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Investigation of Antibiotics Residues in Imported and Locally Produced Red Meat in Muscat, Sultanate of Oman

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ABSTRACT

Background: Antibiotics are heavily used for animal treatment, growth enhancer and protection from disease. The residues of antibiotics are related to antibiotic resistance which is the biggest threats to global health, food security and development today. The aim of this study was to investigate the presence of antibiotic residues in available red meat in the Sultanate of Oman.

Methods: The extraction was done using QuEChERS method by Aglient and 11 standards were used. The extract was analyzed using The Aglient 6460 LC-MS/MS.

Result: The results showed that investigated Omani goat muscle and liver were free of antibiotics as latter they used as blank sample. All investigated Somali goat samples didn't contain Tetracycline, Amoxicillin, Ceftiofur, Oxacillin, Deoxytetracline, Tylosin and Phenoxymethylpenicillin. Only two samples had Tilmicosin with value of 18-32.2 µl/kg which was that was lower than maxiumam residual limit (MRL). Around 48% of total somali goat samples contained Oxytetracycline (OXY) and Chlorotetracycline (CTC) with value lower than MRL. However, two samples had values of CTC and OXY exceeding the MRL. Ciprofloxacin and Enrofloxacine were detected in 9 samples exceeding the MRL. The finding of this study showed unsafe food source supply to Oman as detection of antibiotic residues in meat.

Key words: Antibiotics, Red meat, Residual limit, Residues maximum.

INTRODUCTION

Antibiotics are chemical compounds which are produced by laboratory procedures or naturally by living organisms to inhibit the growth or kill microorganisms. In animal husbandry, antibiotics are used for three purposes; therapeutic, prophylaxis and as growth promoters. The therapeutic purpose is to treat animal diseases such as respiratory diseases, gastrointestinal diseases, mastitis, brucellosis, arthritis and other bacterial infectious diseases (Orwa *et al*., 2017). Whereas the prophylaxis purpose is to prevent diseases or at the outbreak of a disease in a herd. The third use of antibiotic in livestock is as growth promoter by increasing the weight gain in shorter period. The therapeutic use of antibiotics is more specific for the type, quantity, length of use and route of administration and frequency, than prophylaxis purpose. Also, the dose of antibiotics for therapeutic purpose is usually higher than in the prophylaxis purpose. However, around 90% of antibiotics in livestock area are used as prophylaxis and growth promoting purposes (Jayalakshmi *et al*., 2017).

The antibiotics are introduced to animal orally in feed or water, injections (intravenously, intramuscularly and subcutaneously), topically on the skin or by intramammary and intrauterine infusions. It was reported that the minimum withholding period for meat is 28 days after treated with antibiotics purposes (Jayalakshmi *et al*., 2017). Although most of the antibiotics are discharged from animal body, some are absorbed by tissue and tend to be higher in liver and kidney than in muscles (Sajid *et al*., 2016). In general, antibiotics have potential to cause allergic reactions to human body such as penicillin and sulfonamide. Harmful effects of antibiotics at high doses were reported such as hear loss and kidney toxicity, carcinogenicity, effects on thyroid and pituitary functions (Doyle, 2006). Adding to that, the residues of antibiotics might be mutagens, teratogenic, reduction in reproductive performance in human (Singh *et al*., 2014). The most common antibiotics groups used in veterinary field are tertacyclines, fluoroquinolones, b-Lactam and macrolides.

The long term use of tetracycline as sub therapeutic dose increased level of antibiotic-resistant pathogens (Chopra and Roberts, 2001; Economou and Gousia, 2015). Although tetracycline has a short half-life (7-10 h), residues levels were detected in liver, milk, kidney and muscles

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(Orwa *et al*., 2017; Doyle, 2006). Ramalta *et al*., 2017 found that the level of tetracyclines was around 168.02 g/kg which was lower than the acceptable MRLs as recommend by FAO/WHO Expert committee (200, 600 and 1200 g/kg for the liver, muscles and kidney, respectively). However, the long term exposure of antibiotics residues might cause acute or chronic toxicity to the organs and the entire body.

