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Residual evaluation of oxytetracycline in camel edible tissues in Tripoli region, Libya

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ABSTRACT

The deleterious effects of the residues of antibacterial drugs have been considered as one of the most serious problems in the world on the basis of their importance in both veterinary and human fields. Therefore, the present study was planned out to evaluate the residual levels of oxytetracycline in camel tissues in Tripoli area in Libya. Forty samples of slaughtered camel's tissues (10 of muscle, 10 of liver, 10 of kidney, and 10 of fat) were collected from different carcasses at different slaughter houses in Tripoli districts. The samples were homogenized, extracted and residual concentrations of oxytetracycline have been measured using liquid chromatography-Mass spectroscopy (LC-MS) technique and oxytetracycline standard. Oxytetracycline residues have been detected in 60% in muscle, 80% in liver, 90% in kidney and 70% in fat samples. The samples, although positive, yet decided acceptable as the detected levels were less than that were regulated by Codex Alimentarius Commission (CAC) for oxytetracycline maximal residual levels (100, 300, 600 and 100 µg/Kg muscle, liver, kidney or fat, respectively). The authors recommended avoiding irrational use of oxytetracycline in veterinary practice and camel in particular; and sticking to the withdrawal time regulated and labelled for drugs used in therapy among veterinary personnel, organizations, and governmental agencies in Libya.

Keywords: Drug residues, oxytetracycline, camel, Tripoli.

1. INTRODUCTION

Many drug classes are used in animals for prophylaxis, therapy and performance enhancement. This use tends to increase where farm management is not optimum or when endemic diseases are not properly controlled. Several guidelines are available for appropriate use of antimicrobial drugs in animals, but very little is being done in our developing countries [1].

Among food producing animals that are domesticated in Libya is the camel. It belongs to the genus *Camelus* and characterized by bearing a distinctive fatty deposit known as hump on its back. There are two species of camels: the dromedary or Arabian camel has a single hump, and the Bactrian camel has two humps. Both species provide milk and meat, and are working animals. A camel carcass can provide a substantial amount of meat. The male dromedary carcass can weigh 400 kg or more. The brisket, ribs and loin are among the preferred parts, while the hump contains fat. It is reported that camel meat tastes like coarse beef, but older camels can prove to be very tough and less flavourful. Camel meat is low in fat, and can thus taste dry. The meat, therefore, can be mixed with beef or lamb fat, to improve both the texture and taste. However, camel meat is considered as an important source for protein supply to Libyan population [2].

Oxytetracycline is approved and is commonly used in camel in Libya which may carry risk of presence of residues in blood and tissues of administered animals slaughtered carelessly without considering withdrawal times of the given drug.

Nowadays, drug residues are considered as one of the most serious problems in the world. The deleterious effects of the drug residues of antibacterials have been

considered as one of the principal research activities since 1962. In 1983, a group of experts at the FAO and WHO concluded that "illness due to contaminated food was perhaps the most widespread health problem in the contemporary world," and "an important cause of reduced economic productivity". In 1992, a conference on Environment and Development managed by UN declared that food was a major vehicle for the transmission of environmental contaminants, both chemical and biological, to human populations throughout the world, and urged authorities in all countries to take measures to prevent or minimize these threats. In 2000, the World Health Assembly, the supreme governing body of the WHO, adopted a resolution recognizing food safety as an essential public health function [3].

Unsupervised use of drugs especially antibacterials for farm animals result in unacceptable concentrations of these drugs in their carcasses after slaughter constituting public health hazards which are serious in some cases. Residues may have adverse direct effects on consumers, e.g., allergic reactions in hypersensitive individuals [4-5]; or indirect effects through induction of resistant strains of pathogenic bacteria [6].

Rather than allergy and bacterial resistance, other serious health hazards are attributed to antibacterial residues including mutagenesis, teratogenesis, organ dysfunction [7] and carcinogenesis [8].

Heavy responsibility is placed on veterinarians to ensure high quality and safe (residue free) edible animal foods to the public. Thus, studies have been adopted to evaluate the extent of presence of antibacterial residues in many countries worldwide especially in underdeveloped countries. However, there are no parallel studies adopted in Libya despite of its extreme medical importance in both veterinary and human fields.

