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DETERMINATION OF OXYTETRACYCLINE RESIDUE LEVELS IN EDIBLE TISSUES OF SLAUGHTERED CATTLE

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Abstract: *Worldwide, antimicrobials are widely used in food producing animals for treatment and/or prophylaxis of diseases. In Tanzania, irrational administration of antimicrobials, notably oxytetracycline (OTC) is common because of the under-developed animal health service delivery system. The administration of OTC and other drugs is often done by animal keepers, thereby leading to drug residues in meat and milk. The study established the level of OTC residues in edible tissues of cattle slaughtered within Dodoma City Tanzania. This was cross-sectional analytical study design which quantified drug residues in muscle, liver, kidney from licenced and unlicenced premises. Samples from licenced as well as unlicenced premises were collected and used in determining OTC levels using High Performance Liquid Chromatography (HPLC) method. The HPLC method was validated to establish the reliability of the method in quantifying OTC residues. The results indicated that OTC residues were 0.6 mg/kg, 0.25mg/Kg and 1.28 mg/kg for muscle, liver and kidney tissues, respectively against corresponding Maximum Residue Limit (MRL) 0.2mg/kg, 0.6mg/kg and 1.2mg/kg. Regardless of the licensure status and source of the sample, 53% of muscle, 65% of liver and 7.1% of kidney tissues were above MRL. The quantities of OTC residues in samples from licensed and unlicensed food vending settings were not significantly different (p = 0.3676) but above the MRL. The above results show unacceptably high levels of OTC residues in meat with most beef value chain actors being ignorant of the impending public health threat/risks of consuming beef with high drug residues.*

Keywords*:* *toxic/anaphylactic reactions, public health threat, antibiotic resistant strain of bacteria, beef value chain actors*

1. Introduction

Antibiotics are among the most widely used veterinary drugs in food-producing animals for therapeutic purposes and as dietary supplements. They may be administered orally as food additives like oxytetracycline (OTC) and sulfadimidine or directly by injection like OTC injection, peni-streptomycin, gentamycin and sulfadimidine [1]. Among the veterinary antibiotics, oxytetracycline is one of the most widely used antibiotics due to its broad spectrum activity and easy access by practitioners [2] and animal keepers

As pointed out, oxytetracycline works by interfering with the ability of bacteria to add new amino acids to the peptide chain to produce essential proteins. Without these proteins, the bacteria cannot grow, multiply and increase in numbers [3]. OTC therefore stops the spread of the infection and the remaining bacteria are thereby killed by the immune system or eventually

die. Resistance against this antibiotic in humans is often attribute to drug residues in animal products [4].

In developing countries, due to scarcity of veterinary professionals and lack of enforcement of veterinary regulations, the OTC and other veterinary drugs are often administered into sick animals by lay persons. According to Bedada and Zewde [5] the control of drugs from the government authorities and information on the actual rational drug use pertaining to veterinary drug in developing countries is very limited. Some antibiotics are sold casually by street vendors like any other commodity with no regard for their use. Consequently there is a widespread and indiscriminate use of tetracyclines, the once effective broad spectrum antibiotic against both gram negative and positive organisms.

The Codex Alimentarius Commission(CAC)/ Maximum Residue Limit stipulates that once the animal is injected with oxytetracycline, the antibiotic residues remain in the body of an animal for 28 days and during this period, the animal should not be slaughtered for human consumption [6]. This however does not happen as many of these animals are sent for slaughter before the drug washout period has elapsed. Such a malpractice can have a disastrous impact to the health of meat consumers. The beef consumed with high levels of drug residue has cumulative effect of causing toxic and anaphylactic reactions to consumers. This study therefore aims at determining the levels of oxytetracycline residues in beef consumed in Dodoma as part of the desire to formulate appropriate public health strategies.

The oxytetracycline drug residues pose a real public health hazard to consumers. Such hazards include anaphylactic reactions and development of drug-resistant pathogens. According to Codex Alimentarius Commission of FAO and WHO, the current standard maximum residue level (MRL) for OTC in beef muscles is 0.2 microgram per gram of beef [6]. However, due to profit motives and resource poor related challenges, animal keepers and business men do not follow the FAO and WHO guidelines.

