Determination of oxytetracycline and sulphamethazine residues in marketed beef from selected parts of Zambia to assess compliance with maximum residual limits

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ABSTRACT

A cross-sectional study was conducted to determine the levels of oxytetracycline (OTC) and sulphamethazine (SMZ) antimicrobial residues and assess compliance with maximum residual limits (MRL) in beef from selected provinces of Zambia. A total of 224 muscle samples were randomly collected from abattoirs/butcheries from; Central (n = 48), Copperbelt (n = 64), Lusaka (n = 82) and Southern (n = 30) provinces. Samples were analysed using high performance liquid chromatography (HPLC) coupled with a Diode Array Detector (DAD). Results showed that 77 (34.4%; 95% CI: 28.4 - 40.9) and 39 (17.4%; 95% CI: 12.9 - 22.9) of samples contained detectable antimicrobial residues of OTC (Range: 27.26 – 481.61 ng/g, mean = 199.6 ± 46 ng/g) and SMZ (Range: 11.92 – 259.98 ng/g, mean = 86.5 ± 8.7 ng/g), respectively. About 45.5 percent and 12.8 percent of the samples contained OTC and SMZ residues, respectively, that were above the *Codex Alimentarius Commission's* MRL. Similarly, 76.6 percent and 33.3 percent of the samples contained OTC and SMZ residues, respectively, above the European Union MRL. The higher levels of the drug residues detected were probably due to failure by farmers to observe the drug withdraw period and posed a threat of development of antimicrobial resistance in foodborne pathogens and probable risk to human health.

Keywords: Oxytetracycline, Sulphamethazine, Residues, Beef, Zambia

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1. Introduction

Antimicrobials are utilised in livestock farming for therapeutic, metaphylactic or prophylactic and as growth promoters (WHO, 2001). The use of antimicrobial drugs has not only significantly contributed in preventing and controlling microbial infections in livestock (WHO, 2001) but also, improved the production of large quantities of beef to meet the high demand of the market (Hughes and Heritage, 2004). In 2013, the worldwide production of meat was 256 million tonnes of which beef accounted for about 59 million tonnes (FAO, 2013). Furthermore, beef makes up the third largest portion of global meat consumption (FAO, 2013). However, the large scale production of beef animals raises problems concerning the health of the herds since infections and diseases can spread rapidly. In response to this concern, antimicrobials are often widely administered in beef production in order to minimise the risk of outbreaks of livestock diseases (WHO, 2001). The use of antimicrobials in any of the above mentioned ways has the potential of leaving residues in beef products that may be harmful to humans (WHO, 2001).

Though the use of antimicrobial growth promoters is completely forbidden in the European Union (EU) since 2006 (EC, 2005), they are still used in other regions of the world like the United States of America (USA), Africa and Asia where they are added in sub-therapeutic amounts to the feed of the entire flock or herd (EC, 2005). With antimicrobial growth

promoters, it is possible to increase the daily growth rate of livestock by up to 10 % (Hughes and Heritage, 2004).

Antimicrobials are also used in sub-therapeutic amounts for prophylaxis to prevent an infection within the flock or herd (Mensah, 2014). If an infection spreads in the flock or herd, antimicrobials are administered to all animals in therapeutic amounts (Mensah, 2014). Selective treatment of diseased animals does not happen, since the costs for diagnosis for each animal would be too high and the infection might still spread among the animals (Mensah, 2014). Metaphylactically, antimicrobials are used as timely mass medication of a group of animals to eliminate or minimize an expected outbreak of disease (Mensah, 2014).

According to the Centre for Disease Control and Prevention (CDC) at least 17 classes of antimicrobials are approved for farm animal growth promotion in the United States of America, some of which include: Aminoglycosides (gentamycin, neomycin, streptomycin), Penicillin's (amoxicillin, ampicillin), Cephalosporin's third generation (ceftiofur), Glycopeptides (avoparcin, vancomycin), Macrolides (erythromycin, tilmicosin, tylosin), Quinolones or Fluoroquinolones (sarafloxacin, enrofloxacin), Streptogramins (virginiamycin, quinupristin-dalfopristin), Streptomycin, Sulphonamides (sulphadimethoxine, sulphamethazine, sulphisoxazole), Tetracyclines (chlortetracycline, oxytetracycline, tetracycline), Polypeptides (bacitracin) and Lincosamides (lincomycin) (Anderson *et al* 2003).

