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Quantification of enrofloxacin residues in broiler chicken tissues using competitive enzyme-linked immunosorbent assay

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In developing countries, poultry farmers routinely use antimicrobial drugs to prevent diseases and mortality in their flocks. Moreover, their use as growth promoter is also common. As studies claim, 5% increase in weight gain was seen in broiler birds after introduction of sub-therapeutic dose (Silfrany*et al.* 2013). However, antibiotic dosing and their irrational use in poultry production without observing appropriate withdrawal period results in their accumulation in body tissues in the form of residues. Consumption of meat with antibiotic residues could pose serious threats to consumer's health, like allergic reactions, gastrointestinal disorders and potential to develop antibiotic resistant bacteria. Furthermore, residues in animal derived foods have economic importance as well, because their levels above permissible limit constrain export (Sattar *et al.* 2014). Residues of enrofloxacin in edible tissues remain stable while cooking and their levels above permissible limit may affect the consumers' health (Fernández *et al.* 2014). From consumer's health perspective in Pakistan, it is essential to quantify and monitor antibiotic residues in poultry meat. Therefore, aim of this research work was to quantify the incidence of enrofloxacin in broiler liver and meat tissues collected from markets of Jhang, Punjab, Pakistan.

Broiler tissues from 90 samples consisting of 45 breast muscle, 45 pools of livers were gathered from chicken retail shops in Jhang city. Samples of liver and breast muscle were randomly selected from different chicken carcasses. A total of 50 g of every sample was collected in a sterile polyethylene bag and stored at –18°C till further analyses. Tissues were sliced to small fine fragments and defrosted at room temperature for homogenization. Extraction protocol was followed as described by the Max- Signal-Enrofloxacin ELISA Test Kit manual. For the quantification of enrofloxacin residues in samples, commercially available competitive ELISA kit (Max- Signal-Enrofloxacin ELISA Test Kit, Bioo Scientific, USA) was used. The detection limit of kit for meat and liver samples was 1 ng/g, with 100% enrofloxacin specificity. Competitative ELISA was performed according to the manufacturer instructions. ELISA plate reader (ELX50, Bio-Tek Instruments, USA) was used to measure the absorbance at 450 nm. The data were analyzed statistically using IBM SPSS® Statistics 20. Mann-Whitney U test was performed to compare the antibiotic residue concentration in breast muscle and liver tissues.

Results of analyses revealed that from 90 samples, including 45 liver tissues and 45 breast tissues of different broiler birds collected from local shops of Jhang city, 40 (88.8%) and 32 (71.1%) contained enrofloxacin residues, respectively. The median concentration of enrofloxacin residues observed in muscle and liver tissues was 103 ng/kg (inter quartile range [IQR] = 1934), and 1409 ng/kg (IQR = 1824), respectively. Quantification of antibiotic residues distribution in chickens' tissues revealed that the difference between breast muscle and liver tissue was non-significant ($P>0.05$). The median concentration of enrofloxacin residues observed in various tissues when compared with maximum residual limits set by the European Union (200 µg/kg for liver tissue and 100 µg/kg for muscles) revealed that 62% of the liver tissue and 53% of the breast meat contained residues above permissible level (Fig. 1). Possible reasons for occurrence of abnormal level of enrofloxacin appear to be overuse of the antibiotic in broiler production, both for prophylactic and therapeutic purposes, not observing the withdrawal period, malpractice of selling diseased and antibiotic treated birds into the markets, non-existence of restrictive legislation or their inadequate enforcement. Also sub-chronic exposure of aflatoxin B1 prominently affects the level of enrofloxacin and ciprofloxacin residues in tissues of broiler birds (Kalpana *et al.* 2012).

In accordance with our results, Naeem and Rafiq (2006) quantified enrofloxacin residue in poultry products in samples taken from local markets in Lahore, Pakistan. It was found that 55 to 92% of the samples contained enrofloxacin residues and 58 to 85% contained