In addition, the residues of B-lactam compounds were found in chicken meat, milk products and beef (Irum *et al*., 2014; Jammoul and El Darra, 2019). Jammoul and El Darra, 2019 reported that 3 chicken samples out of 80 were contaminated with mean values of amoxicillin (63, 62.5 and 77.5 µg/kg), which were exceeding the MRL (50 µg/kg). Another study showed that 9 to 84 µg/kg of amoxicillin was detected in beef meat and around 40% of meat samples were above MRL (Irum *et al*., 2014).

As the other antibiotics, the use of fluoroquinolones has a side effect in animal such as arthropathy, articular cartilage degeneration, tendonitis and other forms of tendon injury in young animals (Aral *et al*., 2008 and Westropp *et al*., 2012). Adding to that, several studies showed that the use of fluoroquinolones had a toxicity in central nervous system at higher doses as well as toxicity in gastrointestinal system and phototoxicity at therapeutic doses. Also, the pregnant animal with fluoroquinolones at higher dose lead to maternotoxicity and occasionally embryonic dead (Brown, 1996). Another study in (Nigerya, 2015) by Omotoso and Omojola, found the concentration range of ciprofloxacin and norfloxacin in beef meat exceed the MRL; 231.08±564.30 and 173.40±154.57 µg/kg, respectively, whereas ofloxacin concentration was below the MRL; 79.28±183.70 µg/kg. In 2017, a study in Vietnam detected enrofloxacin concentration exceeding 1000 µg kg^{-1} in eggs (Yamaguchi *et al*., 2017). Chowdhury *et al*., (2015) reported that concentration of ciprofloxacin residues in commercial farms was significantly higher than in local.

The classification of macrolides depends on the number of atoms, which comprise the lactone ring, ranging from 12 to 16 members. In the early 1960s, spiramycin was the first macrolide proposed for food animal use and followed by erythromycin and tylosin in the early 1970s. The mechanism of macrolides is to inhibit bacterial protein synthesis either to act as bactericidal or bacteriostatic depending on the concentration (Pyörälä *et al*., 2014). Tylosin is used in treating mastitis in many parts of the world. However, the residues of tylosin and other marcolids were detected in milk and egg. A study was done in Karnataka in India using samples from dairy herds. They investigated residues of the azithromycin and tetracycline in cow milk samples using high-performance liquid chromatography. Azithromycin and tetracycline concentration were high (9708.7 and 5460 µg kg^{-1}, respectively). The azithromycin concentration was decreased when subjected to 70 and 100°C for 24 h (Kurjogi *et al*., 2019).

In Oman, few studies were done in detection of antibiotics residues in red meat. Thus, the aim of this study was to investigate the residues of veterinary drugs in locally and imported red meat.

MATERIALS AND METHODS

A total of 48 samples of fresh Somali goats (24 liver and 24 muscle) were collected between April and November 2021 from Muscat municipality slaughterhouse. The sample of muscle was taken from gluteobiceps muscle area. The samples were collected in sterilized polyethylene bags, labelled and preserved in an ice box and immediately stored in a deep freezer at -20°C. One Omani goat sample (muscle and liver) was purchased from local goat breeder (no supplement was given) and weighed to be used as a blank and for matrix calibration curve. All samples were trimmed of the external fat and fascia cut in cubes and grinded by using food processor. After grinding the sample, the weight was taken and the sample was preserved in a sterilized zipper bag after removing the air to avoid oxidation.

The extraction was done by using QuEChERS extraction method: Agilent Bond Elut EMR-Lipid tubes (p/n 5982-1010) and Agilent Bond Elut Final Polish for Enhanced Matrix Removal-Lipid (p/n 5982-0101) as describe by (Zhao and Lucas, 2015).