Even at the ranch setting where Hazard Analysis Critical Control Points (HACCP) is not applicable, the animals are likely to be sent for slaughter before the recommended withdrawal period. Thus, the meat is likely to enter the market with high OTC residues. In most of developing countries, this is further compounded by the fact that slaughter houses/slaughter slabs do not have facilities for detecting drug residues. Consumption of meat with high drug residues can lead to toxic reactions, anaphylactic reactions and development of antibiotic resistant strains of bacteria.

As stipulated in the Tanzania Food, Drugs and Cosmetic Act [7], the washout periods of drugs are supposed to be observed before animals are sent for slaughter. But due to absence of serious enforcement systems, animals are often slaughtered without taking into account the drug withdrawal requirements; thereby posing a real threat to public health. Despite this threat,to date, little is known on the oxytetracycline residue levels in edible cattle tissues in central Tanzania and the animal keepers' awareness on the withdraw period.

2. Matherials and Methods

Study Site and Setting

Dodoma city is in Dodoma region which is in the central part of Tanzania. Following declaration of Dodoma as as the Capital City of the country there has been a lot of influx of people. This has created a high demand of beef.

Despite the high demand of beef, Dodoma city has only one ultra modern abattoir. The abattoir slaughters an average of 20 animals a day and ante mortem and post mortem inspections are done by municipal meat inspectors.

Study Design

This was an analytical cross-sectional study that was done October and November 2015.

Sample Size and Sampling Method

Sample size

The sample size at confidence interval of 95% with prevalence of positive samples estimated at being 70% [5] was determined using the following formula

$$n = \left(\frac{z}{\Delta}\right)^2 p(1-p)$$

where z=1.96, p=0.7, Δ=0.08

$(1.96/ .08)^2$ x0.3x0.7 +35% = 171

Therefore the sample size was 171 specimens

Sampling Method

This study focused on the laboratory quantification of the levels of OTC residues in 171 beef samples from five wards namely Majengo, Viwandani, Makole, Makulu and Ntyuka which were randomly selected out of 14 wards of Dodoma City from 1st October to 30th November 2015. Of these samples, 128 were collected from licensed vending outlets. This involved samples from 30 butchery shops, five supermarkets and one abattoir) and 43 specimens were from unlicensed vending outlets, especially the pubs and bars where often animals were slaughtered under a backyard system. Each sample collected weighed approximately 250g. The samples as shown in Table 1 were collected using the properly marked sampling bags, stored in a cool box and transported to the laboratory.

Data collection

Growth Inhibition Method

This method which detects antibiotic residues through inhibition of growth of *Bacillus subtilis* on Nutrient Agar at 37ºC when incubated over night was planned to be used to screen positive samples that were then subjected to HPLC analysis. However, due to lack of conclusive answers with this method, in the study all 171 were subjected to HPLC analysis.

High Performance Liquid Chromatography (HPLC)

Frozen samples were initially thawed and cut with scissors to remove fat and fascia before being weighed. The samples were then homogenised using the Ultra-Turrex T25 tissue homogenizer.

The homogenized muscle, liver or kidney samples which weighed approximately 5 grams were then mixed with 30ml of extraction solvent (5% trichloroacetic acid with 0.5% disodium ethylenediaminetetraacetate, EDTA) and acetonitrile-water (3:7) and then centrifuged for 25 min at 5300 rpm. The supernatant was then filtered through a 0.45 µm filter paper into HPLC vial. 20µl of filtered solution of each sample was then injected into HPLC for analysis. Hypersil BDC C18 column was used and OTC was separated at 24^0C using a mobile phase of methanol-acetonitrile-0.2M oxalic acid (1:1:3.5) at pH=2.0 (with 28% aqueous ammonia) at a flow rate of 0.6 ml/min and wavelength detector set at 360 nm. OTC residues were determined by using HPLC (model Shimadzu Class-VP Series, Kyoto, Japan) equipped with SIL-10autoinjector with sample cooler and LC-10 on-line vacuum degassing solvent delivery unit. Chromatographic control, data collection and processing were carried out by using Shimadzu Class VP data software by aid of computer connected to the detector.

The quantities established were compared to the MRL set by CAC and categorized as being within, below or above the MRL.

