

## Tetracycline Residue Levels in Slaughtered Beef Cattle from Three Slaughterhouses in Central Ethiopia

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**Abstract:** A cross-sectional study was conducted from October 2006 to May 2007 to estimate the proportion of tetracycline residue levels in beef at Addis Ababa, Debre Zeit and Nazareth slaughterhouses in central Ethiopia. A total of 384 muscle and kidney samples were randomly collected from slaughtered cattle in the respective slaughterhouses. The samples were qualitatively screened for tetracycline residues by thin layer chromatography (TLC) and presumptive positive samples were then further analyzed by using high performance liquid chromatography (HPLC). A given sample was considered positive for tetracycline if its retention time and peak corresponded to that of the standard. Out of the total 384 samples that we analyzed for tetracycline residues, 71.3% (274/384) had detectable oxytetracycline residues. Tetracycline and doxycycline residues were not detected in any of the samples analysed. Among the meat samples collected from Addis Ababa, Debre Zeit and Nazareth slaughterhouses, 93.8%, 37.5% and 82.1% were positive for oxytetracycline. The mean ( $p > 0.05$ ) residue levels of oxytetracycline for the three slaughterhouses studied in muscle were as follows: Addis Ababa 108.34  $\mu\text{g}/\text{kg}$ , Nazareth 64.85  $\mu\text{g}/\text{kg}$  and 15.916  $\mu\text{g}/\text{kg}$  at Debre Zeit while in kidney samples were ( $p < 0.05$ ): 99.02  $\mu\text{g}/\text{kg}$  in Addis Ababa, 109.35  $\mu\text{g}/\text{kg}$  in Nazareth and 112.53  $\mu\text{g}/\text{kg}$  in Debre Zeit slaughterhouses. The oxytetracycline positive samples, which showed residues of oxytetracycline above maximum residue limits (100  $\mu\text{g}/\text{kg}$ ) in muscle samples, were 58 (48.3%) at Addis Ababa slaughterhouse and 51 (48.1%) at Nazareth slaughterhouse and 1 (0.9%) in the kidney samples of Nazareth slaughterhouse. At Debre Zeit slaughterhouse no samples were above the maximum residue limits (MRL). The result of this study indicated that oxytetracycline residues previously detected in thin layer chromatography were also detected in all samples using HPLC. About 48% of the edible tissues had oxytetracycline residue levels above the recommended maximum residue limits. This suggests that proper withdrawal periods were not respected before slaughter of the animals and indicate the need for regulatory authorities and producers to ensure the proper withdrawal periods for the various antimicrobials administered to food animals before slaughter.

**Key words:** Drug • Addis-Ababa • Nazareth • D/Zeit • Kidney

### INTRODUCTION

In many of the developing countries, where no or limited regulatory legislation or mechanisms are in place for drug approval and use or for residues monitoring the occurrence of residues is not surprising. Drugs, including unapproved and unregulated compounds that may have no acceptable daily intake, can often be easily purchased from village shops and informal vendors [1]. In Ethiopia, the control of drugs from the government authorities and information on the actual rational drug use pertaining to

veterinary drug use is very limited. In addition, misuses of drugs are common among the various sectors including veterinary and public health. Tetracycline is one of the commonest drugs used in animals and humans. It is also common to see drugs including tetracycline being sold in the market without prescription and along the roads by informal vendors.

In addition there is lack of awareness and preparedness among the controlling authorities and producers in dealing with the risk of indiscriminate use of antimicrobials to the livestock and to the consumers.

Food animals slaughtered for domestic and export purposes in the country are not screened for the presence of residues in any of the slaughterhouses in the country. No formal control mechanisms exist to protect the consumers against the consumption of meat and milk products containing harmful drug residues in the country. To our knowledge no study has been done to determine the drug residue levels in edible tissues above the maximum residue levels (MRLs) including tetracycline in Ethiopia. Therefore, the present study was designed to estimate the residue levels of tetracyclines (oxytetracycline, tetracycline and doxycycline) in slaughtered beef cattle from three different slaughterhouses (Addis Ababa, Debre Zeit and Nazareth slaughterhouses) in Central Ethiopia.