The livestock sector in Zambia is segmented in two distinct and poorly coordinated channels: the commercial livestock sector and traditional or smallholder livestock sector. The smallholder sector accounts for 80% of the total livestock in the country and only 20% is under the commercial sector. (Lubungu and Mukuka 2012). The cattle population in the smallholder sector increased from 2001 to 2008. However, between 2008 and 2012 there was a decline in cattle population from 2,815,583 to 2,162,357. (Lubungu and Mukuka 2012). As

of 2012, the smallholder livestock sector in Zambia consisted of approximately 2,162,357 cattle and the distribution of cattle across the four provinces under the study were 273,382 cattle in Central province, 43,118 cattle on the Copperbelt province, 84,590 cattle in Lusaka province and 857,570 cattle in Southern province (Lubungu and Mukuka 2012). There is no real national cattle breed in Zambia but a mixture of plenty of breeds resulting in Zebu and Sanga types such as Tonga, Ngoni and Barotse for the traditional sector. (Breeding Impuls Zambia, 2014)

The major challenge that the livestock sector faces in the country is low productivity characterised by slow growth rate of about 1.2% for cattle, mortality rates of 13%, livestock diseases and poor accessibility to livestock services. There was high prevalence of animal diseases between May, 2011 and April, 2012 and over 60% of the cattle in the traditional livestock sector were affected by disease. (Lubungu and Mukuka 2012). The important livestock diseases in Zambia are Contagious Bovine Pleural Pneumonia (CBPP), Foot and Mouth Disease (FMD), African Swine Fever (ASF), East Coast Fever (ECF) and Newcastle disease. Others include tick-borne disease such as Trypanosomiasis and Anthrax which result in low animal productivity and high mortality rates. The overall disease death rate per 1000 animals among traditional livestock farmers is estimated at 127 for cattle, with Central, Copperbelt, Lusaka and Southern provinces having 279, 80,148 and 93 disease death rates per 1000 animals respectively (Lubungu and Mukuka 2012). Disease control is a major challenge in livestock production. Disease among cattle are prevented with vaccines or treated. Over 80% of traditional livestock farmers use veterinary drugs to control disease outbreaks. (Lubungu and Mukuka 2012).

Among antimicrobial drugs, oxytetracycline (OTC) and sulphamethazine (SMZ) are frequently used in veterinary management of livestock diseases in Zambia (Mainda, 2014). Oxytetracycline is a member of the tetracycline group of antimicrobial drugs (Marilyn and

Chopra, 2001). It has been successfully used worldwide in both veterinary and aquaculture fields because it is cheap and has broad antimicrobial spectrum against both gram positive and gram negative bacteria (Marilyn and Chopra, 2001). Because of its widespread application, the Codex Alimentarius Commission (CAC) has published maximum residue levels (MRL's) for tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC) and their 4-epimers, which are 200 ng/g for muscle, 600 ng/g for liver and 1200 ng/g for kidney tissue (CAC, 2014). Sulphamethazine, also referred to as sulphadimidine, belongs to the broadband sulphonamide group of antimicrobials (Riviere and Papich, 2009). The MRL's according to the CAC are 200 ng/g for muscle, kidney and liver tissue (CAC, 2014).

In Zambia, beef production is high and holds an important position in the daily diet (ZDA, 2011). However, little is known about the magnitude of OTC and SMZ residues in marketed beef in Zambia. The objective of this study was, therefore, to determine the levels of OTC and SMZ residues in beef from selected provinces of Zambia and assess compliance with MRLs.

2. Materials and Method

2.1. Study areas, design and sample size estimation

A cross-sectional study was conducted in four (4) provinces (Central, Copperbelt, Lusaka and Southern provinces) of Zambia over a period of 3 months, from September to December 2015. The four (4) provinces were purposively selected based on the fact that they had high beef consumption ratios and their abattoirs and butcheries received a good number of animals for slaughter which were later distributed to other parts of the country for sale (ZNFU, 2012).

