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PREVALENCE OF ENROFLOXACIN AND ITS PRIMARY METABOLITE CIPROFLOXACIN RESIDUES IN BROILER MEAT AND ORGAN SAMPLES OF FIELD ORIGIN

V. Sureshkumar[1] and Ghadevaru Sarathchandra[2]
[1]Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tirunelveli – 627 358.
[2]Pharmacovigilance Laboratory for Animal Feed and Food Safety, Directorate of Centre for Animal Health Studies, TANUVAS, MMC, Chennai – 600 051.
Corresponding author email: gsarathchandra@rediffmail.com

ABSTRACT
The administration of fluoroquinolones to food animals without an adequate withdrawal time may lead to violative concentrations of residues in foods destined for human consumption. To ensure control over the presence of antibiotic residues in food stuffs of animal origin, European Union and Japan have set maximum residue limits for antibiotic residues in edible animal tissues. Since 2005 the U.S. Food and Drug Administration has banned the use of enrofloxacin in poultry/ food animals. In view of this, the prevalence of enrofloxacin and its primary metabolite ciprofloxacin residues in broiler meat and organ samples of field origin was explored in the present study. Liver (18.18%), kidney (16.67%) and skin (13.33%) samples had enrofloxacin residues higher than the MRLs and found to be violating the regulations of Japan. Indeed, all muscle samples were found to be safe as per EU and Japan MRLs.

KEY WORDS: Enrofloxacin residues, broiler tissues, field samples, HPTLC.

INTRODUCTION

The widespread use of fluoroquinolones as therapeutic and prophylactic agents, in food animals particularly in intensive poultry production, has become a matter of considerable concern in recent years due to the identification of resistant *Campylobacter spp*., *Escherichia coli* and *Salmonella serovars* in meat and possible transfer to humans via the food chain (Petrovic *et al*., 2005). This resistance has led to lowered therapeutic efficacy of these compounds in human infections, suggestive of direct impact on public health (EMEA, 2006). Enrofloxacin, a fluoroquinolone developed exclusively for veterinary use is advocated in poultry in large-scale for treatment of chronic respiratory disease, colibacillosis, salmonellosis and fowl cholera. After administration, enrofloxacin is metabolised in the liver via de-ethylation into pharmacologically active metabolite ciprofloxacin. Ciprofloxacin is one of the most commonly advocated antimicrobial in human medicine worldwide. The principal risk is associated with the drugs used both in human and veterinary medicine and with those that produce similar metabolites.

In cognizance to this, since 2005 the U.S. Food and Drug Administration (FDA) have banned the use of enrofloxacin in poultry /food animals (U.S. FDA, 2005). European Union (EU) has set the MRLs for sum of enrofloxacin and ciprofloxacin residues as 100µg/Kg in muscle, skin and fat, 200µg/Kg in liver and 300µg/Kg in kidney (EMEA, 2002). Whereas, Japan has established much lower MRLs (10µg/Kg) in chicken muscle, liver and kidney (Ministry of Health and Welfare, Japan, 2005). In view of this, the prevalence of enrofloxacin and its primary metabolite ciprofloxacin residues in broiler meat and organ samples of field origin was explored in the present study.

MATERIALS & METHODS

Sampling protocol

A total of 180 numbers of broiler chicken edible tissue samples (liver, kidney, breast muscle, thigh muscle and skin each 36 numbers) were randomly purchased from various retail outlets in Chennai, India. Sampling was carried out as per the guidelines prescribed by Codex Alimentarius (FAO/WHO, 1995).

Sample Preparation and HPTLC Quantification

Liver, kidney, muscle and skin samples were extracted as described by Verdon *et al*. (2005) and subjected to enrofloxacin and ciprofloxacin quantification by validated High Performance Thin Layer Chromatography (HPTLC) - Fluorescent Densitometry.

RESULTS & DISCUSSION

The results of the present study revealed that 30.56% of the samples investigated were found to contain detectable levels of enrofloxacin residues at the time of marketing. The incidence of enrofloxacin residues were highest in liver (61.11%) followed by kidney (50.00%) and skin (41.67%). Whereas, the residues of enrofloxacin were not detected in any of the breast and thigh muscle samples investigated in the present study (Table-1). The incidence and decreasing order of abundance of enrofloxacin and ciprofloxacin residues as found in broiler tissue wise was as follows; liver>kidney>skin. However, the residues of enrofloxacin were not detected in any of the breast and thigh muscle samples investigated. These observations are in accordance with Naeem *et al*. (2006), who reported that

the amount of residual quinolones in liver and kidney were more than those in muscle and egg samples randomly purchased from super and local markets in Lahore, Pakistan. Further, Prescott *et al*. (2000) substantiated that enrofloxacin was distributed widely into body tissues and found in high concentration in the excretory organs especially the liver and in the bile.

