely to be observed as well as withdrawal period before slaughter. This misuse of antibiotics is a potential hazard to human health [15]. Improper dosages of tetracyclines especially subtherapeutic doses may lead to the emergence of resistant bacteria. The organisms may become resistant to tetracyclines and to other agents [30,33]. Resistant strains of *Staphylococci*, *Coliforms*, *Bacilli*, *Pheumococci*, *Haemolytic streptococci*, strains of *Haemophilus influenczae* and *Clostridium welchii* have been [22,12].

Human health problems resulting from intake of subchronic exposure levels of tetracyclines include gastrointestinal disturbances [25,2], poor foetal development [5] and hypersensitivity]23] and other toxic effects. Tetracyclines in meat potentially may stain teeth of young children.

In order to safeguard human health, the World Health Organisation (WHO) and the Food Agriculture Organisation (FAO) have set standards [13] for acceptable daily intake and maximum residue limits in foods *inter alia*. These limits apply to both the parent drug or chemical and its metabolites that may accumulate and be deposited or stored within the cells, tissues or organs following the administration of the compound.

The acceptable maximum residue residue limit for tetracyclines as recommended by the joint FAO/WHO Expert Committee on Food Additives (1999) is 200 μg/kg, 600 μg/kg and 1200 μg/kg for beef, liver and kidney respectively.

Several methods like fluorimetry [32] and chromatography [28] have been used to detect antibiotic residue levels in feeds and animal tissues but each method has its own limitations. The easiest, fastest and cheapest method is the microbiological assay, using *bacillus cereus*

type ATCC 11778 as the test organism. Several workers have used this microbiological assay on their studies with slight modifications [14].

Tetracycline levels above maximum residue limit have been reported in eggs and chicken tissue in Kenya [20], however there are no reports for tetracyclines levels Kenyan beef. The purpose of this study was therefore to investigate residue levels of tetracyclines in beef slaughtered in Nairobi slaughterhouses. The slaughtered animals were obtained from various parts of the country.

Material and Methods

Area of study

A total 250 samples were obtained from beef carcasses in five major slaughter plants in Nairobi and its environs. Fifty carcasses were sampled at each station. These slaughter plants included Athi River abattoir, Dagoretti, Dandora, Ngong and Kiserian slaughter plants. Records indicated that the animals slaughtered in these slaughter plants originated form Nakuru, Kajiado, Narok, Laikipia and Machakos Districts while the while the other districts mentioned above supplied slaughter animals for Dagoretti, Ongata Rongai and Kiserian slaughterhouses.

Sampling procedure

Approximately 50 to 100 grams of labelled liver, kidney or muscle samples obtained from each carcass was wrapped in polythene bags and put in cool boxes with dry ice or freezer packs at 4°C. The samples were subsequently transported to our laboratories. The samples were stored at –20°C until time of analysis.

Preparation of the standard curves

Sigma Chemical Co., St. Louis MO. USA, supplied analytical standards of oxytetracycline and chlortetracycline chlorides. For each tetracycline, 100 mg was accurately weighed and put in a 100 ml volumetric flask, the powder was dissolved in 100 ml of methanol to make a stock solution of 1,000 ppm. Several serial dilutions of the stock solutions were carried out to give the following dilutions: 1 : 100 (10 ppm), 1 : 200 (5 ppm), 1 : 400 (2.5 ppm), 1 : 500 (1.25 ppm), 1 : 1000 (0.1 ppm), 1 : 10000 (0.01 ppm), These final concentrations were used to prepare the standard curves. The corresponding concentrations of these dilutions (ppm were: 10, 5, 2.5, 0.1, and 0.01) were used as working standards. The detection limit for oxytetracycline was 0.01 ppm.

For the standard curves, the best line of fit was calculated by a curve fitting programmes (Macintosh SE), using the following equation, $Y = a + b \log X$. When Y = length of the peak (mm), a = Y-intercept, b = the slope, X = concentration of the oxytetracycline (ppm).

Sample preparation

Five grams of each organ to be analysed was weighed using a balance and then cut into very small pieces and subsequently ground into fine powder using sartorius mincer. This was then blended three times with 20 and 30 ml aliquots of McIlvaine buffer (pH 4.0) : methanol (3 : 7) using a high speed Elmore Parker blender and then centrifuged with Heraeus-Christ GMBH, Hannover, centrifuge at 2000 × g for ten minute. This was then filtered using whiteman filter paper. The filtrate was collected in clean beaker and the supernatant discarded. The filtrate was then applied on a Baker 10 C18 cartridge, activated with water and methanol and the cartridge was washed twice with 20 ml of water. The tetracyclines were eluted with 10 ml of 0.01 µl methanolic oxallic and solution and collected in 10 ml volumetric flask.