Since there was no documented information or study that had been undertaken in Zambia to determine the levels of antibiotic resistance or residues in marketed beef, the assumptions were that the apparent levels of drug residues in each abattoir be 50% (i.e. 0.5) and the maximum sample sizes be collected. The sample size to estimate the apparent level of antibiotic residues across the selected provinces given that the abattoir beef population officially recorded stood around 11, 800 carcasses per annum (CSO, 2009), was calculated using the simple random formula (Krejcie *et al.*, 1970).

Thus, the total sample size was estimated at 224 carcasses be collected across the four provinces of Zambia. Given that, each province had different abattoir throughputs (CSO, 2009), the provinces were weighted according to the socio-economic demographical survey data. Lusaka Province, being the highest in the country in terms of beef consumption and the available abattoirs (CSO, 2009), was weighed at an arbitrary scale of five (5). Luapula, Muchinga and North-Western had low abattoir throughputs (CSO, 2009) and these were weighed at one (1). Using the ratios obtained against the calculated sample size and after adjusting to the mentioned scale, the provincial sample sizes were tabulated as given in Table 1, with the sample size for Lusaka estimated at 80 carcasses, and the least provinces sampling about 16 animals. Thus, a total number of 224 samples were collected and analysed.

Table 1: Provincial sample size estimations

Variable	**Lsk**	**C/belt**	**C/ral**	**S/thn**	**E/ten**	**N/thn**	**M/nga**	**L/la**	**W/thn**	**N/W**	**Total**
Given ratio	5	4	3	2	2	2	1	1	2	1	**23**
Sample size (*n*)	80	64	48	32	32	32	16	16	32	16	**368**

2.2. Sample collection

Two hundred twenty-four (224) cattle meat samples were collected from abattoirs/butcheries of four different provinces of Zambia (Central, Copperbelt, Lusaka and Southern provinces). Selection of animals to be sampled was done randomly. In most cases the daily slaughter was less than 10 animals, in which all were sampled. But in cases of larger slaughter size, a minimum of 10 animals or a fixed proportion of 20% were sampled. Samples were taken from the gluteal muscles which are predominant injection sites. About two hundred grams (200g) of muscle tissue was collected from each animal and put into sterile histo-pack bags and transported in Cooler boxes containing frozen ice packs to the laboratory at Central Veterinary Research Institute (CVRI), Chilanga, Lusaka. At the laboratory, samples were stored in a freezer set at -20^{o}C until analysis.

2.3. Determination of OTC and SMZ

Samples were analyzed in the chemistry laboratory at Zambian Agriculture Research Institute (ZARI) using a modified certified method according to Froehlich (2013).

2.3.1 Sample extraction and clean up

All glassware used were washed three times with tap water, three times with deionized water (Zambia Agriculture Research Institute) and one time with acetone (Sigma-Aldrich, Steinheim, Germany). The HPLC vials (Agilent Technologies, USA) for sample analysis were washed with 0.02 mol/L EDTA-solution and dried in open air. Samples were removed from the -20^{o}C freezer and were thawed. Approximately 10 g of muscle was weighed and mixed with 25 mg EDTA (Sigma-Aldrich, Steinheim, Germany) per gram sample. The

sample and the EDTA were homogenized for one (1) minute using a blender (1L FRI 15023050M: Firma, Villa Verucchino Remin, Italy).

One gram (1g) of the homogenized sample was accurately weighed into a 15 mL polypropylene centrifuge tube. To the sample, 50 µL of 50 µg/mL caffeine solution (Agilent Technologies, C = 50 µg/mL in H2O, ≥ 99%), equivalent to 2500 ng caffeine was added as an internal standard. Five millilitres (5 mL) of acetonitrile (Sigma-Aldrich, Steinheim, Germany) was added using a 5 mL volumetric pipette and the mixture was vortexed for 1 minute. The sample was centrifuged (REMI-R-24: Asia, Mumbai) for 10 minutes at 7000 rpm. The supernatant was collected into a separate 15 mL centrifuge tube by decantation. Five millilitres (5 mL) of acetonitrile was added to the residue, the mixture was vortexed (Cat S8220, 60 Cycles, USA) for 1 minute. The sample was again centrifuged for 10 minutes at 7000 rpm. Both supernatants were combined into a 15 mL centrifuge tube, briefly mixed using a vortex and gently dried under a stream of nitrogen compressed gas (Afrox, Zambia) to 2 mL. After drying, 0.5 mL of HPLC grade water (Sigma-Aldrich, Steinheim, Germany) and 30 µL of formic acid (98%, Sigma-Aldrich, Steinheim, Germany) were added, making the mixture 1.2 % acidic. Fifteen milligrams (15 mg) of Supelclean ENVI-carb active coal (HPLC grade, Sigma-Aldrich, Steinheim, Germany) was added, the sample was mixed for 30s using a vortex and centrifuged for 10 minutes at 7000 rpm. The supernatant was collected into a separate 15 mL centrifuge tube and dried under a stream of nitrogen compressed gas to 0.5 mL. The dried sample (0.5mL) was transferred into the HPLC glass vial and was measured using the HPLC/DAD (HP 1200 series, Agilent Technologies, USA).