Chromatographic condition

The separation of the antibiotic residues was performed using a Poroshell EC-120 EC-C18 analytical column (2.1 mm inner diameter I.D × 150 mm length, 2.7 µm particle size; Agilent). The separation of antibiotic was accomplished at 40°C. The flow rate and injection volume was 0.5 mL/min and 15 µL, respectively. Two mobile phases were used; (A) 0.1% formic acid and water and (B) Acetonitrile. The gradient elution program was planned as follows: starting with 98% A and 2% B for 1 min, then 85% A and 15% B for 1.5 min. After that, 70% A and 30% B for 2.5 min, followed by 55% A and 45% B for 6 min. Then, 20% A and 80% B for 8.5 min, later 100% B for two stages 10 min and 11 mins. Final run method is 98% A and 2% B for 11.2 min.

Calibration curve

The 11 standards were used in this study as showed in Table 1. The linear curve was obtained from individual standards using six concentrations of 2 ppb, 5 ppb, 10 ppb, 20 ppb, 50 ppb and 100 ppb. For the preparation of the work solutions (1 mg/ml in LC-MS -grade methanol/Acetonitrile) the antibiotics were diluted to several concentrations by using methanol as a diluent. All concentrations were prepared in the dark and immediately stored at -20°C.

Matrix match calibration

Matrix-matched calibration standards (STD) prepared with standard working solutions were post-spiked, corresponding to 0.5, 1.0, 10, 25, 50 and 100 ng/mL in muscle and liver extract. The blank matrix was choosen after comparing the meat and liver with solvent. The antibiotics free matrix was chosen as blank matrix.

Statistical analysis

Excel program was used for statistical analysis for all obtained data from LCMSMS as descriptive statistics.

RESULTS AND DISCUSSION

The total number of samples which have been analyzed were 48 Somali meat (24 muscles and 24 liver in triplicates). Adding to that, Omani goat meat (muscle and liver) sample was collected from Omani shepherd. All samples were investigated for Tetracycline (TC), Amoxicillin (AMOX), Ceftiofur (CEF), Oxacillin (OXA), Deoxytetracline (DOX), Tylosin (TYL), Phenoxymethylpenicillin (PCV) Oxytetracycline (OXY), Chlorotetracycline (CTC), Ciprofloxacin (CPFX) and Enrofloxacine (ENR) using Aglient 6460 LC-MS/MS analysis.

In Table 2, the recovery of spiked antibiotics in muscle and liver of Omani goat was compared with spiked solvent. The result showed that the matrix did not affect the recovery of the spiked antibiotics as the actual values of antibiotics compounds were negligible or false positive. Therefore, Omani goat was used as blank matrix as it was free of investigated antibiotics in this study.

Table 1: Different standards were used under each category.

Category	Compounds names
Group 1-Beta-lacatm	Ceftiofur
	Amoxicillin trihydrate
	Oxacillin sodium salt monohydrate
	Penicillin V potassium salt
Group 2-Fluoroquinolones	Ciprofloxacin
	Enrofloxacine
Group 3-Macrolide	Tilmicosin
	Tylosin tartrate
Group 4-Tetracycline	Oxytetracycline hydrochloride
	Chlorotetracycline hydrochloride
	Deoxytetracycline

Out of 24 Somail goats, five goats reported residues of CEF and ENR. The total range of CEF and ENR was 47.9 µl/kg to 2056.8 µl/kg in liver and 196.1 µl/kg to 3547.4 µl/kg in muscle (Fig 1). Four out of five goats had total values of CEF and ENR exceeding the MRL as shown in Fig 1. The MRL of CEF and ENR in goat muscle and liver were 100 µl/kg and 300 µl/kg, respectively (the MRL GSO 2481/2015, CX/MRL 2-2018 and EU 37/2010. Adding to that, Fig 1 showed that the value of CEF and ENR was higher in the liver compared to the muscles. Omotoso and Omojola in Nigerya reported similar results where they found the concentration range of ciprofloxacin and norfloxacin in beef meat exceeded the MRL; 231.08±564.30 and 173.40±154.57 µg/kg, respectively. Also in 2017, a study in Vietnam detected enrofloxacin concentration exceeding 1000 µg kg^{-1} in eggs (Yamaguchi *et al*., 2017). Moreover, (Chowdhury *et al*., 2015) reported that concentration of ciprofloxacin residues in commercial farms was significantly higher than in local. Whereas in Iran, the residuals of fluoroquinolones were detected in beef meat by ELISA techniques with concentration lower than MRL (Baghani *et al*., 2019). These detections of concentrations exceeding the MRL indicates of potential threats to human health as well as animal health.

