OPEN ACCESS
DOI: 10.5455/javar.2015.b101

J. Adv. Vet. Anim. Res., 2(3): 321-325.
Available at- *http://bdvets.org/JAVAR*

JAVAR Journal of Advanced Veterinary and Animal Research

ORIGINAL ARTICLE

Volume 2 Issue 3 (September 2015)

Oxytetracycline residues in Sheep meat in Khartoum State, Sudan

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ABSTRACT

In the last two decades, the chemical and biological contaminants in livestock and livestock products worldwide have been considered as one of the causes that threatens life of both man and animals. In Sudan very little work has been done in this field. This study was carried out to detect Oxytetracycline (OTC) residues in sheep liver and muscle samples in Khartoum State, Sudan. A total of 150 samples comprising of liver (n=75) and muscle (n=75) of sheep were collected from three different localities in Khartoum State during the period from March to September 2013. The OTC residues were detected using high performance liquid chromatography (HPLC) and one plate test (OPT). The results of HPLC showed that the level of OTC residues in liver samples above maximum residue limit (MRL) was 4% (n=3/75) and under MRL was 96%. Regard to muscle samples, the level of OTC residues above MRL was 60.3% (n=35/58), whereas, under MRL was 39.7%. Using OPT, 14.7% (n=11/75) and 6.7% (n=5/75) of liver and muscle samples showed inhibition zone, respectively. It is concluded that OTC residues are detected in sheep liver and muscle sample with different levels in Khartoum state. Further investigation on level of OTC residues in other farm animals and other organs throughout the country is recommended.

Keywords

HPLC, Liver, Muscle, Oxytetracycline, Sheep, Sudan

ARTICLE HISTORY

Received : 24 May 2015, **Revised**: 23 June 2015,
Accepted : 5 July 2015, **Published online**: 5 July 2015.

INTRODUCTION

Sudan is considered as one of the wealthiest African and Arab countries in term of animal resources and their products which include meat, meat products, milk, eggs, fish and poultry. The quantity of antibiotics consumed in the animal production sector in production of these foodstuffs is subject to unforeseeable economic and health hazards. However, Livestock producers used a variety of products, amongst which antibiotics, to control diseases and increase yields, sometimes under veterinary control and sometimes not. Unfortunately the problem of antibiotics residues has increased significantly due to the indiscriminate and frequent use of antibiotics in clinical practice. Antibiotics are used for two main purposes; as preventive and curative drugs and as feed additives or growth promoters. These drugs are quickly excreted from the animal; others are not readily metabolized or excreted and so, their residues will persist in the animal tissues and hence enter the human food chain constituting health risks to the consumers. Figures concerning the quantities of antibiotics consumed in the animal production sector in Sudan are not always accurate. Moreover, the regulations governing the storage, distribution and use of veterinary medicinal products are a long way behind European rules.

Many livestock producers treat their animals by themselves. Even if they use the same drugs as veterinarians, they have little understanding of the conditions and quantities to administer or the waiting periods. In addition, there are cases of veterinary medicinal products intended for ruminants being administered to other species (poultry). The uncontrolled use of anti-infectious agents in general and antibiotics in particular, can lead to residues in

animal products, especially when users fail to respect waiting periods. The risks of residues in foodstuffs of animal origin could be reflected into several forms, carcinogens, allergies toxicity alteration of the intestinal flora, selection of bacteria resistant to antibiotics (Khan, 1975; Wageh et al., 2013). In addition to the health risks, the presence of veterinary drug residues in foodstuffs of animal origin can affect its exportation to foreign countries however, the efforts currently being made on food safety only related to products exported to Middle East countries and local consumers are left to fend for themselves. On the other hand, there is currently little data on the presence of antibiotic residues in the meat sold in Sudan; this lack of knowledge has prompted investigations.