2.3.2 Method validation

Validation was carried out to conform to the Germany Society of Toxicology and Forensic Chemistry (GTFCh, 2009) by determining the levels of acceptable analytical confidence for

all analysed compounds. Accuracy was determined by spiking two (2) replicates of the blank matrix with 400 and 2500 ng of OTC and SMZ analytical standards and analysed for three (3) consecutive days. The precision was tested by spiking 5 replicates of the blank matrix with 400 ng of OTC and SMZ standard while the limit of detection (LOD) and quantification (LOQ) were measured by forcefully defining peaks from 5 replicates of the negative control sample. The standard deviation of the resulting concentration was then the basis for calculating the LOD and LOQ. The recovery rates of OTC and SMZ were determined by spiking three (3) replicates of the beef blank matrix with 400 ng of OTC and SMZ standards, a solvent with 400 ng of OTC and SMZ standard and 3 replicates of the beef blank matrix remained unspiked. The linearity was established through an internal calibration curve obtained by analysis of OTC and SMZ at 5 concentrations (200, 400, 800, 1200 and 2500 ng/g) in the blank meat matrix. The areas under the curve to determine the concentrations of OTC and SMZ were calculated based on the equations generated by the calibration curves.

2.3.3. Sample analysis using HPLC

The extracted beef samples were quantified for OTC and SMZ residues using a HPLC. The HPLC System, HP1200 series (Agilent Technologies, USA) comprised of an automatic degasser, quaternary pump, autosampler, column thermostat, a fluorescent detector (FLD) set at 420nm Emission and 270nm Excitation and a Diode Array Detector (DAD) set at 275nm and 355nm wavelengths. The analytical column was an Eclipse XDB C-18, 150 x 4.6mm, 5µm. The column temperature was maintained at 25^{o}C. Mobile phase A (0.1% Formic acid in water) and mobile phase B (0.1% Formic acid in acetonitrile) were pumped at a flow rate of 0.5mL/min. The gradient mobile phase was pumped through the analytical column with the programme presented in Table 2.

Table 2: HPLC mobile phase gradient programme

Time (min)	**Flow rate**(mL/min)	**Eluent A** (%)	**Eluent B** (%)
0	0.5	85	15
10	0.5	40	60
10	0.5	95	5
17	0.5	95	5
18	0.5	85	15
21	0.5	85	15

Eluent A: water + 0.1% formic acid
Eluent B: acetonitrile + 0.1% formic acid

A 100µl of each sample was injected to obtain chromatograms with mean peak areas of positive samples corresponding to retention times of 7.4 to 7.7 minutes of the reference standard for OTC and 9.0 to 9.4 minutes for SMZ. The tests were performed according to the manufacturer's instructions and used in a semi-quantitative way by the calculation of an internal calibration curve (Area under the curve versus Concentration).

2.4. Data analysis

Data were summarized and analyzed using Excel 2010® and Stata® version 13.0 software, respectively. Since the data was normally distributed, the one-way Analysis of Variance (ANOVA) test was performed to compare the mean differences of OTC and SMZ levels within and between the four provinces. In order for the analyte to be considered found, its peak must have appeared on both UV wavelengths (275nm and 355nm) and the fluorescence wavelength (520nm for Oxytetracycline and 420nm for Sulphamethazine respectively). The UV spectra of the peak must resemble the UV spectrum of the analyte. The purity factor must exceed the calculated threshold. The quantification was done using the wavelength of 275nm.

If two peaks are merged together, the peak for the analyte at 355nm was used to define the peak at 275nm. The fluorescence detector was only used for qualifying the analytes.