In addition, there was a detection of tilmicosin in two muscles samples with values 18 and 32.2 µl/kg which were less than the MRL (50 µl/kg and 100 µl/kg in muscle and liver respectively (the MRL GSO 2481/2015, CX/MRL 2-2018 and EU 37/2010) as shown in Fig 2. Also, in this study, tetracycline (TC), amoxicillin (AMOX), ceftiofur (CEF), oxacillin (OXA), deoxytetracline (DOX), tylosin (TYL) and phenoxymethylpenicillin (PCV) were not detected in any sample as it is presented in Fig 2. Conflict result was reported in 2006, which was done on the Somali goat in Oman. They reported a presence of TC with mean of 49.8 µl/kg using ELISA (Mahgoub *et al.,* 2006).

In this study, around 48% of total Somali goat samples contained Oxytetracycline (OXY) and Chlorotetracycline (CTC) with range of 6.04-6.23 µg/kg and 5.48-8.35 µg/kg,

Table 2: Effect of matrix on antibiotic compound recovery in muscle and liver.

Compounds	Muscle			Liver		
	Blank meat	Blank solvent	Actual result of compounds (ppb)	Blank meat	Blank solvent	Actual result of compounds (ppb)
Amoxicillin (AMOX)	2.7573	2.4774	0.2799	0	0	0
Ceftiofur (CEF)	2.0605	0.4368	1.6237	0.9943	0.9932	0.0011
Chlortetracycline (CTC)	3.64	1.6279	2.0121	2.1705	2.2027	-0.0322
Ciprofloxacin (CPFX)	6.6961	6.4519	0.2442	1.6206	1.7092	-0.0886
Enrofloxacine (ENR)	4.007	3.6651	0.3419	1.477	1.6337	-0.1567
Oxacillin (OXA)	2.353	0.7604	1.5926	0.6634	0.6671	-0.0037
Oxytetracycline (OTC)	4.323	2.4997	1.8233	1.3251	1.4049	-0.0798
Phenoxymethylpenicillin (PCV)	2.4281	0.8377	1.5904	0.9853	0.9848	0.0005
Tilmicosin (TIL)	3.7983	4.3231	-0.5248	0.535	0.5369	-0.0019
Tylosin (TYL)	1.442	0	1.442	0.6009	0.6014	-0.0005

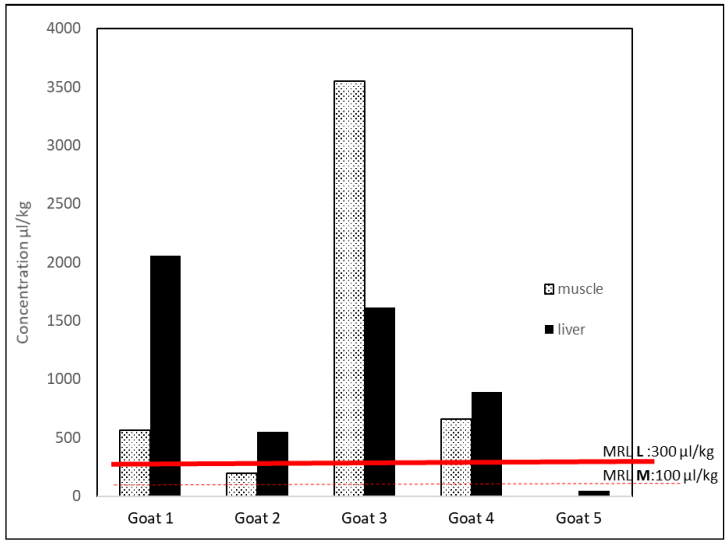

Fig 1: Residual Ciprofloxacin (CPFX) and Enrofloxacine (ENR) concentrations in Somali muscle and liver from Muscat Slaughterhouses.