Therefore, the present study was planned out to evaluate the residual levels of oxytetracycline in camel tissues that are heavily consumed in Tripoli area in my country Libya.

2. MATERIALS AND METHODS

2.1 Study area and time

This study was carried out during the time period 2012 - 2014 in Tripoli area, Libya. Samples were collected from different slaughter houses and meat shops located in Al-Hadba, Souq-Al-Jomaa, Hay Al-Andalus, Gorjy, Ed-Dereby, El-Fallah, Gergaresh, Al-Mansoura, Ad-Dahra and Mizran.

2.2 Feedback information

A questionnaire survey was conducted by personal interviews with the meat shops' owners, veterinarians and farmers in different districts in Tripoli. It was carried out to determine associations between the occurrence of antibiotic residues in camel edible tissues and various risk factors like management practices, treatment factors and residues prevention methods. In management practices of the meat shops' owners, the information collected were the sources of purchasing camels be slaughtered, use of any drugs for keeping animal live status. Information on treatment factors from field veterinarians included sources of antibiotics, type person who administered antibiotics to camels, route of antibiotic administration, record keeping. Regarding residue prevention methods, the information gathered were marking of treated camels and use of antibiotic test kit and knowledge of withdrawal periods of antibiotics.

2.3 Experimental design

Samples were collected randomly from the above mentioned districts in Tripoli area for the detection of residues of oxytetracycline. Samples were prepared for detection of residues of oxytetracycline by liquid chromatography – Mass spectrometry (LC-MS). Data were statistically analysed and incidence of residues was determined as a percentage. Acceptability or not of the positive samples was decided according to Codex Alimentarius Codex (CAC) [9].

2.4 Collection of samples

Forty random samples of slaughtered camel's tissues (10 of liver, 10 of muscle, 10 of kidney, and 10 of fat) were collected from different carcasses at different slaughter houses in Tripoli districts mentioned above. Each sample was put in a separate clean plastic bag, labelled and transferred to the laboratory and frozen at -50 °C till analysis.

2.5 Equipment

The following devices have been used in the present study:Homogenizer (PRO Homogenizer, Pro scientific, Oxford, USA), Vortex (Maxi mixII, Thermo scientific, Watham, MA, USA), Balance (Vibra HT, Intelligent Weighing Technology, CA, USA), Centrifuges (Pro-SepE, Centurion, West Sussex, UK), Elmasonic X-tra ultrasonic cleaner (Laval Lab Inc., Quebec, Canada), Filtration device (Puradisc TM 25NYL, Whatman, Kansas, USA), Rotary evaporator (Büchi, Switzerland) and SPE sorbent (Strata® SPE, Phenamenex, CA, USA).

2.6 Reagents and Chemicals

All reagents and chemicals were of analytical grade. Reference standard of the tested antibacterial drug has been purchased from Pharma Swede, 10th of Ramadan city, Egypt. The standard was of 99% purity or higher. It was used for calibration curve preparation (see below). Acetonitrile, methanol and other chemicals were supplied by Sigma, Saint Louis, USA. Ultrapure water was generated by a Milli-Q system (Millipore, Billerica, Massachusetts, USA).

2.7 Sample preparation

One gram of homogenized camel meat, fat, kidney or liver was extracted using 9 mL of a 2: 8 (v/v) mixture of water and acetonitrile (forming a total volume of 10 mL) and vortexed for 5 minutes in 50 mL

polypropylene centrifuge tube. The sample was then centrifuged at 5000 rpm for 5 minutes and the supernatant was decanted into a 15 mL tube containing 500 mg Strata® C18E SPE sorbent. The sample was shaken and again briefly vortexed for 30 seconds and centrifuged again for 1 minute. A 5 mL aliquot of the resulting supernatant was transferred to a graduated tube and the contents evaporated down to less than 1 mL. The sample was then brought up to 1 mL total volume with water, and filtered (0.45 µm PVDF; polyvinyl difluoride) prior to performing LC-MS analysis on the same day as the extracts were prepared [10].

2.8 Standard curve and calibration

Individual stock solution (1000 ng/mL *viz* 1000 ppb) was prepared in the solvent mixture (acetonitrile: water, 80: 20, v/v). Working diluted concentrations have been prepared for each antibiotic under investigation ranging between 0.5 and 150 ppb according to the expected residue levels.

