

## RESIDUAL ANALYSIS OF OXYTETRACYCLINE IN MARKETED PORK OF NORTH-EAST INDIA

D. C. Roy\*, A. Kafle, S. K. Laskar and R. K. Roy

Department of Pharmacology and Toxicology, College of Veterinary Science, A.A.U.,  
Khanapara, Guwahati-781022, Assam, India.

Article Received on  
29 October 2018,

Revised on 19 Nov. 2018,  
Accepted on 09 Dec. 2018

DOI: 10.20959/wjpr20191-13891

### \*Corresponding Author

**Dr. D. C. Roy**

Department of  
Pharmacology and  
Toxicology, College of  
Veterinary Science, A.A.U.,  
Khanapara, Guwahati-  
781022, Assam, India.

### ABSTRACT

The present study was undertaken to monitor the residue of oxytetracycline in pork samples marketed in various district of Assam, Nagaland, Manipur, and Mizoram. A total of 720 nos. of pork samples comprising of Liver, Kidney, and muscle, 60 each, were collected from the local vendors of the respective markets. The samples were collected in a sterile container and transported to the laboratory under deep freeze condition. Samples were analyzed using an Ultra-High-Performance Liquid Chromatography equipped with diode array detector. 57 samples (out of 180) were found to contain the residue of oxytetracycline, however, none of the samples showed residue above Maximum Residue Limit (MRL). Hence, it can be well inferred from the present study that there is no risk of public health hazard from

oxytetracycline in the selected states so far.

**KEYWORDS:** Residue, Oxytetracycline, Pork, HPLC, MRL.

### INTRODUCTION

Antibiotics are commonly used for the treatment of livestock as prophylactic as well as control measures. Antibiotics residues are the most common artificial inhibitory substances in product obtained from livestock and imparts negative impact on processing, quality of the product and ultimately to the human health.<sup>[1]</sup> Indiscriminate use of such antibiotics has been taking pace in developing countries particularly due to lack of complete information or due to self-medication. All drugs whether antibiotics or not do come with certain constraints and among those, the most important is their residue in the edible part (of livestock) meant for human consumption. Residues are parent compounds and/or metabolites of biological and

chemical substances routinely used in prescribed concentrations and in certain phases of animal production.<sup>[2]</sup> Food Safety and Standards Act, 2006 defines veterinary drug residues as “the parent compounds or their metabolites or both in any edible portion of any animal product and include residues of associated impurities of the veterinary drugs concerned.”<sup>[3]</sup> Owing to low cost and broad spectrum of activity tetracycline class of antimicrobial agents are one of the most commonly used antibiotics in livestock production.<sup>[4]</sup> Nevertheless, tetracycline group of drugs acts a miracle as adjunct to main therapy in hemoprotozoal infection as well. However, it is also true that tetracycline group of antimicrobial agents readily binds to divalent cations (specially calcium ions) on the body and able to deposit and persist in tissue for fairly long period of time.<sup>[5]</sup> The main concern of modern era is indiscriminate use of antibiotics to livestock without considering the fate of these administered antibiotics. The antibiotic residue when consumed along with meat for instance, leads to the problem of antibiotic resistance in human population. So, the present study was initiated with the aim to evaluate tissue from marketed pork samples for the presence of oxytetracycline residues and to estimate the level of oxytetracycline in liver, kidney and muscle of the pork sample using Ultra High-Performance Liquid Chromatography.

## **MATERIALS AND METHODS**

### **Chemical and reagents**

Oxytetracycline standard (sigma aldrich), HPLC grade Acetonitrile (Fisher), Methanol (Fisher) and HPLC grade water (Fisher) were used for the study.

### **Sample collection**

A total of 720 samples of pork were collected from local vendors of different districts of North-Eastern states for residue analysis. The samples were collected aseptically in a clean sterile container and were transported to the laboratory at -20°C till analysis.