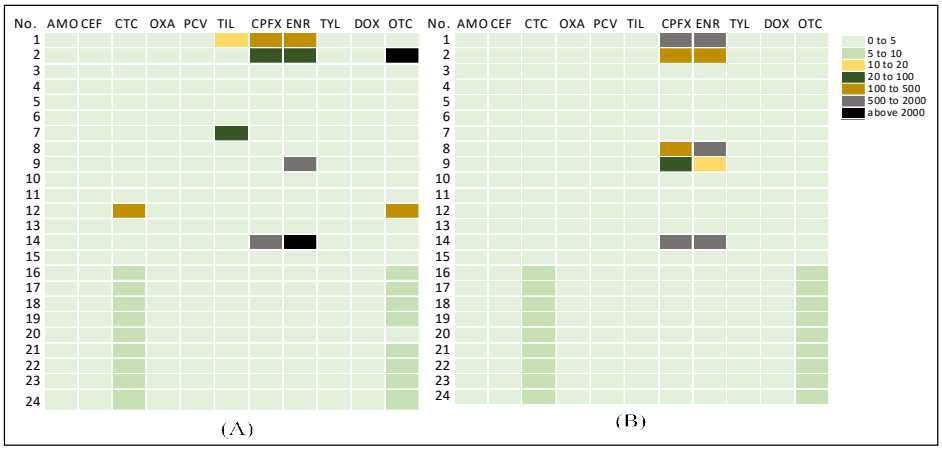

Fig 2: Heatmap of detected antibiotics in (A) Muscles, (B) liver.

respectively. Only two samples had value of CTC and OXY exceeding the MRL. One muscle got CTC and OXY as 274.8±87 µl/kg and 403±234 µl/kg, respectively. On other hand, one liver got highest residues level of CTC which were 2628.4± 325.13 µl/kg. In 2019, the range of TCs was 10.4-40.2 µg kg^{-1} (Cammilleri *et al*., 2019) which was higher than the detected range in this study. Other studies using different techniques such as ELISA and HPLC found the concentrations not reaching the MRLs as recommended by FAO/WHO Expert committee (200, 600 and 1200 g/kg for the liver, muscles and kidney, respectively) (Al-Amri, 2021, Ramalta *et al*., 2017). Although these studies showed the low concentration of TCs in meat, the health of consumer will be affected by long-term exposure to these concentrations.

These results might indicate the usage of veterinary drugs in production of Somali goat in Somalia. In addition, the concentration exceeding the MRL might be related to withdrawal time of the samples.

CONCLUSION

In this study, four groups of antibiotics were investigated in the liver and the muscle of Omani and Somali goats using LC-MS/MS. The Omani goat can be considered as a safe source of meat as it didn't contain any residues of antibiotics. Several antibiotics namely tetracycline, amoxicillin, ceftiofur, oxacillin, deoxytetracline, tylosin and phenoxymethylpenicillin were not detected in both types of goats. However, the investigated Somali goat (imported) had residues of several antibiotics such Tetracycline, Oxytetracyline, Ciprofloxacin and Enrofloxacine exceeding the MRL which might be related to withdrawal time or due to uncontrolled used of antibiotics. The study, however reported higher concentrations of antibiotics found in liver compared to muscles. Therefore, more numbers of samples and different livestock suppliers need to be investigated which can help to identify the good source of meat.

Conflict of interest: None.

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