3. Results and Discussion

3.1. Method validation

The results of the repeatability experiments (Table 3) showed a mean ± standard deviation (SD) concentration of 294 ± 29 ng/0.5mL for SMZ. This concentration is much lower than what was initially spiked (400 ng/g). The results for OTC shows a mean of 353 ± 82 ng/0.5mL.This elucidates that OTC had a much better extraction efficiency as compared to SMZ. On the other hand, the standard deviation for SMZ is smaller than that of OTC. This explains why SMZ had peaks with high intensity as compared to OTC.

Table 3: Results of repeatability of the negative samples (Blank matrix) spiked with 400ng of OTC and SMZ standards

Sample Number	OTC (ng/g)	SMZ (ng/g)
1	263	328
2	418	279
3	266	265
4	432	274
5	383	323
Mean± SD	353± 82	294± 29

The accuracy of the method was tested for a concentration of 400 and 2500 ng/g of each analyte and analysed for 3 consecutive days. The concentration of 400ng/g was chosen

because it lays between the MRLs for tetracyclines in muscle (200 ng/g) and in the kidney (600 ng/g) (CAC, 2014). The concentration of 2500 ng/g was chosen to test the performance of the method for high concentrations.

The accuracy was checked for the calibration at 275 nm and 355 nm. Oxytetracycline showed a mean of 397 ± 40 ng/g for a spiked concentration of 400 ng/g at 275 nm. The confidence interval (P = 95%) overlapped with the spiked concentration, showing that the method accurately described the concentrations for OTC in the lower concentration range of the calibration. For 2500 ng/g, OTC showed a mean of 2951 ± 625 ng/g at 275 nm. The confidence interval (P = 95%) overlapped although it was very broad. At 355 nm, OTC showed a mean of 331 ± 40 ng/g for a spiked concentration of 400 ng/g. The confidence interval overlapped with the spiked concentrations for both P = 95 % and P = 99 %.The results for the accuracy for = 275 nm at 400 ng/g (Table 4) for SMZ showed a mean accuracy of 349 ± 32 ng/g. The confidence interval for P = 95% and P = 99% overlapped with the spiked concentration of 400 ng/g and 2500 ng/g. This clearly showed that according to the acquired data and the requirements for the accuracy described in the GTFCh, the method was fit for the intended purpose.

The recovery test showed a recovery rate of 104% and 90% for OTC and SMZ, respectively. The recovery rate for OTC was considerably above 100%, which might be due to the active coal clean up. The active coal clean-up was conducted under acidic conditions which increased the UV absorbance especially for tetracyclines. The recovery rates for all analytes were close to 100 %, which showed that the method had a very good recovery for all target analytes. Sulfamethazine had the lowest LOD and LOQ with 7 ng/g and 20 ng/g as compared to OTC (Table 4).

Table 4. Method validation results

Parameters	Oxytetracycline		Sulphamethazine	
LOD (ng/g)	30		7	
LOQ (ng/g)	89		20	
Recovery rate (%)	104		90	
Accuracy	Sample spiked with 400ng/g OTC	Sample spiked with 2500ng/g OTC	Sample spiked with 400ng/g SMZ	Sample spiked with 2500ng/g SMZ
Mean±SD (ng/g)	397±40	2951±625	349±32	2264±339
SD at 95% C. I.	42 ng/g	655 ng/g	34 ng/g	356 ng/g
SD at 99% C. I.	66 ng/g	1028 ng/g	53 ng/g	559 ng/g
	Overlap of C.I. of Mean with spiked Concentration			
C. I. at 95%	YES	YES	YES	YES
C. I. at 99%	YES	YES	YES	YES