## MATERIAL AND METHODS

**Study Design:** A cross-sectional study was under taken in Addis Ababa, Debre Zeit and Nazareth slaughterhouses from October 2006 to May 2007. Individual animals to be sampled were selected using random sampling technique. From each selected slaughter cattle, kidney and muscle samples of 50-100 g were aseptically collected in separate sterile sample containers and transported in ice box packed with ice to the laboratory. The samples were stored at freezer temperature (-20°C) before analysis. All samples were processed and analyzed separately. Both qualitative tests and quantitative tests were undertaken at the Ethiopian Drug Administration and Quality control Laboratory (DACA), Addis Ababa, Ethiopia.

**Detection of Tetracycline Residue in Meat Using Thin Layer Chromatography (TLC):** TLC method was used as a screening test as it is a sensitive and specific method for monitoring low amounts of different biological and chemicals. Illumination of tetracyclines against UV light helps as a simple detector for the analysis. In this study TLC was used for the detection of tetracycline residues following the techniques recommended by Tajick and Shohreh [2].

**Tetracycline Extraction:** 10 grams of ground kidney tissues of cattle in 10 ml of 96% ethanol was crashed and squeezed fine in a Chinese mortar. The solvent was transferred into 15 ml falcon centrifuge tubes and centrifuged at 4000 rpm for 10 minutes. The clear supernatant was transferred to clean glass test tubes and evaporated in water bath at 80°C. After full drying, the

deposits resolved in 0.2 ml methanol. The samples were ready to point on silica plates (Tajick *et al.*) [3].

**Preparation of Silica Plates:** Glass plates (10 × 20 cm dimensions) were washed in acetone bath. For each plate 2 g of silica gel 60 plates (Merck, Germany) mixed in 5 ml distilled water and shacked thoroughly to produce fine paste. Clean glass plates were coated with silica paste by TLC gel spreader system (CAMAG, USA) in 0.25 mm thickness. Plates were activated in 120°C for two hours [4]. The pH of disodium EDTA was adjusted to 7.0 by a 10% w/v solution of 10 molar sodium hydroxide and sprayed evenly onto the plates (about 10 ml for a plate of 100 x 200 mm). The plates were then allowed to dry horizontally for at least 1 hour. Before use the plates were allowed to dry at 110°C for an hour [5].

**Standard Preparation:** For comparison of extracted residues with raw antibiotics, reference standard (Sigma Chemical Co., St. Louis MO. USA) supplied analytical standards of oxytetracycline, tetracycline and doxycycline of 1µg/ml was dissolved in methanol and prepared as described previously [6].

**Pointing, Running and Detection:** About 10 µl of methanol dissolved deposits were pointed on silica plates and treated plates transferred to TLC tank containing a saturated mixture of dichloromethane, methanol and water (59: 35: 6 by volume) as mobile phase. After receiving of solvent front to end of plates they were removed off and dried in a current air and examined under ultraviolet light at 366 nm [3,5].

**Determination of Tetracycline Residue Levels with High Performance Liquid Chromatography (HPLC):** Samples that were previously considered as positive by thin layer chromatography were subsequently analyzed by high performance liquid chromatography following the techniques recommended by Agence Franaise de Securite sanitarie des aliments [7] for determination of tetracycline residues in kidney and muscle by high performance liquid chromatography.

**Sample Pretreatment:** The samples were kept at -20°C until analysis. Before analysis samples were allowed to defrost at room temperature. Each sample to be analyzed was ground into fine powder using sartorius mincer and 5g was weighed using a balance into a centrifuge tube. 25 ml McIlvaine buffer-EDTA solution was added to the tube and was blended 30 seconds by shaking and then

tube vortex-mixed for 15 minutes and centrifuged 10 min at 4000g at 4°C. The supernatant was transferred to a beaker on a magnetic stirrer and 2.5 ml trichloroacetic acid was added slowly with constant stirring. It was centrifuged again for 5 minutes at 3000g. Then single GF/B filter paper was fixed in buchner funnel moisturized with McIlvaine buffer-EDTA and the supernatant was filtered through funnel.