Data Analysis

The laboratory data were analyzed as follows: Continuous variables (quantities of OTC residues in mg/kg) slightly failed to be symmetrical, but right skewed. The assumption of normal distribution was made based on the smallness of sample size (171 samples). The test for normality by the Shapiro-Wilk W test (quantile-quantile plot) was done. Since there was no evidence of gross deviation from normal distribution of the continuous variables, the descriptive statistics for continuous variables was hinged on pairwise comparison of the means between the Licensed and Unlicensed premises. The pairwise comparison of the means was done using Benferron's and Student-Newman Keuls' methods. The generalized linear model was used to select the best predictors of OTC residues in our samples after subjecting the predictor variable to univariate and multivariate analyses. All analyses were done by using Stata software version 13.

3. Results and Discussion

In this study, one hundred seventy one (171) beef samples were collected from five (5) wards within Dodoma municipality catchment area. The concerned wards were Majengo, Viwandani, Makole, Makulu and Ntyuka.

Table 1

Types of Beef Samples

Sample Type	Number of sample per ward	Samples from LICENSED[1] premises from 5 wards	Samples from UNLICENSED[2] premises from 5 wards	TOTAL NUMBER OF SAMPLES
Muscles	27	94	43	137
Liver	4	20	0	20
Kidney	3	13	1	14

[1]Officially recognized beef vending outlets like butchery shops and supermarkets; [2]Unofficially recognized beef vending outlets like bars and under the tree.

Table 1 shows the results of the samples which were subjected to Growth Inhibition Technique using nutrient agar seeded with *Bacillus subtilis*. For muscles from licensed premise, there were 60 samples, 21 samples from unlicensed premise, 18 samples of liver and 14 samples of kidneys which inhibited growth but at this stage it was not possible to know the type of antibiotic and quantities till the next method of high performance liquid chromatography (HPLC).

The antibiotic residues pose a real public health threat. This is consequent to the lack of adequate control on the rational use of these antibiotics and lack of observation of drug withdrawal periods. Such a scenario has made the antibiotic residues find their way into the food value chain. This study established 53% of muscle tissues regardless of the licensure status and source of the sample to have levels of OTC residues above the maximum residue limit (MLR) of 0.2mg/Kg. Only 34.1% (47/137) of the muscle tissue had residues below the maximum residue limit whereas 12.4% (17/137) had OTC residues within the limit. These results were similar to the results of the study which was conducted in Ethiopia where out of the total 384

samples analyzed for tetracycline residues, 71.3% had detectable oxytetracycline levels [5]. These results are comparatively higher in relation to Canada where there is zero tolerance of drug residues in food products [8].

Table 2

Growth Inhibition Method Results *(Bacillus subtilis)*

	SAMPLE TYPE	SAMPLE SOURCE	NO GROWTH	GROWTH	TOTAL SAMPLES
A.	Muscles	Licensed Premise	60	34	**94**
		Unlicensed Premise	21	22	**43**
B.	Livers	Licensed Premise	18	2	**20**
		Unlicensed Premise	0	0	**0**
C.	Kidneys	Licensed Premise	14	0	**14**
		Unlicensed Premise	0	0	**0**
	TOTAL				**171**

Table 2 shows the OTC residue results for muscle, liver and kidney samples by HPLC whereby 73 muscle samples, 13 liver samples and 1 kidney sample were above the set residue limit.

Table 3

OTC Residue Quantification in beef by HPLC

	TYPE	MRL1	RANGE OF RESULTS	SOURCE OF SAMPLES					
				LICENSED		UNLICENSED		TOTAL	
				N	%	N	%	N	%
A.	Muscle	0.2mg/Kg	i. Below limit (<0.2mg/Kg)	27	19.71	20	14.6	47	34.31
			ii. Within limit(0.2mg/Kg)	12	8.76	5	3.65	17	12.41
			iii.Above limit (>0.2mg/Kg)	55	40.15	18	13.14	73	53.28
B.	Liver	0.6mg/Kg	i. Below limit (<0.6mg/Kg)	7	35	0	0	7	35
			ii.Above limit (>0.6mg/Kg)	13	65	0	0	13	65
C.	Kidneys	1.2mg/Kg	i. Below limit (<1.2mg/Kg)	13	92.86	0	0	13	92.86
			ii.Above limit (>1.2mg/Kg)	1	7.14	0	0	1	7.14

1Maximum Residue Limit as set by Codex Alimentarius Commission according to different tissue types.