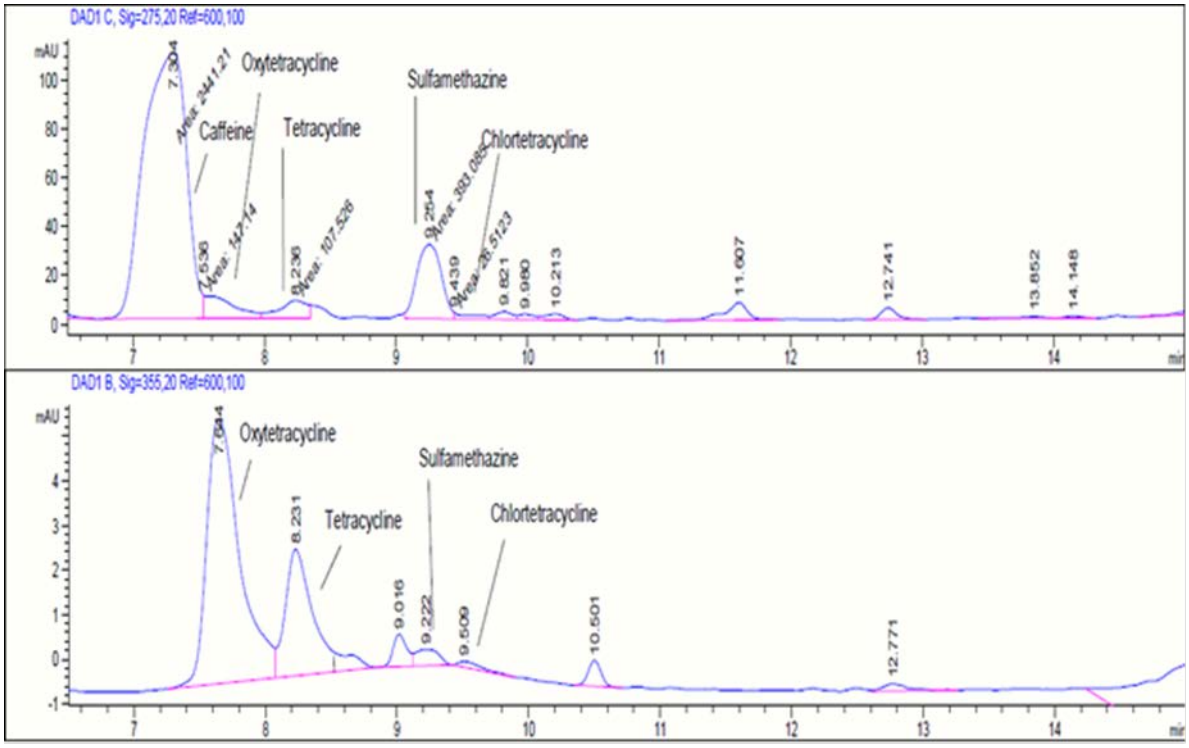

Figure 1: Chromatograms of the representative positive sample. [UV detector at 275nm wavelength for sulfamethazine (top) and 355nm wavelength for oxytetracycline (bottom); Flow rate: 0.5mL/min; Column: Eclipse XDB C-18, 4.6 x 150mm, 5µm I.D, temperature 25°C; Mobile phase: gradient of water/acetonitrile containing 0.1% formic acid].

3.2. Levels of oxytetracycline and sulphamethazine in samples

A total number of two hundred twenty-four (224) samples were analysed for OTC and SMZ antimicrobial residues, of which the highest number of samples were from Lusaka province (82) followed by Copperbelt province (64) while the lowest number of samples were from Central province (48) and Southern province (30).

Out of 224 samples analysed, 77 (34.4%) [95%; CI: 28.4 – 40.9] had detectable levels of OTC residues while 39 (17.4%) [95%; CI: 12.9 – 22.9] had detectable levels of SMZ residues. The mean concentrations for OTC and SMZ with detectable levels of residues were 199.6 ± 122.6 ng/g [95%; CI: 171.8-227.5] and 86.5 ± 75.9 ng/g [95%CI: 61.9-111.1], respectively; whilst the concentration range for OTC and SMZ residues was 27.26 ng/g to 481.61 ng/g and 11.92 ng/g to 259.98 ng/g (Table 5), respectively.

In a study conducted in Central Ethiopia, Bedada *et al.* (2012) reported a proportion of 71.3% samples with detectable OTC residues with a mean of 109.4ng/g. These values are higher compared to our study. This could be attributed to many factors including, unauthorised use of the antimicrobials, failure to follow label instructions or inappropriate withdrawal period before slaughtering of animals, failure to consult a veterinarian before using the antimicrobials and lack of prior training in animal husbandry. However, the concentration range (11.5 to 429.3 ng/g) from the same study is close to our findings (Bedada *et al.*, 2012).