**Sample Clean up by Solid Phase Extraction:** Solid phase extraction (SPE) cartridge was conditioned with 1ml methanol, 1ml McIlvaine buffer solution and 1 ml of HPLC grade water. The final extract was applied onto the cartridge. When the extract loading completed, tetracycline was eluted with 1 ml of 0.01molar oxalic acid in methanol and next with 1ml HPLC grade water. Determination of the tetracycline residues was done using a high-pressure liquid chromatography (model Shimadzu Class-VP Series, Kyoto, Japan) equipped with SIL-10 autoinjector with sample cooler and LC-10 on-line vacuum degassing solvent delivery unit. Chromatographic control, data collection and processing were carried out using Shimadzu Class VP data software, a constant flow pump and a variation wavelength UV detector set at 355 nm was used for analyzing data. The separation was done on Nucleosil C18 (5µm, 250x 4.0 mm I.D.E Merck) column with acetonitrile and 0.01M aqueous oxalic acid solution by gradient mode as the mobile phase flow-rate of 0.8 ml/min at room temperature and the sensitivity range was 0.08 ppm. HPLC analysis was performed in 20 minutes.

**Preparation of Standard Stock and Working Solution:** Sigma Chemical Co., St. Louis MO. USA, supplied analytical standards of oxytetracycline, tetracycline and doxycycline. Stock solution of 100µg of tetracycline was prepared by diluting 100 mg of reference standard to 100ml with methanol. The intermediate solution of 100 µg/ml was prepared by diluting of 10 ml of stock solution to 100 ml with methanol. The working solution of 1µg/ml was prepared by diluting 50µl of 100 µg/ml solution to 5ml methanol. Standard solutions were prepared from 0.125 to 1 µg/ml and from 0.75 to 6 µg/ml for muscle and kidney respectively and were kept at -20°C.

## RESULTS

**Qualitative Analysis (TLC):** Analyses of kidney with TLC showed that majority of the samples have variable amounts of oxytetracycline residues. Out of the total 384 kidney samples analyzed using TLC in this study,

Table 1: The mean, range and numbers of the samples (kidney and muscles) positive for oxytetracycline residues

		Tissue types	
Slaughter houses		Beef	Kidney
Addis Ababa	Positive	120/128 (93.7%)	120/128 (93.7%)
	Mean	108.349	102.49
	Range	11.5- 429.3	9.73- 449.6
	Std. deviation	61.321	49.67
Nazareth	Positive	106/128 (82.8%)	106/128 (82.8%)
	Mean	64.852	113.18
	Range	6.7- 67.3	63.1- 260.6
	Std. deviation	35.469	69.88
Debre Zeit	Positive	48/128 (37.5%)	48/128
	Mean	15.916	116.53
	Range	3.0- 145.8	56.0- 740.6
	Std. deviation	12.288	34.02

274 (71.4%) had detectable levels for oxytetracycline residues. Tetracycline and doxycycline were not detected in any of the samples. In every sample where kidney sample had been positive for oxytetracycline by TLC, muscle samples were also positive by HPLC. In Addis Ababa, Debre Zeit and Nazareth slaughterhouses 120 (93.8%), 48 (37.5%) and 106 (82.81%) kidney and beef samples were positive for oxytetracycline respectively. Details of the results are presented in Table 1.

**Quantitative Analysis by High Performance Liquid Chromatography (HPLC):** Those samples positive with the thin layer chromatography were further analyzed using HPLC for confirmation and quantifications. The results of this study indicated that oxytetracycline residues previously detected in TLC were also detected in all samples by HPLC. A given sample was regarded as positive for tetracycline if its retention time and peak corresponded to that of the standard. Retention time was considered a reasonably unique identifying characteristic of a given analyte. Figures 1 and 2 show chromatograms (the visual output of the chromatograph) in which x-axis is the retention time and the y-axis is a signal obtained by UV diode array detector corresponding to the amount of oxytetracycline existing in the system. The peaks are characteristic of their identity, with a distribution around the mean position (apex of the peak) that is characteristic of the kinetic properties of the chromatographic system. The area inscribed by the peak is proportional to the amount of substance separated in the chromatographic system. To get the concentration of oxytetracycline sample, a reference standard of a known concentration had been injected into the HPLC and concentration of the sample was extrapolated from the curves peak area.