2.9 LC-MS Analysis

The chromatographic system consisted of an Agilent 1100 series (Agilent Technologies Inc., CA, USA) including binary pump (G1312B), equipped with on-line solvent degasser (G1379B), Auto-sampler (G1367E), thermo-stated column (G1316A; kept at 40°C) and temperature module (Palo Alto, CA, USA). This chromatographic system was interfaced with an Applied Biosystems 4000 QTRAP® LC-MS system with Turbo V™ ion source. The whole system was controlled using Analyst® software version 1.4.1 [11]. The chromatographic conditions were as follows:

- Column: Kinetex 2.6 µm C18
- Dimensions: 50 x 2.1 mm
- Part No: 00B-4462-AN
- Mobile phase: A: 0.1 % Formic Acid in Water and B: 0.1 % Formic Acid in Methanol
- Injection volume: 10 µL
- Flow rate: 0.5 mL/min
- Temperature: 40 ºC
- Detector: Mass spectrometer (MS)

The MS determination was performed in electrospray (ESI) positive or negative mode combined with monitoring of the most abundant MS/MS (precursor → product) ion transitions (dwell time of 75 ms for each transition). The following MS conditions were used:

- Entrance potential: 10 V
- Ion spray voltage: 4500V
- Ion source temperature: 525 °C
- The curtain gas regulator was set at 40 psi with optimum setting (in the Analyst software).
- The nebulizer and collision gas regulators were set at 90 psi with optimum settings.

At the above mentioned settings, oxytetracycline was detected after retention time of 3.71 min.

2.10 Data management and statistical analysis

The obtained results were statistically analysed using SPSS software. The number of positive samples against the total number of examined samples was calculated as %. Among the positive samples, those which were found exceeding the MRL (maximum residual level) were considered significant. The overall mean ± STDEV of the detected residue values have been calculated for each type of samples (muscle, liver, kidney and fat).

3. RESULTS AND DISCUSSION

Camel meat is not universally eaten. However, camel is a good source of meat and milk in areas where the climate adversely affects other animals [12]. In the pastoral communities, camel meat is only eaten on special occasions. These include festive gatherings following the return of the herd from grazing [13]. However, in Libya, meat from camel, especially Youngers (GAOOD) is considered as an important source for protein supply to Libyan population.

A variety of antimicrobial agents have been administered to camelids (Camel, Llama and Alpaca). However, most have not been studied scientifically, and the attending veterinarian must assume the responsibility for extra-label drug use and potential adverse effects on the animal. As a general rule, antibiotics appear to have longer elimination half-lives in camelids than in domestic ruminants, potentially prolonging their therapeutic effect but also increasing their risk of toxicity. This may be due to a lower rate of urine production in camelids [14], which may increase half-life of antibiotics excreted primarily through the kidneys (e.g., penicillins, aminoglycosides). As another general rule, volume of distribution varies tremendously among individual camelids. Higher dosages are generally recommended to avoid subtherapeutic drug concentrations in some camelids. The unsupervised extra-label use of these drugs may carry the risk of presence of residues in blood and tissues of administered animals slaughtered carelessly without considering withdrawal times of the given drugs. The present study revealed the findings stated and discussed below.

3.1 Questionnaire information

Camels are imported from Sudan, and drugs are given by herd managers including, benzylpenicillin, oxytetracycline, tylosin, neomycin, sulphonamides, chloramophenicol, enrofloxacin, amoxicillin and ivermectin. Usually, because of unavailability of doses for camels, those of ruminants are applied without differentiation. Moreover, dosage “dose regimen” including frequency of dosing and period of medication are inaccurate and mostly performed in a haphazard manner. The aforementioned drugs are used for treatment of infections that occur accidentally to animals and/or for prophylaxis. Most interviewees said that there is no veterinary inspection procedures are being undertaken at the Libyan ports. Additionally, animals are being slaughtered directly without residual

check, where there were no routine tests are carried out at abattoirs before slaughter. Almost all farmers, breeders and some of Veterinarians are unaware and are not updated about the maximum residue levels (MRLs) and withdrawal times of the used drugs. Taking history of medication and calculating withdrawal times are almost neglected at slaughter houses.