On the contrary, other studies conducted in Nigeria (Olatoye and Ehinmowo, 2010) revealed very high ranges of 424 to 2370 ng/g of detectable OTC residues in edible cattle.

Similar studies carried out by Abasi *et al.* (2009) in Iran, Muriuki *et al.* (2001) in Kenya and Abavelim *et al.* (2014) in Ghana reported 43%, 44% and 50% of detectable OTC in beef, respectively, which are relatively higher compare to our findings. However, Mor *et al.* (2012) in Turkey reported 5.73% of detectable SMZ in beef, a percentage much lower than what was found in this study but which still indicates that compliance to drug withdraw periods is a problem in many countries, and further underscores the need for greater enforcement of regulations.

Table 5: Proportion of positive for oxytetracycline and sulphamethazine residues in beef samples per province

	Oxytetracycline (n = 224)		**Sulphamethazine (n = 224)**	
	Positive (%)	95% CI	Positive (%)	95% CI
Copperbelt	23 (10.3)	49.6–261.1	12 (5.4)	37.3-134.6
Central	17 (7.6)	111.2–279.0	10 (4.5)	30.3-133.9
Lusaka	26 (11.6)	140.0-280.1	9 (4.0)	28.2-133.1
Southern	11 (4.9)	94.6-328.2	8 (3.5)	37.6-175.6
TOTAL	**77 (34.4)**	28.4 – 40.9	39 **(17.4)**	12.9 – 22.9
MEAN	19.2 (8.6)		9.8 (4.4)	
Conc. range (ng/g)	27.3 – 481.6		11.9 – 259.9	
Mean conc. (ng/g) ± SD	199. 6 ± 122.6		86.5 ± 75.9	

3.3. Distribution of OTC and SMZ positive samples above Codex and EU MRLs

Of the 77 samples with detectable levels of OTC residues, 35 (45.5%) [95% CI: 34.5 - 56.9] were above the MRLs for Codex Alimentarius Commission's standard of 200 ng/g while 59 (76.6%) [95% CI: 65.6 - 84.9] were above MRLs for the EU standard of 100 ng/g (Table 6).

Out of the 39 positive samples for SMZ residues, 5 (12.8%) [95% CI: 5.2 - 28.2] were above the MRLs for Codex Alimentarius Commission's standard of 200 ng/g; while 13 (33.3%) [95% CI: 19.9 -5.0] were above MRLs for the EU standard of 100 ng/g (Table 6).

Table 6: Distribution of positive beef samples to OTC and SMZ residues according to the MRLs per provinces

Province	Number (%) positive OTC samples				Number (%) positive SMZ samples			
	Positive & Below Codex MRL	**Positive & Above Codex MRL**	Positive & Below EU MRL	**Positive & Above EU MRL**	Positive & Below Codex MRL	**Positive & Above Codex MRL**	Positive & Below EU MRL	**Positive & Above EU MRL**
C/belt	14 (18.1)	**9 (11.7)**	10 (13.0)	**13 (16.9)**	10 (25.6)	**2 (5.0)**	8 (20.5)	**4 (10.2)**
Central	9 (11.7)	**8 (10.4)**	3 (3.9)	**14 (18.1)**	9 (23.1)	**1 (2.6)**	7 (17.9)	**3 (7.7)**
Lusaka	13 (16.9)	**13 (16.9)**	2 (2.6)	**24 (31.2)**	8 (20.5)	**1 (2.6)**	6 (15.4)	**3 (7.7)**
Southern	6 (7.8)	**5 (6.5)**	3 (3.9)	**8 (10.4)**	7 (17.9)	**1 (2.6)**	5 (12.8)	**3 (7.7)**

Total	42 (54.5)	**35 (45.5)**	18 (23.4)	**59 (76.6)**	34 (87.2)	**5 (12.8)**	26 (66.7)	**13 (33.3)**
	77 (100.0)		**77 (100.0)**		**39 (100.0)**		**39 (100.0)**	
Range %	7.8-18.1	**6.5-16.9**	2.6-13.0	**10.4-31.2**	17.9-25.6	**2.6-5.0**	12.8-20.5	**7.7-10.2**
Mean %	13.6	**11.4**	5.8	**19.2**	21.8	**3.2**	16.7	**8.3**

A study conducted by Emiri *et al.* 2014 in Albania showed as low as 11% of detectable OTC residues of which, all were below both the Codex Alimentarius and the EU standards. This could be due to good observation of drug withdrawal periods, non-extended usage of antimicrobials, existence of restrictive legislation, adequate enforcement of food safety monitoring plans, good records of treatment, ability to identify treated animals and good consumer awareness about the magnitude and human health hazards associated with antimicrobial residues in the food of animal origin.