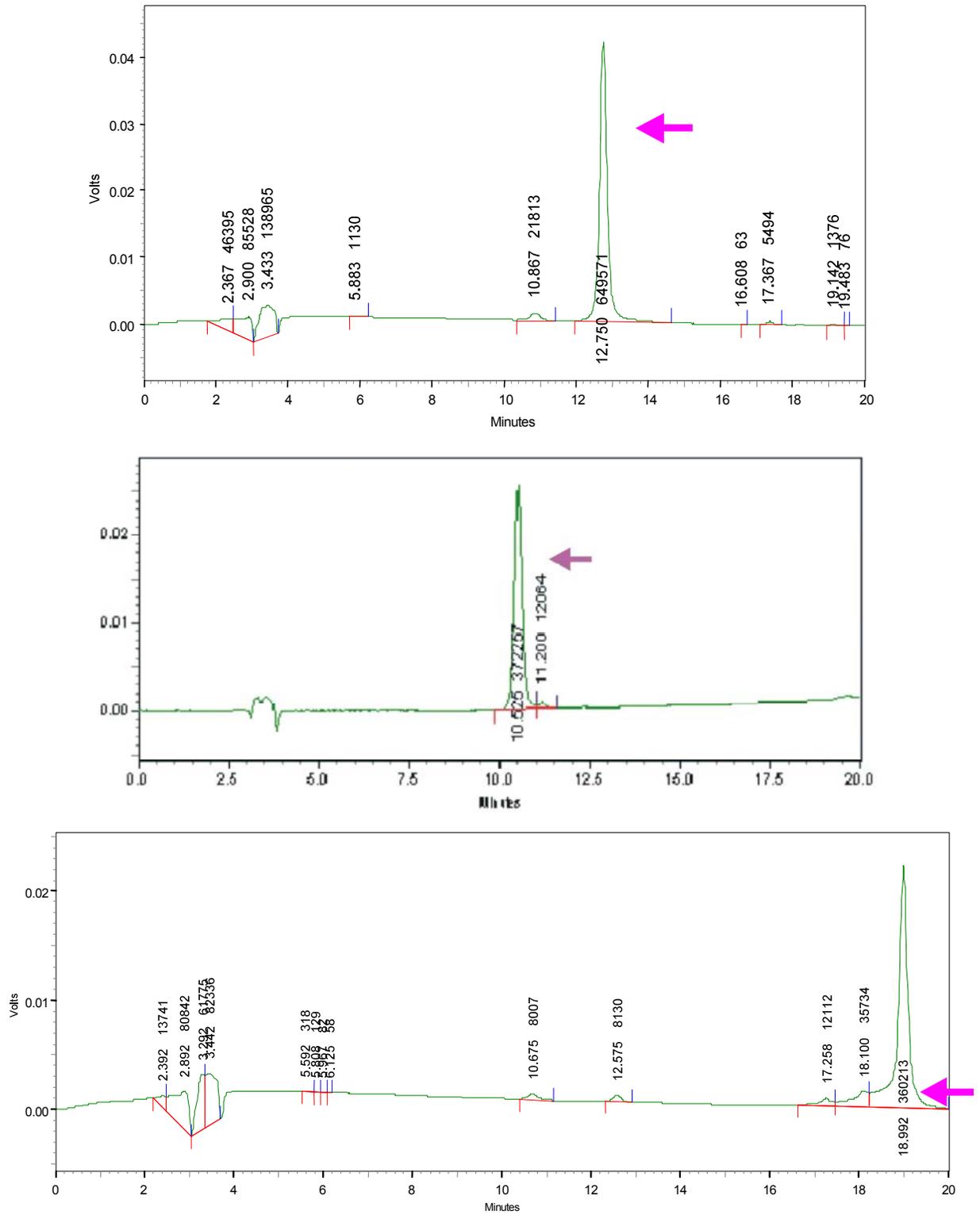


Fig. 1: Chromatograms of reference standards: tetracycline, oxytetracycline and doxycycline (from top to the bottom). The arrow indicates the peak, peak area and its retention time.