3.2 Standard calibration curve

Standard calibration curve for Oxytetracycline is depicted in Figure 1; the curve was linear throughout the prepared concentrations.

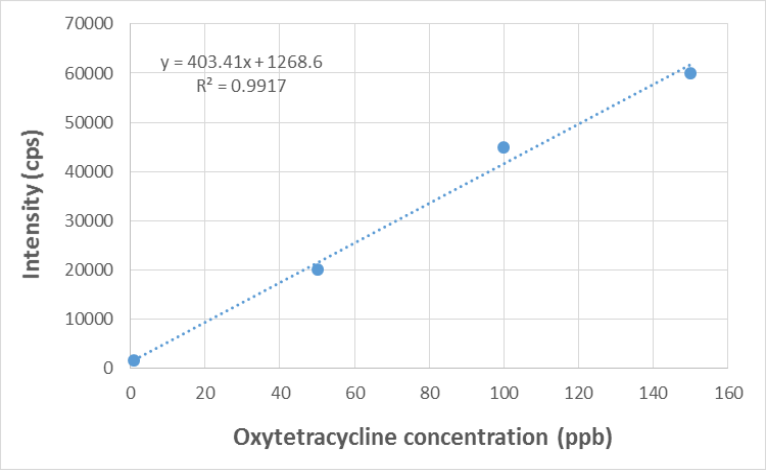


Figure 1: Standard calibration curve for Oxytetracycline.

3.3 Residual concentrations of Oxytetracycline

Results of the residual analysis of Oxytetracycline (ppb) in the examined camel samples (n= 40), including muscles (M), livers (L), Kidneys (K) and fat (F) were shown in table (1).

Table 1: Residual analysis of Oxytetracycline (ppb) in the examined camel samples.

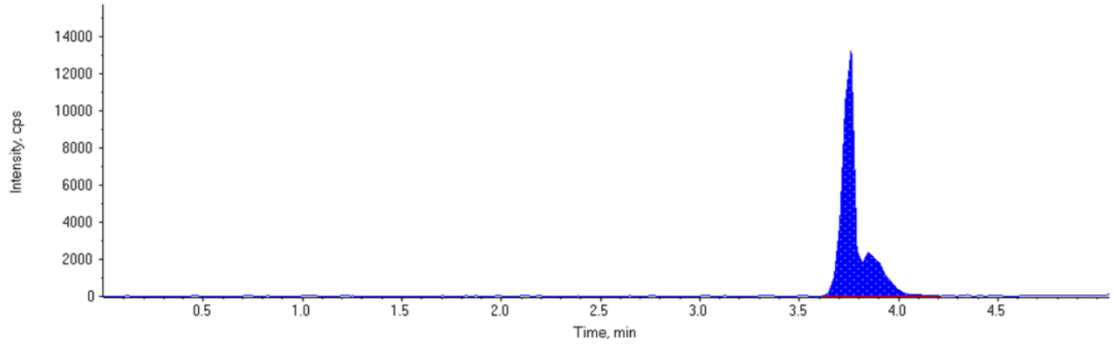
Sample #	Muscle	Liver	Kidney	Fat
1	0.563	0.797	35.70	7.600
2	0.375	0.070	0.141	3.560
3	0.281	0.070	0.094	0.844
4	0.211	0.375	0.422	0.211
5	0.610	0.141	0.141	0.188
6	13.20	0.188	0.141	4.500
7	ND	0.750	1.500	0.281
8	ND	1.24	0.188	ND
9	ND	ND	0.235	ND
10	ND	ND	ND	ND
Negative samples	4 (40%)	2 (20%)	1 (10%)	3 (30%)
Positive samples	6 (60%)	8 (80%)	9 (90%)	7 (70%)
Minimum value	0.211	0.797	0.094	0.188
Maximum value	13.2	1.240	35.70	7.600
Mean ± SD	2.540 ± 5.225	0.454 ± 0.430	4.285 ± 11.789	2.455 ± 2.869