TABLE 1. Incidence of enrofloxacin and ciprofloxacin residues in broiler tissue samples procured from various retail outlets in Chennai

Tissue sample	Number of samples analysed	Number (%) of samples found positive			Number (%) of violative samples	
		Enrofloxacin	Ciprofloxacin	Enrofloxacin + Ciprofloxacin	As per EU MRL	As per Japan MRL
Liver	36	22 (61.11)	13 (36.11)	22 (61.11)	0	4 (18.18)
Kidney	36	18 (50.00)	9 (25.00)	18 (50.00)	0	3 (16.67)
Skin	36	15 (41.67)	7 (19.44)	15 (41.67)	0	2 (13.33)
Muscle-Breast	36	0 (0)	0 (0)	0 (0)	0	0
Muscle-Thigh	36	0 (0)	0 (0)	0 (0)	0	0

TABLE 2. Enrofloxacin and ciprofloxacin residue levels (µg/Kg) found in broiler tissue samples procured from various retail outlets in Chennai, (Mean ±SE)

Tissue sample	Enrofloxacin	Ciprofloxacin	Enrofloxacin + Ciprofloxacin
Liver	6.53 ±0.45	5.77 ±0.22	9.93 ±0.73
Kidney	6.46 ±0.48	5.98 ±0.32	9.45 ±0.78
Skin	6.32 ±0.43	5.57 ±0.17	8.92 ±0.66
Muscle-Breast	ND	ND	ND
Muscle-Thigh	ND	ND	ND

ND – Not detected
Limit of detection – 2 ng/band for enrofloxacin; 3 ng/band for ciprofloxacin
Limit of quantification – 5 ng/band for both compounds
Analysis carried out in triplicate

Mean concentrations of enrofloxacin residues observed in liver, kidney and skin samples were 9.93 ±0.73, 9.45 ±0.78 and 8.92 ±0.66 µg/Kg respectively (Table-2). Similarly, Salehzadeh *et al*. (2007) also reported mean concentrations of enrofloxacin residues in muscle, liver and kidney samples collected from Tehran slaughter houses in Iran as 18.32 ±32.29, 18.34 ±12.36 and 26.06 ±19.52 µg/Kg respectively. Even though the mean concentrations of enrofloxacin residues were found to be below the MRLs prescribed by European Union and Japan regulations, the results of the present study indicated that 4 liver (18.18%), 3 kidney (16.67%) and 2 skin (13.33%) samples had enrofloxacin residues higher than the MRLs and found to be violating the regulations of Japan. Indeed, all muscle samples investigated were found to be safe as per European Union and Japan MRLs. These results confirm that the enrofloxacin was extensively used in broiler chicken and suggest that the recommended withdrawal time was not strictly adhered. In veterinary field mainly poultry and swine receive the majority of antibiotics for therapy. They are also given prophylactically to entire groups of undiseased animals, typically during periods of high risk for future infection such as after weaning or transport (Ungemach, 2000). Because of lack of awareness farmers buy antimicrobial drugs from veterinary pharmacy shops and treat the chicken themselves. When such drugs are administered by non-professionals correct dosages and withdrawal period are unlikely to be adhered for products like meat (Nonga *et al*., 2009). Kishida (2007) also added that the residues are mainly found in chicken meat due to the over dosage in the farm and slaughtering the chicken before withdrawal period.

CONCLUSION

The mean concentrations of total enrofloxacin residues were found to be below the MRLs prescribed by European Union and Japan regulations; however 18.18% liver, 16.67% kidney and 13.33% skin samples had enrofloxacin residues higher than the MRLs and found to be violating the regulations of Japan. Indeed, all muscle samples were found to be safe as per EU and Japan MRLs. The present study has reflected the general lack of implementation of recommended withdrawal times.

RECOMMENDATION

This study therefore stresses the need for adhering the withdrawal period as prescribed by various regulatory bodies (FDA, EU and Japan) and the need for stringent regulation for the use of antimicrobial drugs in the poultry industry as well as the inspection of chicken for antimicrobial residues prior to marketing in India.

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