Table 3 shows to what level the mean OTC residues are above the maximum residue limit (MRL) in muscles and liver while below the limit in the kidney. The anomaly of the kidney to have residues below the limit is explained in the subsequent discussion.

Table 4

Mean Levels of OTC Residues according to Tissue Type

Tissue Type	Number of samples	Mean (SD)[1]	MRL[2]
Muscle	137	0.60(1.19)	0.20
Kidney	14	0.25(0.43)	0.60
Liver	20	1.28(1.03)	1.20

[1] Mean residues levels (Standard Deviation) in mg/Kg; [2] Maximum Residue Limit as set by CAC in mg/Kg

Table 5

OTC residue quantification by Tissue, Licensure and Sample Source

Variable		Mean (SD)[1]	MRL(MAR)[2]
	Muscle	0.60(1.19)	0.20(10.72)
Tissue Type	Liver	0.25(0.43)	0.60(3.00)
	Kidney	1.28(1.03)	1.20(1.67)
Licensure Status	Licensed	0.63(0.89)	(15.94)
	Unlicensed	0.73(1.70)	(10.72)
	Butchery	0.64(0.83)	(5.34)
	Supermarket	0.77(1.12)	(5.94)
Sample Source	Slaughterhouse	0.39(0.80)	(2.95)
	Bars	0.37(0.38)	(1.35)
	Emergency slaughters	2.29(3.64)	(10.72)

[1] Mean residues levels (Standard Deviation) in mg/Kg; [2]Maximum Residue Limit (MRL) as set by CAC in mg/Kg against the Maximum Actual Residues (MAR) as found in the samples in mg/Kg based on tissue, licensure and sample source.

Table 6

Comparison of the Mean OTC Residues by Licensure

Licensure Status	Mean Residues[1]	SD[2]	Maximum[3]	95% Confidence Interval (CI)
Licensed	0.629	0.8962	5.944	-.302388 .498497
Unlicensed	0.727	1.7030	10.716	

[1]Mean residues in mg/Kg; [2]Standard Deviation; [3]Maximum residue in mg/Kg

Table 6 shows the mean residues of the samples from the two groups based on licensure status whereby the samples from unlicensed premise had slightly higher OTC residues (0.73mg/Kg) compared to those from licensed premises(0.63). But

the comparison of the mean residues from the two groups by Benferron's and Student-Newman Keuls' methods revealed no significant difference between the two groups at 95% Confidence Interval (-.302388, .498497).

As evidenced from the results, most of the muscle tissues and the liver tissues had mean residues above the MRLs. But most of the kidney tissues had low mean residues (Table 6). There was one muscle sample with unexpectedly high levels of OTC residues up to 10.72mg/Kg. The sample was taken from unlicensed premise (emergency slaughter) at Makulu ward. This is explained by confession of the interviewed farmers who treated the animals but when death was eminent they resorted to slaughtering of the animal. Given the pharmacokinetics of OTC, the sick animal has high concentration of OTC due to alteration of haemodynamics especially drop in blood pressure, cardiac output, glomerular filtration, renal blood flow and decreased hepatic drug metabolism all of which bottleneck to lower clearance rate and longer half-life. The kidney tissues which were expected to have comparatively higher levels of OTC residues were found to have low levels of OTC due to the similar reason of pharmacokinetics of the drug. Another reason behind high levels of residues in that muscle sample is the injection sites through which the drugs are administered into the animals which are neck and buttocks muscles. The sample from the injection site of an animal which has been injected recently is expected to have high levels of residues compared to muscle away from the injection site. Given the current animal marketing system even at the slaughterhouse, the traceability of the slaughtered animal was difficult to underscore with the aim of feedback to the culprits for corrective action. Based on the licensure status of the premise, the mean residues of the samples from the two sources, the samples from unlicensed premise had slightly higher OTC residues (0.73mg/Kg) compared to those from licensed premises (0.63) as shown in Table 8. But the comparison of the residues among the two groups by Benferron's and Student-Newman Keuls' methods revealed no significant difference between the two groups at 95% Confidence Interval (-.302388, .498497).

From the licensed premises the residues ranged from 0 to 5.94mg/Kg whereas from unlicensed premises the residues ranged from 0 to 10.72mg/Kg. The results are indicating how people who are sourcing beef from licensed and unlicensed premises are all exposed to consumption of drug residues.