ND: Not detected

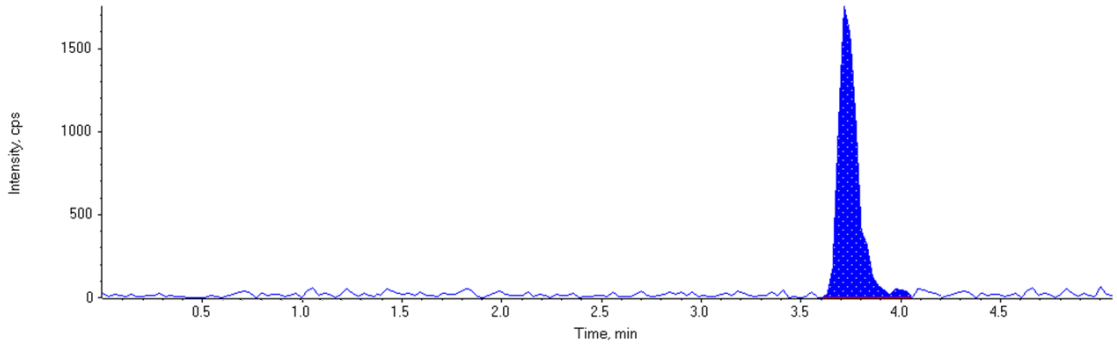
Table 2: Maximum residue levels (MRLs) of Oxytetracycline.

Tissue	MRL (ppb)*	Accepted samples		Unaccepted samples	
		No	%	No	%
Muscle	100	6	100	0	0
Liver	300	8	100	0	0
Kidney	600	9	100	0	0
Fat	100	7	100	0	0

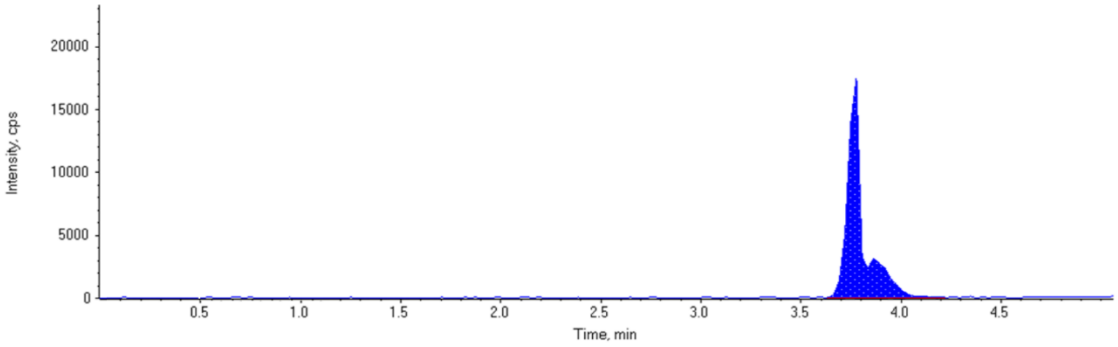
Table (2) shows acceptability of the examined camel tissue positive samples based on their maximum residue levels (MRLs) of Oxytetracycline according to Commission of the European Communities; Veterinary drug residues, 2nd Ed. 1994 [9]. Figure (1) represents LC-MS chromatograms of maximum residual amounts of oxytetracycline detected in camel muscle (A), liver (B), kidney (C) and fat (D) samples (n = 40).



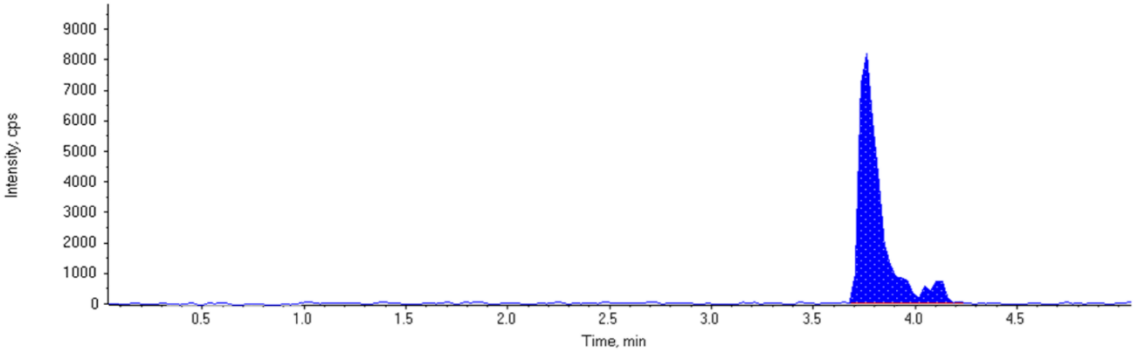
(A)