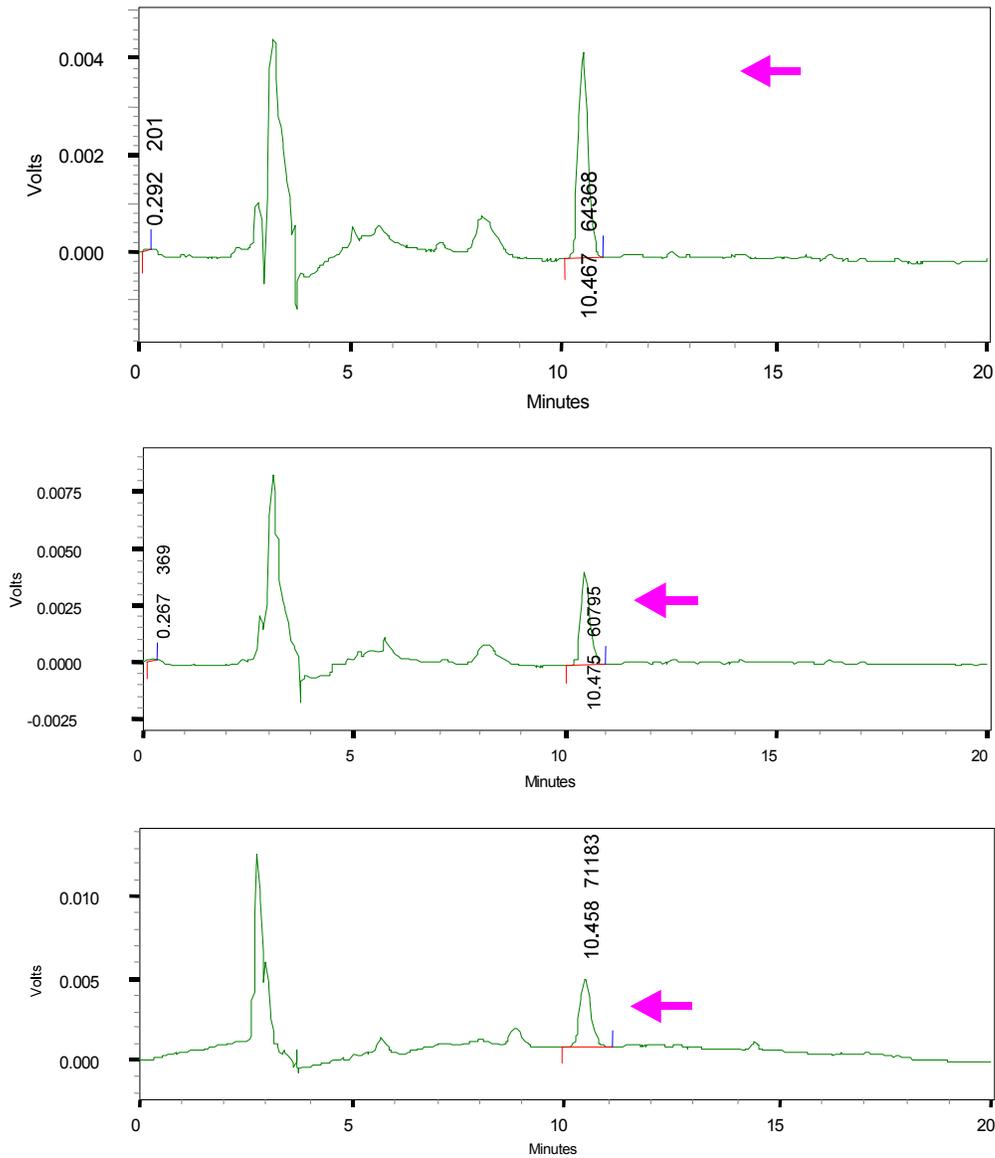


Fig. 2: Chromatograms of representative samples that were positive for oxytetracycline: Addis Ababa, Debre Zeit and Nazareth slaughterhouses (from top to the bottom). Arrow indicates the peak, peak area and retention time.