Oxytetracycline (OTC) is a common antibiotic used in livestock for prophylactic, therapeutic treatment, and as a growth promoter due to its broad spectrum activity. It is mainly bacteriostatic active against large number of gram-positive and gram negative pathogenic bacteria (Chee-Sanford et al., 2001). The acceptable Maximum Residue Limit (MRL) for OTC as recommended by the joint FAO/WHO Expert Committee on Food Additives (1999) is 200, 600 and 1200 µg/kg for meat, liver and kidney, respectively (Bogaard and Stobberingh, 2000).

Owing to meagre data available on chemical and biological contaminants in livestock and livestock products in Sudan that threaten life of both man and animals, this study was carried out to investigate and evaluate the magnitude of the OTC residues in sheep meat in Khartoum State, Sudan.

MATERIAL AND METHODS

Sample collection: A total of 150 samples (75 liver and 75 muscle samples) were collected from sheep carcasses from slaughter houses in three major localities in Khartoum State during the period from March to September 2013. Fifty samples (25 liver and 25 muscle samples) were collected from each locality including El Huda Slaughter house (Khartoum), El Kadro Slaughter house (Bahri) and El Sabaloga Slaughter house (Omdurman). Approximately 50 to 100 gm of labeled liver and muscle samples obtained from each sheep carcass was wrapped in polythene bags and put in cool boxes with dry ice or freezer packs at 4°C. The samples were stored at -20°C until analyzed.

One Plate Test (OPT): Samples were screened for antibiotic residues using the microbial inhibition plate test. The standard test organism *Bacillus subtilis* strain NCTC8236 was obtained from National Laboratory for Public Health, Ministry of Health, Sudan. The test depends on bacterial growth inhibition. The sample was considered to be positive when the inhibition zone appeared around the well.

Test Procedure: 1 mL of standard organism was added to 20 mL of nutrient agar in each Petri dish. Then mixed and left for 10 min to solidify on a leveled surface bench. Four cups (10 mm in diameter) were cut using sterile cork borer (No.4) and the agar discs were removed. Alternate cups were filled with 0.1ml each of samples using automatic micro pipettes and allowed to diffuse at room temperature for 2 hours. Plates were incubated at 37°C over night until growth is visible within. Zone inhibition was observed around the wells when the sample containing antibiotic and was measured in millimeter using a ruler. Negative samples did not show such clear zone.

High performance liquid chromatography (HPLC) analysis:

Sample preparation: Five grams of each organ to be analyzed was weighed using a balance and then cut into very small pieces and subsequently grounded into fine paste using Sartorius mincer. This was then blended three times with 20 mL aliquots of Mcllvaine buffer (9.15 mL citric acid with 90.85 mL Na_2HPO_4) (pH 4.0) methanol (3:7) using a high speed Elmore Parker blender and then centrifuged using cold centrifuge (Hettich, ROTANT 460R, UK), centrifuge at 2000 g for ten minutes. This was then filtered using filter paper (Whatman No 1). The filtrate was collected in falcon tube (50ml). The filtrate was then applied on Solid phase extraction (SPE) C18 cartridge (for cleanup), that were activated with water and methanol and washed twice with 20 mL of water The OTC were eluted with 10 mL of 0.01 M methanolic oxalic acid solution and collected in 10 mL vial. The extracted OTC were analyzed, identified and quantified by HPLC.

Test procedure: Determination of the OTC residues was done using a high-pressure liquid chromatography equipped with a constant flow pump and a variation wavelength PDA detector set at 350 nm. The separation was done on shim-pack C18 (250×4.6 mm) 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 oxalic acid). Flow-rate of 1 mL/min at room temperature concentrations: 2.5, 5 and 10 ppm were injected manually using 25 µL syringes to prepare standard curves (**Figure 1**). HPLC

results, were recorded only if the retention time was equal to 4.6 minutes which was the retention time for OTC (**Figure 2**).

Statistical analysis: Data collected were subjected to Statistical analysis using SPSS version 20 software. Chi square test was used to find the *P*-value, often known as pearson's Chi square Karl pearson.