(B)



(C)



(D)

In the present study, oxytetracycline residues have been detected in 60% (muscle), 80% (liver), 90% (kidney) and 70% (fat) of examined samples. The samples, although positive, yet decided acceptable as the detected levels were less than those regulated by CAC for oxytetracycline maximal residual levels (100, 300, 600 and 100 µg/Kg muscle, liver, kidney or fat, respectively). The percentage of oxytetracycline incidence as residue in different tissues of slaughtered camels are much higher when compared with data reported by [15]; they found that oxytetracycline residues were detected at percentages of 4 and 6% in muscles and 21 and 12% in kidneys picked from cattle and buffaloes, respectively, in Assiut city, Egypt. A study conducted by [16] revealed presence of oxytetracycline residues in slaughtered camel liver and kidney samples at a percentage of 13.5% exceeding MRLs; the study was conducted in Riyadh region, KSA.

A study conducted by [17] to investigate the proportion of tetracycline residues in marketed pork in suburb and urban districts in Hanoi. A total of 290 raw muscle samples were randomly collected from open markets in these districts. The samples were qualitatively screened for tetracycline residues using the agar inhibition test, and *Bacillus cereus* (ATCC 11778) as the reference strain. The inconclusive samples were then analysed using HPLC. Overall, 5.5% of all collected samples were positive for tetracycline residues. Although the detected percent was much lower than detected in our study, yet the authors implied that it is a high proportion that may pose a potential hazard to public health, particularly since they might induce drug resistance of pathogenic micro-organisms. A study conducted by [18] to evaluate oxytetracycline in edible tissues of cattle revealed the presence of oxytetracycline as (kidney: 9.47 µg/kg ± 3.24 µg/kg; liver: 12.73 µg/kg ± 4.39 µg/kg; muscle: 16.17 µg/kg ± 5.52 µg/kg); however, the detected levels were lower than the decided MRLs there. Kim et al. (2013) [19] surveyed the presence of some commonly used antibiotics randomly collected pork (97 samples) and chicken (83 samples) meat from three different provinces (Hanoi, Hai Duong and Thai Binh) in Vietnam; the study revealed that 10 samples (all pork) were confirmed containing tetracyclines (chlortetracycline, oxytetracycline, tetracycline, doxycycline), from which 3 samples were found to contain tetracycline residues with a concentration higher than the respective MRLs. As mentioned earlier, the study conducted by [20] revealed that oxytetracycline was detected in food product samples obtained from different regions in Korea. Although, the residues were detected only in 6.5% of samples, the levels were below the MRL. Gratacós-Cubarsí et al., (2007) [21] evaluated the tetracycline and its degradation products 4-epitetracycline, anhydrotetracycline and 4-epianhydrotetracycline formation in thermally treated chicken breast, pig loin, and pig loin with added back-fat; they stated that even if the potential toxic effects of these breakdown compounds should be further investigated, their formation in cooked meat should be taken into account when MRLs are established.

4. CONCLUSION

It could be concluded that, oxytetracycline residues were detected at higher incidence rates that reached 90% in the kidney tissues in Tripoli, Libya. Although the detected levels were lower than the legislated MRLs, yet we imply that they are of unsafe drawbacks as they may pose a potential hazard to public health as they might induce drug resistance of pathogenic micro-organisms or teratogenic effects if the residue-contaminated camel tissues are heavily consumed by pregnants. Therefore we strongly recommend making Libyan individuals and organizations aware of the problem through education by veterinary personnel, organizations, and literatures and governmental agencies. Avoiding irrational use of antibiotics in field veterinary practices and sticking to the withdrawal times regulated and labelled for the drug. Additionally, developing and application of simple and economic residue identification field tests for routine and rapid screening procedures for the analysis of antibiotic residues and instant grading and prohibition of food containing residues even if their levels are lower than MRLs.

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