The extracted tetracyclines were analysed, identified and quantified by use of the HPLC method.

Analysis for tetracyclines

Determination of the tetracycline residues was done using a high-pressure liquid chromatography equipped with a constant flow pump and a variation wavelength UV-detector set at 350 nm. The separation was done on Lichrosorb RP-18 (10 µm, 250 × 4.0 mm I.D.E Merck) column with methanol-acetonitrile-0.01 M aqueous oxalic acid solution pH 2.0 (1 : 1.5 : 2.5) as the mobile phase (methanol-acetonitrile-0.01 M flow-rate of 2 ml/min at room temperature and the sensitivity range was 0.0 8ppm.

For determination of tetracyclines, several blanks (methanol only) and OTC and OTC standard solution (25 µl) % concentrations: 10.5, 2.5, 1.25, 1.0, 0.5, 0.25 and 0.1 ppm were injected manually using 10 µl syringe in a descending order and their corresponding areas (concentrations), were recorded only if the retention time was equal to 4.5 minutes which was the retention time for oxytetracycline. This was done in triplicates for the samples. Results for the positive samples were plotted automatically on the inegrater whose attenuation was 128. To get the concentration of a given sample, a reference standard of a known concentration was injected into the HPLC and concentration of the sample was extrapolated from the curves peak height. This was done in triplicate each. A given sample was regarded as positive for tetracyclines if its retention time and peak corresponded to that of the standard. The recorder was operated at 10 mv with a chart speed of 5 min/min. Since the concentration of standard was known, calculations to get the concentration of the samples was carried out as follows:

$$\text{Sample (y) Conc.} = \frac{\text{Area of sample peak (Y cm)} \times \text{X ppm} \times 100\%}{\text{Area of standard peak (X cm)}}$$

X cm of the standard represents x ppm. Y cm of a given sample (component) represents y ppm, where x and y are peak height (cm) of the standard and component with the same retention time.

Statistical analysis

Statistical analyses of the data was carried out by use of one way analysis of variance (ANOVA) using Macintosh ll SE Computer with Stastimew 512 + TM Statistical programme.

Results

Out of a total of 250 meat samples analysed during this study 114 (45.6%) had detectable levels for tetracycline residues. The two tetracycline groups that comprised the positive samples were oxytetracycline, which was found in 110 (44%) and chlortetracycline in 4 (1.6%) of the samples. The mean, range and numbers of the samples (kidney, liver and muscles) positive for tetracycline residues are shown in Table 1.

In Athi River slaughterhouse 75% of kidney, 50% of liver and 30% of beef were positive for tetracyclines. In Dagoretti market 53.3% of kidney, 32% of liver and 20% of beef were also positive for tetracycline. From Dandora slaughterhouse 80% of kidney, 32% of liver and 13.3% of beef were positive for tetracycline while from Kiserian market slaughterhouse 53.5% of kidney, 52% liver and 405 beef were positive for tetracycline residue.

The ranges for tetracycline residue levels from individual organs were: 50 to 845 µg/kg for kidney, 60 to 573 µg/kg for liver and 70 to 355 µg/kg for muscle in Athi River plant; 60 to 2 6 7µg/kg for kidney, 50 to 43 5µg/kg for liver and 23 to 370 µg/kg for muscle in Dagoretti market slaughter houses; 80 to 432 µg/kg for kidney, 50 to 430 µg/kg for liver and 100 to 320 µg/kg in the muscle in Dandora; 70 to 451 µg/kg for kidneys, 80 to 334 µg/kg for liver and 60 to 238 µg/kg for muscle in Ngong slaughter houses and 70 to 572 kidney, 50 to 247 µg/kg liver and 50 to 5 6 0µg/kg muscle in Kiserian slaughter houses.

Mean oxytetracycline residue levels from the five slaughterhouses were not significantly different ($p>0.05$). The mean values of the 5 slaughterhouses were Athi River 1060 µg/kg, Ngong 701 µg/kg, Dagoretti 648 µg/kg, Dandora 594 µg/kg and Kiserian 524 µg/kg (Table 1).