RESULTS AND DISCUSSION

In the present study the results showed that OTC was detectable in all examined samples by HPLC and microbiological assay (OPT).

Microbiological assay (OPT): 11 Out of 75 liver samples (14.7%) showed inhibition zone (1-3 mm diameter) whereas, 5 out of 75 muscle samples (6.7%) showed inhibition zone (1-2 mm diameter) (**Figure 3**). Chi square results reveald that no statistical significant difference (*P≥0.05*) between prevalence of OTC residue in liver and muscle samples using microbiology assay.

HPLC results: All liver samples had detectable level of OTC residues by HPLC analysis while the relevant muscle samples were not all positive (**Figure 4**). 3 out of 75 (4%) liver samples had OTC levels above permissible MRL (**Figure 5**) distributed as follows: 2 out of 25 (8%) in Khartoum locality, 1 out of 25 (4%) in Bahri locality and 0 out of 25 (0%) in Omdurman locality (**Figure 6**). The levels of OTC in liver sample ranged between 142-841 µg/kg (mean 378.66±117.74 µg/kg). No significant difference (*P ≥ 0.05*) was found between the localities. 58 out of 75 (77.3%) muscle samples had detectable OTC residues. Of these, 35 (60.3%) had OTC levels above the permissible MRL (**Figure 5**) distributed as follows: 19 out of 25 (76%) in Khartoum locality, 10 out of 25 (40%) in Bahri locality and 6 out of 25 (24%) in Omdurman locality (**Figure 7**). The levels of OTC in muscle samples ranged between 0.0–631 µg/kg (mean 217.33±161.39 µg/kg). Significant difference (*P≤0.05*) was detected between the three localities. Chi-square results showed statistically significant (*P≤0.05*) differences between prevalence of OTC residues in liver and muscle samples using HPLC. The level of OTC residues above MRL in examined sheep muscle samples is high 35/58 (60.3%) compared with examined liver samples 3/75 (4%) by using HPLC (**Figure 5**).

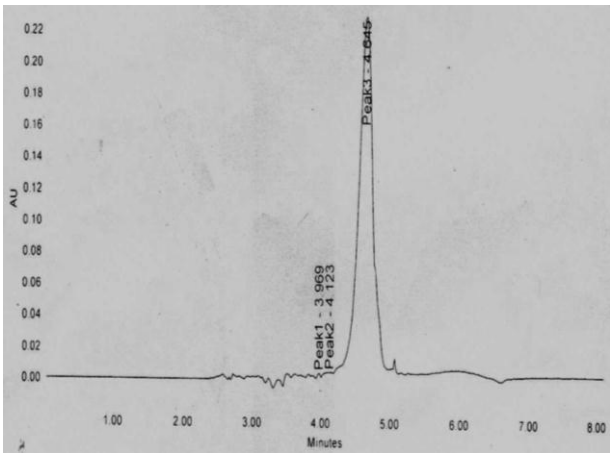

Figure 1. Chromatogram of reference standard of oxytetracycline. The arrow indicates the peak, peak area and its retention time.

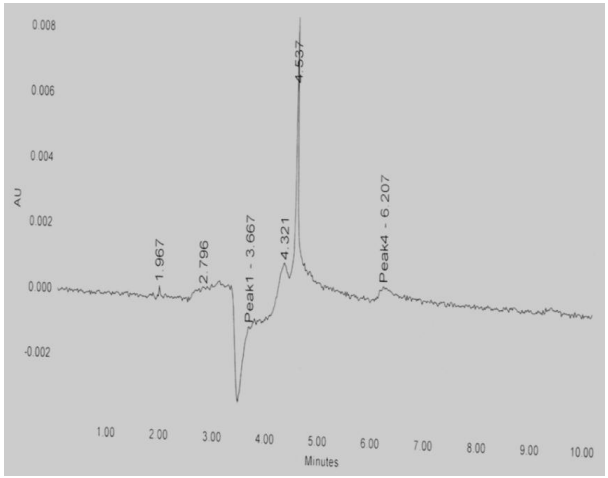

Figure 2. Chromatogram of representative meat sample that was positive for oxytetracycline in Khartoum State, Sudan. The arrow indicates the peak, peak area and its retention time.