This study shows that 34.4% of samples had detectable residues of OTC, out of which 45.5% were above Codex Alimentarius and 76.6% above EU MRLs. In addition, 17.4% of samples had detectable residues of SMZ, out of which 12.8% were above Codex Alimentarius and 33.3% above EU MRLs. From the above results, it is evident that a number of farmers do not follow the drug withdraw period, therefore, putting the human population in the four provinces where samples were collected at risk of exposure to unsafe levels of OTC and SMZ residues. This situation could lead to the development of adverse effects considering the fact that beef muscle is the most consumed tissue from slaughtered cattle (World Bank, 2011). Previous studies conducted in Spain have demonstrated that high intake of beef contaminated

by antimicrobials, even at relatively low-level, increases the risk of selecting for resistant bacteria (Baquero *et al*., 2010). The situation is more critical when we consider that meat is among the most preferred foodstuffs in Zambia (World Bank, 2011). The antimicrobial residues in food of animal origin has rarely been a serious concern in developing countries (Mensah *et al.,* 2014). This is because the major course of antimicrobial residues in beef in developed countries is their use as growth promoters which are, however, used at a low level. The high prevalence of tick-borne diseases such as theileriosis, anaplasmosis and heartwater in Sub-Saharan Africa including Zambia (Mainda, 2014) has made therapeutic use of antimicrobials, especially, OTC a major course of concern in relation to antimicrobial residues in beef (Mainda, 2014). Antimicrobial residues in food of animal origin can in general cause allergies, cancer, alteration in the intestinal flora, bacterial resistance and inhibition of fermentation in the dairy industry (Mensah *et al.,* 2014). Besides health risks to the local population, the presence of residues could jeopardise international trade in the wake of the world trade organisation (WTO) agreement on the application of sanitary and phytosanitary measures. On the other hand, SMZ residues contained in meat preserved with sodium nitrate may develop a triazine complex that has a considerable carcinogenic potential in humans (Aamer *et al.*, 2000).

Studies conducted in the State of Kuwait reported that adhering to withdrawal periods and the enforcement of restrictive legislation significantly reduces the levels of antimicrobials in beef (Muhammad *et al.,* 1997). In contrast, failure to respect waiting time before slaughtering cattle leads to a high risk of exposure to antimicrobial residues. The enactment of farmer friendly regulations in Zambia could reduce the indiscriminate use of antimicrobials and lead to the implementation of a plan for the control and surveillance of residues from veterinary medicinal products in foods of animal origin.

In order to assure food safety and penetrate the globalised international market, farmers in Zambia and other developing countries must offer products that are competitive in terms of quality and safety. The problem is that, in most African countries, there is no proper control of the distribution of veterinary pharmaceutical and phytosanitary products (Messomo, 2006). Worse still, no appropriate legislation yet exists to guarantee the quality of various products released onto the African market (Messomo, 2006).

4. Conclusion

This study has revealed the wide occurrence of OTC and SMZ antimicrobial residues in beef from the four provinces of Zambia with OTC being more prevalent. There was no significant difference in the proportion of residues of the two antimicrobials between provinces. The study further showed a high incidence of positive samples for OTC residues above the MRL for both Codex Alimentarius Commission and EU standards. Although in low proportion, positive samples to SMZ residues above the MRL for the Codex Alimentarius Commission and EU standards are reported in our study. This situation constitutes a health hazard for the Zambian population.

We therefore recommend that sensitization of farmers about the dangers of antimicrobial residues in food and the need to follow the withdraw period be undertaken; Government should implement the monitoring plan for veterinary residues and enforce relevant legislations to control the abuse and miss-use off veterinary drug. Further studies should be conducted on the pharmacokinetics of antimicrobials in animals infected with various infectious diseases in Zambia in order to ascertain that the withdraw period recommended by drug manufacturers is not influenced by any of the local conditions.

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Ethical Issues

Non to be declared

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