Adjusting for the licensure status and source of the samples, 65% (13/20) of the liver tissues were found to have OTC residue levels above the MRL of 0.6mg/Kg while 35% (7/20) had residues below the limit. These results are in line with the results of the the similar study done in Akure, Nigeria by Olatoye and Ehinimowo [9] where most of the tissues with residues above MRL were the liver tissues. This is due to the fact that the liver is the organ for metabolism and excretion of these drugs. These tissues are preferred by many, hence a lot of consumers at high public health risk.

The kidney tissues were found to have 7.1% (1/14) of the samples above the MRL of 1.2mg/Kg whereas 92.9% (13/14) of the samples were below the MRL. But these results were not like the results of the study done by Olatoye and Ehinimowo [9] where more kidney tissues had residues above the limit.

Viewed from the source of the sample without taking into account of tissue types and licensure status, the butcheries, supermarkets and slaughterhouses had mean residues of 0.64mg/Kg, 0.77mg/Kg and 0.39mg/Kg respectively. The samples from the bars and emergency slaughters

(slaughter under the tree) had OTC residues of 0.37mg/Kg and 2.30mg/Kg respectively. From the above findings, the entire source of beef is not safe in terms of drug residues because the samples from the entire source are all above the MRLs. The samples from the emergency slaughter had the highest mean of OTC residues. Remarkably, the emergency slaughter source was the one with the sample with highest levels of OTC residues of 10.72mg/Kg which is unacceptably high. With such high levels of residues, it was quite evident that in the bars most of the beef from unlicensed premised managed to penetrate into the food chain. This presents a threat to people who go to the bar to enjoy bites with liquor or soft drinks. The beef products with high levels of drug residues were likely to have been sourced from unlicensed premises more precisely the slaughter under the tree and emergency slaughters. Alarmingly, the public health is at risk. This needs joint efforts from regulators and stakeholders to address the problem. The fight against the problem demands all the key players along the value chain addition to work together to fight against this public health threat.

Table 7

Determinants of OTC residues

Predictors	Univariate Analysis		Multivariate Analysis	
	P Value	Confidence Interval	P Value	Confidence Interval
Tissue Type	0.051	-.0009856 .507935	0.028	0.03118 0.56067
Licensure	0.629	-.2995204 .495629	0.850	-0.8853 0.72952
Source	0.424	-.0757791 .180373	0.399	-0.1475 0.37022

Table 7 shows the predictor variables for OTC residues after subjection to univariate and multivariate analysis whereby the tissue type was found to be a significant predictor variable (p value = 0.028).

The generalized linear model was run through the variables to detect the best OTC residue predictor variable between tissue type, licensure and source. The univariate analysis of the variables revealed the tissue type to be a significant predictor at p value 0.05 while licensure and source were not significant at p values 0.63 and 0.42 respectively. When considering the predictor variables of OTC residues holistically (tissue type, licensure and source) by using the generalized linear model (Table 7), it was established that the tissue type was a significant predictor at p value = 0.028 at 95% CI (0.03118 0.56067) at multivariate analysis. This study has managed to unveil the truth about residues and knowledge of stakeholders and other beef value chain actors. However, the levels of residues to which the consumers are constantly exposed to have been brought to light. It is also undeniable fact that Dodoma Capital City is centrally located at the heart of Tanzania. It is receiving animals from different parts of Tanzania. Hence the samples taken represent the true picture of the other parts of Tanzania.

4. Conclusion

This study established the beef food chain in Dodoma to be contaminated by OTC residues at unacceptably high levels. This in turn poses a real public health threat to the consumers who are likely to succumb to toxic/anaphylactic reactions, drug resistance eratogenic risks to the foetus and can cause discolouration of primary and secondary teeth as well as hypoplasic development of teeth when consumed by infants or pregnant mothers during the last 2 terms.

It is highly recommended for the Ministry of Livestock Development and Fisheries to intervene in screening the animals at the market and enhance traceability of the

animals by improving the animal marketing system in Tanzania. The law enforcers should also actively seek find and take action against the practice of slaughter under the tree. It is also recommended to have effective control of veterinary drugs so that some of them like antibiotics should be bought from licensed shops with prescription.

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