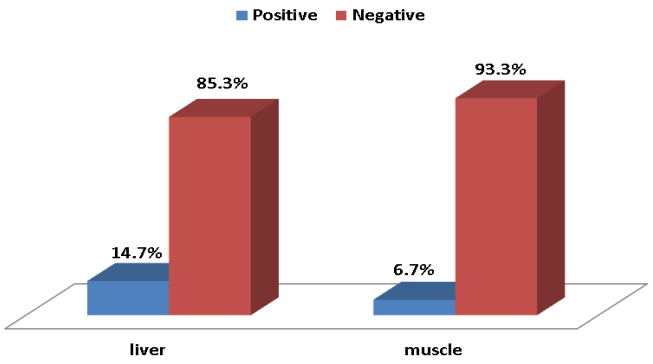

Figure 3. Total positive and negative results of oxytetracycline residues in liver and muscle samples using Microbiological assay (OPT) in Khartoum State.

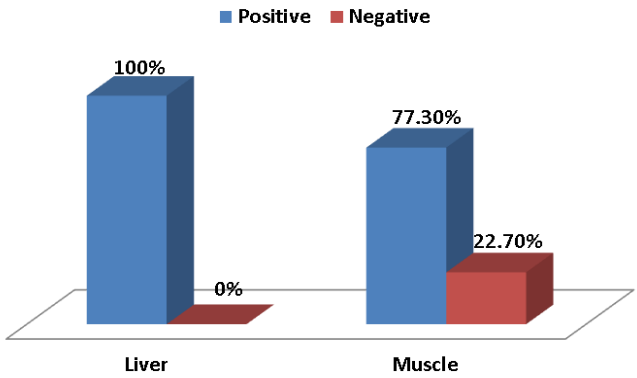

Figure 4. Total positive and negative results of oxytetracycline residues in liver and muscle samples using HPLC analysis in Khartoum State.

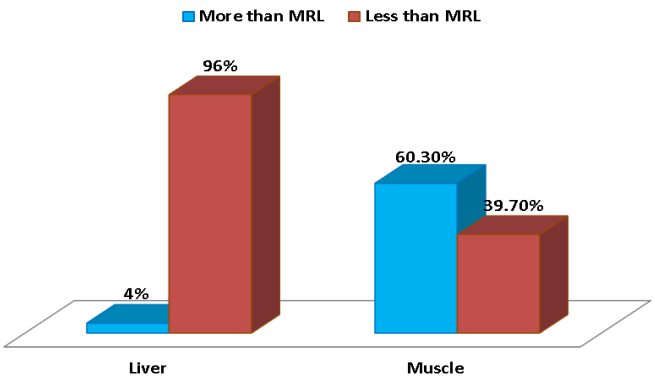

Figure 5. Total maximum residue limit (MRL) of oxytetracycline in liver and muscle samples by HPLC analysis in Khartoum State.

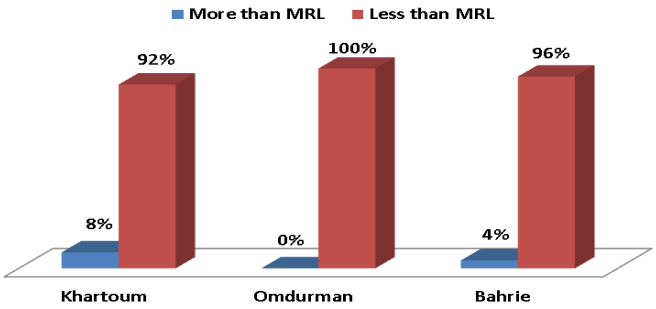

Figure 6. Maximum residue limit (MRL) of oxytetracycline in Liver samples in three localities of Khartoum State.