### **Chromatographic conditions**

The levels of oxytetracycline were determined using a UHPLC system of Make: Dionex equipped with an autosampler and Diode Array Detector (DAD) operated at 350 nm. The samples were separated on an RP-C 18 column and were eluted with a mobile phase of a mixture of water and Acetonitrile in the ration of 67:33 v/v. The isocratic mode was run at a flow rate of 1.0 ml/min with Coefficient of Determination of STD curve 99.0%. Further the Limit of Detection (LOD) and Limit of Quantification (LOQ) were 0.005µg/g and 0.008µg/g respectively. The repeatability percentage was 88.0-98.0.

### Preparation of samples

The fascia and fat of pork samples were removed and then cut into small pieces. 10 g of each sample was taken in a blender and to it added an equal volume of distilled water. 10 g of each blended sample was transferred to a centrifuge tube. After a few minutes 10 ml of acetonitrile was added. The sample was ultrasonicated and left undisturbed for 10 min. The samples were centrifuged and the collected supernatant was filtered. The filtrate was then passed through C18 polymeric cartridge after which it was further filtered using 0.22 µm filter paper.

### RESULT

The level of Oxytetracycline residue in the states of Assam, Nagaland, Manipur and Mizoram has been presented in Table no 1, Table no 2, Table no 3 and Table no 4, respectively.

**Table 1:**

Region: Assam							
Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL /MPL Ug/g As per Codex Alimentarius
Oxytetracycline	Kidney	60	8	0.068-1.018	ND	ND	1.2
	Liver	60	6	0.014-0.047	ND	ND	0.6
	Muscle	60	5	0.017-0.144	ND	ND	0.2

**Table 2:**

Region: Nagaland							
Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL/MPL Ug/g As per Codex Alimentarius
Oxytetracycline	Kidney	60	6	0.483-1.016	ND	ND	1.2
	Liver	60	5	0.121-0.394	ND	ND	0.6
	Muscle	60	5	0.064-0.104	ND	ND	0.2

**Table 3:**

Region: Manipur							
Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL/MPL Ug/g As per Codex Alimentarius
Oxytetracycline	Kidney	60	5	0.273-1.009	ND	ND	1.2
	Liver	60	3	0.008-0.289	ND	ND	0.6
	Muscle	60	3	0.039-0.134	ND	ND	0.2

Table 4:

Region: Mizoram							
Name of the residue/toxic metal analyzed	Sample	No. of samples analyzed	No. of + samples	Range conc. (ug/g)	No. of + samples above MRL/MPL	Mean conc. of samples which are above MRL/MPL	Approved MRL/MPL Ug/g As per Codex Alimentarius
Oxytetracycline	Kidney	60	4	0.275-1.009	ND	ND	1.2
	Liver	60	4	0.008-0.300	ND	ND	0.6
	Muscle	60	3	0.040-0.140	ND	ND	0.2

## DISCUSSION

The highest level of oxytetracycline was found in the kidney as the main organ for the elimination of oxytetracycline is the kidney. Tetracycline undergoes glomerular secretion.<sup>[6&7]</sup> and this is the basis of using oxytetracycline for treating urinary tract infection,<sup>[8]</sup> in contrast to its counterpart, doxycycline which is unique in its elimination pathway (bile). Kidney receives the metabolites of drug from liver as the latter serves a gateway for metabolism of almost all xenobiotics. Hence, the levels of residues were also detected in liver. Both enteral and parenteral route has been used for administering oxytetracycline to livestock. Presence of food in stomach reduces absorption of oxytetracycline to 50% while cations like calcium on muscle (serve as injection site) binds with the tetracycline and the drug-cation complex owing to their larger size unable to diffuse out easily.<sup>[9&10]</sup> The complex has a tendency to remain at the injection site for considerable period which possibly could be the reason for the presence of oxytetracycline residue in muscle in the present study. The present author also reported similar findings while studying the residue of tetracycline in pork of Assam.<sup>[11]</sup>

## CONCLUSION

From the present study it can be concluded that tetracycline residues although present in pork samples in all the four states screened, the levels were below the permissible limits. The findings possibly reflect a strict implementation of withdrawal period before slaughtering of the animals (28 days for oxytetracycline). To safeguard public health the same should be strictly followed and moreover the presence of residue of drug meant for treating livestock into the food chain, should not be underestimated.

## ACKNOWLEDGEMENT

The authors are grateful to ICAR and AAU for their financial help and technical supports.

**CONFLICT OF INTERST**

None.

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