The ranges for tetracycline residue levels from individual organs were: 9.732 $\mu\text{g}/\text{Kg}$  to 449.65 $\mu\text{g}/\text{Kg}$  for kidney and 11.5 $\mu\text{g}/\text{Kg}$  to 429.289 $\mu\text{g}/\text{Kg}$  for muscle at Addis Ababa slaughterhouse; 56.037 $\mu\text{g}/\text{Kg}$  to 740.59 $\mu\text{g}/\text{Kg}$  for kidney and 3.01 $\mu\text{g}/\text{Kg}$  to 145.87 $\mu\text{g}/\text{Kg}$  for muscle at Nazareth slaughterhouse and 63.12 $\mu\text{g}/\text{Kg}$  to 260.56 $\mu\text{g}/\text{Kg}$  in kidney and 6.68 $\mu\text{g}/\text{Kg}$  to 67.34 $\mu\text{g}/\text{Kg}$  in the muscle at Debre Zeit slaughterhouse.

Mean oxytetracycline residue levels in muscle from the three slaughterhouses were not significantly different ( $p > 0.05$ ). However, oxytetracycline residue levels in kidney samples were significantly different ( $p < 0.05$ ). The

mean, range and numbers of the samples (kidney and muscles) positive for oxytetracycline residues are shown in table one.

Oxytetracycline positive samples which showed residues of oxytetracycline above maximum residue limits (MRLs) were 58 (48.3%) at Addis Ababa slaughterhouse and 51 (48.1%) at Nazareth slaughterhouse in muscles and 1 (0.9%) in kidney samples at Nazareth slaughterhouse. At Debre Zeit slaughterhouse no samples were above the maximum residue limits. Details of the results for each slaughterhouse are presented in Figure 3, 4 and 5.

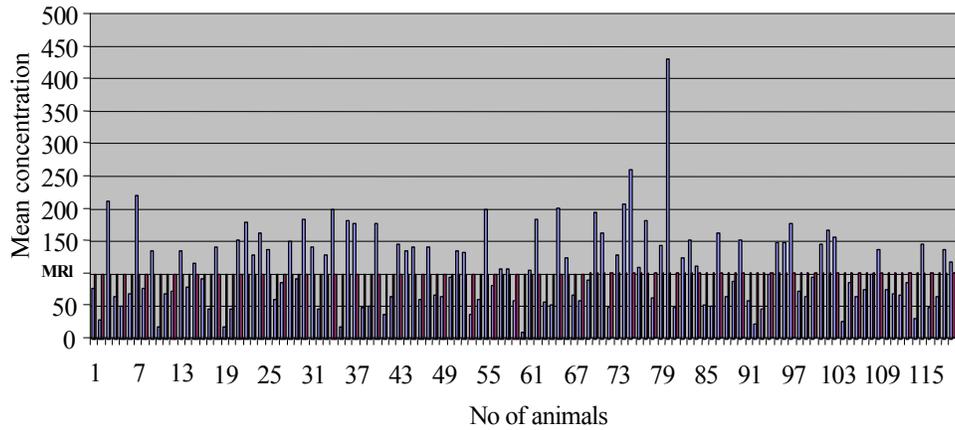


Fig. 3: Mean detectable concentrations ( $\mu\text{g}/\text{kg}$ ) of oxytetracycline in muscle samples in comparison with the maximum residue limit (MRL=100  $\mu\text{g}/\text{kg}$ ) (Addis Ababa slaughterhouse)