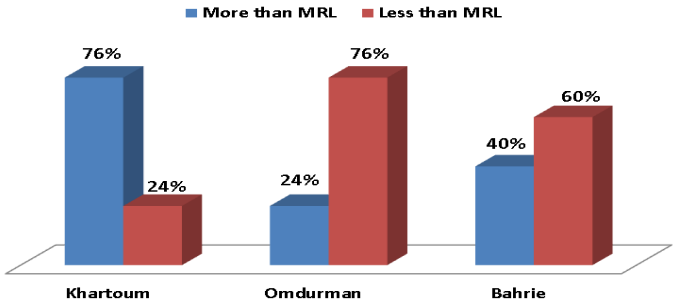

Figure 7. Maximum residue limit (MRL) of oxytetracycline in muscle samples in three localities of Khartoum State.

The residues of antibiotics or it's metabolites in Livestock meat and other foods animal cause adverse toxic effects on consumer's health. The current study detected hazardous levels of OTC in sheep meat in Khartoum State for human consumption.

In the present study 133 out of 150 (88.7%) had detectable levels for OTC residues, from which 38 (28.6%) had detectable levels above permissible MRL of OTC residues which indicated a widespread misuse of veterinary drugs by food animal producers. In addition, the results suggested that OTC was indiscriminately used in sheep in the study areas.

This is in agreement with Hala (2006) who observed a high percentage (16.87%) of antibiotics residues in Khartoum State, Sudan.

The results indicated that the recommended withdrawal time was either not respected or extra label recommended doses for treatment might have been used. A greater proportion of livestock in most developing countries such as Sudan are reared by the nomadic herdsmen who administer chemotherapeutic agents without veterinary prescription. Therefore, correct dosages are unlikely to be administered and the withdrawal periods are usually not observed (Olatoye and Ehinmowo, 2009). The animal owners in Sudan believed in the miracle effect of OTC and therefore refer to it as (Gameea Alamrad) they normally treated their animals by themselves as they obtain different drugs without veterinary prescription or supervision. Moreover, 32% of the owners slaughtered their animals during the course of antibiotic treatment and 66% of them before completing withdrawal periods (Mohamed et al., 2011). This type of practice normally resulted in promiscuous use of drugs which have direct health impact for animals and their products as well as consumer (human). In addition, human exposure to animal products containing significant level of OTC residues may demonstrate 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, OTC 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 (Walton et al., 1994). Moreover, using OTC during the second month of

pregnancy has a teratogenic effect to the fetus (Czeizel and Rockenbauer, 2000).

Mohamed et al. (2011) conducted a study to detect antibiotics residues in beef in Ghanawa Slaughterhouse, Khartoum State, Sudan. Questionnaire survey revealed that 86% of the veterinarians did not determine the weight of animals exactly when describing doses which lead to over-dosing or sub-dosing, and there was no following up of cases after leaving the clinic or pharmacy. Furthermore, 76% of the veterinarians leave dose restriction and administration to owners.

CONCLUSSION

Oxytetracycline residues are detected in sheep liver and muscle samples with different levels by using HPLC and OPT in Khartoum State. HPLC method is more suitable than microbiological assay (OPT) because it is qualitative and quantitative technique, and suitable for detection the permissible MRL level. Therefore, further investigation on level of OTC residues in other farm animals and other organs at the country level is suggested.

ACKNOWLEDGEMENT

The authors wish to thanks Dr. Hanan Moawia Ibrahim, Head and member of Bacteriology Department and HPLC Units, Central Laboratory for their valued assistance, comments and observations. We are grateful to Dr. Wafa Emam Ibrahim for helping in sample collection. The authors wish to express their appreciations to Miss Azaa Babiker Musa, Central Laboratory, for helping in statistical analysis.

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