Discussion

About 20% of the total number of samples detected for tetracyclines had residue levels, above WHO (1999) standard. The group maximum residue limit (MRL) for teracyclines is 200 µg/kg, 600 µg/kg and 1200 µg/kg for beef, liver, and kidneys (WHO, 1999). The number of samples positive for tetracyclines was higher than that obtained (WHO 1999) in most countries in which such studies have been reported [21,31,26,27]. A similar study carried out by [16] reported that beef in Nairobi and surrounding area had violative levels of antibiotics and significant amounts of trypanocides. Their finding showed that 20% of Athi River slaughter beef had antibiotics and 55% of the beef from Dagoretti, Kiserian, and Dandora had violative residues of veterinary drugs. The current study revealed mean sum values for tetracyclines levels from the five slaughter houses in ascending order as 524

Table 1. Mean, range and proportion of positive samples for tetracycline (µg/kg) in Athi River, Dagoretti, Dandora, Ngong and Kiserian slaughterhouses

Area of study (slaughter house)		Tissue types		
		Kidney	Liver	Beef
Athi River	Positive	15/20	10/20	3/10
	Mean	330	270	280
	Range	50-850	60-570	36-70
Dagoretti	Positive	8/15	8/25	2/10
	Mean	290	250	110
	Range	36-60	50-350	20-370
Dandora	Positive	12/15	8/25	2/15
	Mean	240	190	160
	Range	80-430	50-430	100-320
Ngong	Positive	13/25	5/10	4/15
	Mean	270	230	120
	Range	70-450	80-330	50-560
Kiserian	Positive	8/15	13/25	4/10
	Mean	250	150	120
	Range	70-570	50-250	50-560

µg/kg in Dandora, 648 µg/kg in Dagoretti, 701 µg/kg in Ngong and 1,046 µg/kg in Athi River. All these results show high and violative levels for tetracyclines residues. In a previous study in our laboratory [20] reported relatively low levels of oxytetracycline in chicken eggs. Shorter withdrawal period following tetracycline therapy could account for observed increased levels of tetracyclines in these samples.

Our findings show that oxytetracycline residues were kidneys: 1,380 µg/kg, liver 1090 µg/kg and muscle 790 mg/kg respectively. This was not unusual since the liver and the kidney are the major storage and excretory organs for tetracyclines and are parenchymatous in nature [29]. No previous reports are available on tetracycline levels in Kenyan beef apart from that of Mdachi *et al.*, (1991) for other veterinary drugs. The HPLC method used in this study was found to be sensitive, precise, specific and convenient analytical method for the screening, detection and quantification of tetracycline residues in biological specimens. One of the major advantages over other microbiological method is that the lower detection limit of about 0.05-0.1 ppm makes it a high precision instrument. At levels above 0.5 ppm, the method is semiquantitative and below 0.5 ppm only quantitiative [21].

Other methods which have been used for determination of oxytetracycline include: fluorimetry [32], chromatography [28,21,4,19], radiommunoassay method [10,3]. Of the methods, the fluorimetric and microbiological methods lack specificity among tetracyclines and employ laborious sample preparation.

Several workers have used the high-pressure liquid chromatography analytical method for tetracyclines in various samples: beef [7,34], human serum [18]; honey [6] and liver and kidneys [24]. Although they worked on different products one common finding in the use of the HPLC was that the method was simple, accurate and reliable analytical method. The detection levels were very low which is indicative of high sensitivity. The recovery of tetracyclines in fortified tissues using HPLC may reach 90% with coefficients of variation of 1.8-7.5% and detection limit of 5/10 µg/kg [17].

The chlortetracyclines were low and were detected only in4 liver samples out of the total 250 samples. These samples were from Dagoretti slaughterhouses and their levels were below 0.1 ppm. This indicates that this group of tetracyclines is not very widely used compared to the oxytetracyclines and thus their risks to the consumers is minimal.

The wide variation in residue levels even from the same slaughterhouse indicates differences in animal husbandry practices from different farms and areas. Some farmers especially pastoralists generally have access to tetracycline and could treat their animals and thus misuse, over dose and failure to observe the withdrawal periods can be common.

The present study indicates the presence of tetracycline residues in edible tissues from the various slaughterhouses and as such regulatory authorities should constantly conduct surveillance on withdrawal period before slaughter.

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