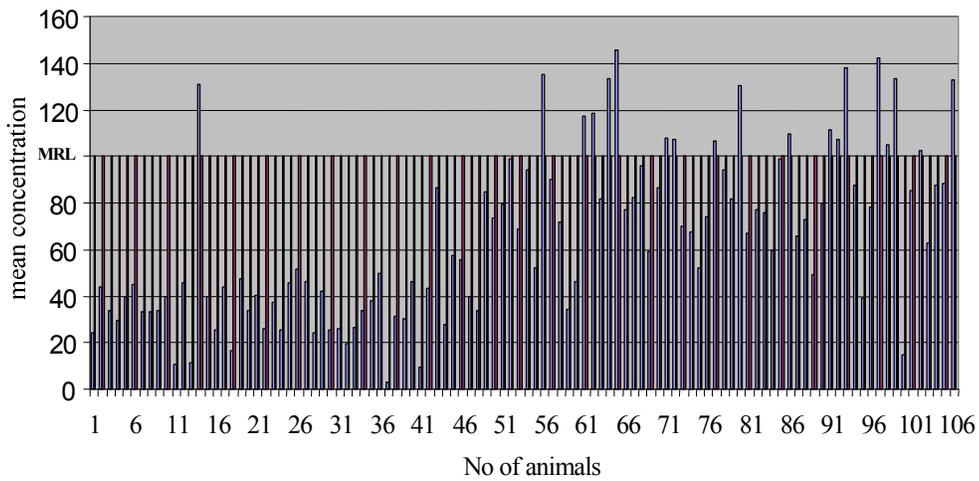


Fig. 4: Mean detectable concentrations ( $\mu\text{g}/\text{kg}$ ) of oxytetracycline in muscle samples in comparison with the maximum residue limit (MRL=100 $\mu\text{g}/\text{kg}$ ) (Nazareth slaughterhouse)

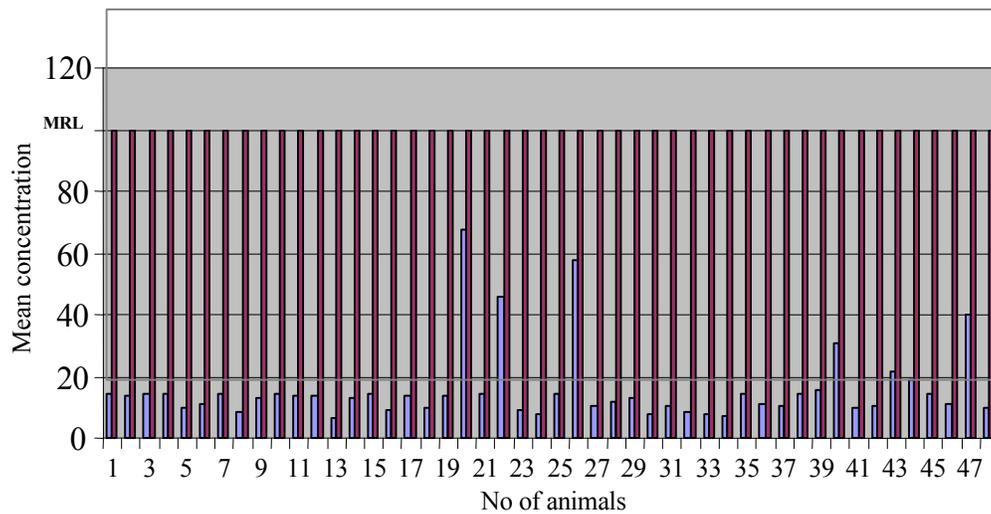


Fig. 5: Mean detectable concentrations ( $\mu\text{g}/\text{kg}$ ) of oxytetracycline in muscle samples in comparison with the maximum residue limit (MRL=100 $\mu\text{g}/\text{kg}$ ) (Debre Zeit) slaughterhouse).

## DISCUSSION

Out of the total 384 meat samples analyzed during this study, 274 (71.4%) had detectable levels for oxytetracycline residues. Tetracycline and doxycycline were not detected in beef and kidney samples. The proportions of oxytetracycline positive samples were much higher as compared to other reports elsewhere. A similar study carried out by Muriuki *et al.* [8] in Nairobi (Kenya) and surrounding areas reported that out of a total of 250 beef samples analyzed during this study, 114 (45.6%) had detectable levels for tetracycline residues. A study undertaken in Vietnam [9] indicated that 5.5% of the pork samples were positive for tetracycline residues which were much lower than the findings of present study. Another study conducted in Iran also indicated that [2] samples from edible tissues of 86 (95.55%) of samples of chickens of broiler farms in Tehran, had residues of oxytetracycline which are comparable with the results of this study.

Research results undertaken elsewhere where stringent control for drug residues are in place such as that of the FSIS-USDA National Residue Program for combined meat and poultry samples in USA [10] revealed that, among monitoring samples the average annual incidence, during this decade, of violative chemical residues in meat and poultry tissues was about 0.23% (1 in 435). It was also reported that in the UK [11], 44 out of over 12,300 samples collected from pigs contain chlortetracycline residues and in Ireland 42% and 12% of the pork samples were positive to chlortetracycline for the period of 1996/1997 and 1997/1998, respectively [12]. The results obtained in this study suggest that oxytetracycline was indiscriminately used in the cattle in the study areas also indicate that the recommended withdrawal time was either not respected or extra label treatment of this drug might be used.

All beef samples had detectable level of oxytetracycline residue by HPLC analysis in which the relevant kidney samples had been positive for oxytetracycline by thin layer chromatography. FAO/WHO Expert committee on Food Additives established MRL for oxytetracycline of 600 µg/kg in kidney; 300 µg/kg in liver; 100 µg/kg in muscle [13]. This study revealed that out of the 274 samples positive for oxytetracycline residues, 58 (48.3%) and 51 (48.1%) beef samples in Addis Ababa and Nazareth slaughterhouses and 1 (0.9%) kidney samples at Nazareth slaughterhouse had residues of oxytetracycline above MRLs. Similar study in Kenya also showed that about 55% of the beef sampled had violative residues of

veterinary drugs [14]. The number of samples above MRLs for tetracyclines was higher than that obtained in most countries in which such studies have been reported [15,16]. The findings of the present study was also higher than the study conducted in Iran<sup>[2]</sup> which indicated that the oxytetracycline positive samples, which showed residues of oxytetracycline above MRLs, were 25 (27.8%) and 17 (18.9%) in muscles and kidney samples respectively.

The use of antimicrobial agents in food animals has caused concern regarding the impact these uses have on human health. Use of antimicrobial agents in animals and humans results in the emergence and dissemination of resistant bacteria. Resistant bacteria from food animals may be passed through the food chain to human resulting in resistant infections. Increasing resistance to antimicrobial agents that are important in the treatment of human diseases, such as tetracyclines fluoroquinolones and third-generation cephalosporins for the treatment of *Salmonella* and *Campylobacter* infections, have significant public health implications [17]. The study also showed that oxytetracycline is imprudently used in the study. Antimicrobial resistance including to tetracyclines has been reported in various pathogens of veterinary and public health significance in Ethiopia including *Salmonella* [18,19] which could partly be associated with the indiscriminate use of antimicrobials in food animals for various purposes. The high prevalence of *Salmonella* isolates resistant to tetracyclines, relatively cheaper and commonly available antimicrobials, is disturbing because of the limited access and high cost of newer cephalosporins and quinolone drugs [20] for developing countries like Ethiopia. In addition, human exposure to animal products containing significant level of antibiotic residues may prove immunological response in susceptible individuals and cause disorders of intestinal flora. Some individuals may have an allergic reaction to these compounds. As undesirable side effects, tetracyclines not only discolor the primary and permanent teeth but also causes hypoplasia in developing teeth when administered to infants, mothers during the last two trimesters of pregnancy and children under 12 years of age [21]. It has also been suggested that discoloration caused by tetracyclines occurs in adult dentition [22].

Drug residue remains very significant from the prospective of international trade and consumer confidence, because it results in international trade barrier. As tariffs are removed and goods flow freely between countries, importing countries must be in confident that goods available for purchase are safe and in addition to this, from time to time, there is pressure to

use antimicrobial residues on non tariffs barrier to import <sup>[23]</sup>. One of the requirements in the international trade of meat and meat products is that antimicrobial residues in food should be below MRLs.

In conclusion, results of the present study indicated that oxytetracycline residues previously detected in thin layer chromatography were also detected in all samples using HPLC. The proportion of oxytetracycline positive samples which showed residues of oxytetracycline above the maximum residue limits (MRLs) were high (48%). This suggests that proper withdrawal periods were not respected before slaughter of the animals and indicate the need for regulatory authorities and producers to ensure the proper withdrawal periods for the various antimicrobials administered to food animals before slaughter.

#### ACKNOWLEDGEMENTS

The authors thank the Ethiopian Drug Administration and Quality Control Laboratory (DACA), Addis Ababa, Ethiopia for allowing us to use the facilities during the study period. Managers and technical staff of the slaughterhouses are acknowledged for their support and collaboration during sample collections.

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