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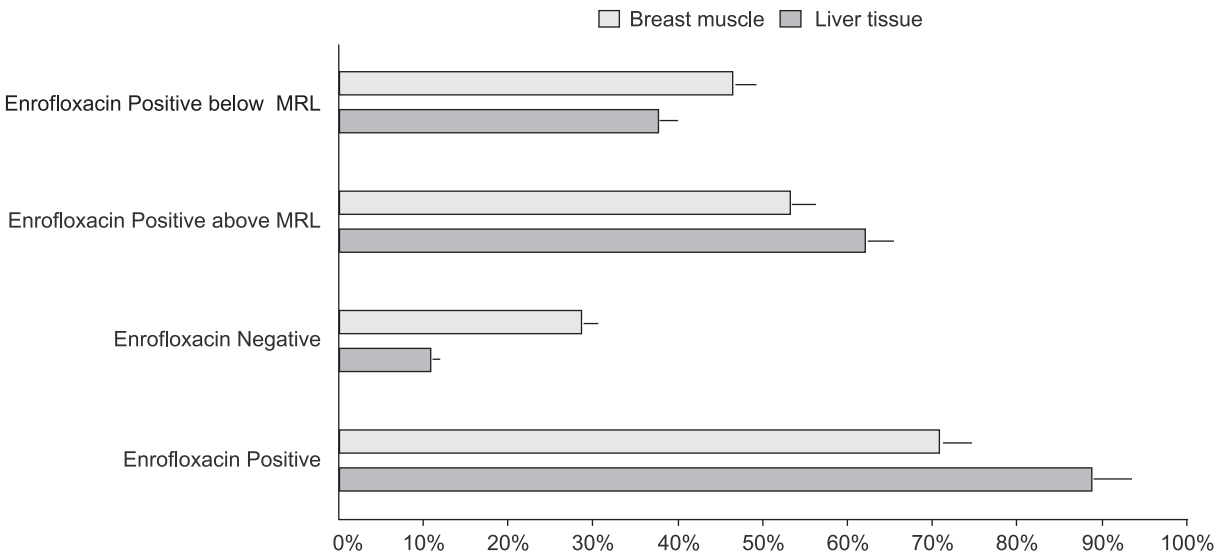

Fig. 1. Results of enrofloxacin residues screening in chicken tissues collected from different local meat shops of Jhang city Pakistan.

ciprofloxacin residues, thus violating the regulations. Similarly, Tavakoli *et al.* (2015) in Tehran established that about 88% of poultry samples were positive for enrofloxacin residues. In another study (Attari *et al.* 2014) carried out in Iran, ELISA was used to investigate residues of enrofloxacin in various tissues of broiler birds, gathered from Tabriz city markets. It was found that 91.1% of the samples contained antibiotic residues. The proportion of samples containing abnormal concentration of residues in Iranian studies (Tavakoli *et al.* 2015, Attari *et al.* 2014) was relatively higher than current study. This may be attributed to different in usage of antibiotics and time zone in which studies are carried out. In an investigation (Sultan 2014) conducted in Mosul, Iraq, it was reported approximately 56.66% of the poultry liver samples were positive for enrofloxacin residues that exceeded the MRLs set for poultry meat. In another field study in Chittagong, Bangladesh, Sattar *et al.* (2014) measured the tetracycline, amoxicillin, ciprofloxacin, and enrofloxacin residues in broiler and layer meat. Results have shown that enrofloxacin residues were higher in liver (40%) followed by kidneys (34%), thigh muscles (22%) and breast muscles (18%) samples. Similarly in a research (Er*et al.* 2013) carried out in Turkey, 45.7% of meat samples of chicken were affirmative for quinolones residues. In 100 randomly collected samples from El-Sharkia, Egypt market, about 66.7% samples of fresh and frozen broiler fillet contained antibiotic residues above MRL (Hussein and Khalil 2013) which may be attributed to misuse of antibiotics and violation of withdrawal period. Our findings that concentration of residues in liver and muscle tissues do not differ statistically is consistent with results of other studies (Naeem and Rafiq 2006, Sultan 2014). Assessment of enrofloxacin residues in present study was done by ELISA kit based on competitive enzyme-linked immunosorbent assay. Different studies (Panzenhagen *et al.* 2016, Gouvêa *et al.* 2014) confirmed the efficacy of the ELISA test, and advocate its use as antimicrobial residues screening method for poultry tissues due to its sensitivity, low price and ease of applicability. Given cross-sectional nature of the study, we could not capture temporal changes in residues. This information should be considered while using and interpreting the findings of the research. The findings of this study have provided evidence that broiler chicken meat being sold in open markets of Jhang city contained abnormal level of enrofloxacin residue thus exposing the consumers to its deleterious effects. Based on our findings, it is recommended that poultry farmers and veterinarians make rational use of enrofloxacin in broiler production and observe recommended withdrawal period. Moreover, MRL should be set and enforced for enrofloxacin and other antibiotics of public health importance. In future, further studies are suggested to evaluate other antibiotic residues and their risk in relation to food animals.

SUMMARY

Irrational use of antimicrobial agents' results in their accumulation in poultry eggs, meat and other byproducts. Less is known regarding antimicrobial residues in broiler meat, specifically for Jhang, Punjab, Pakistan. Thus, the main purpose of this research was to evaluate the prevalence of enrofloxacin residues in broiler chickens tissue. A total of 90 samples of chickens' tissue were collected randomly from different local meat shops of Jhang city and were analyzed using competitive colorimetric enzyme-linked immunosorbent assay kit. Out of 90 samples analyzed, 53% of the breast muscle and 62% of the liver tissues samples contained residues of enrofloxacin higher than maximum residue limits. Median concentration of enrofloxacin residues in breast muscle and liver tissue was 103 ng/g and 1409 ng/g respectively. Quantification of antibiotic residues distribution in chickens' tissues revealed that the difference between breast muscle and liver tissue was non-significant. Results of study revealed that broiler chickens being sold in Jhang city contained the enrofloxacin residues and may

exposethe consumers to their deleterious effects.

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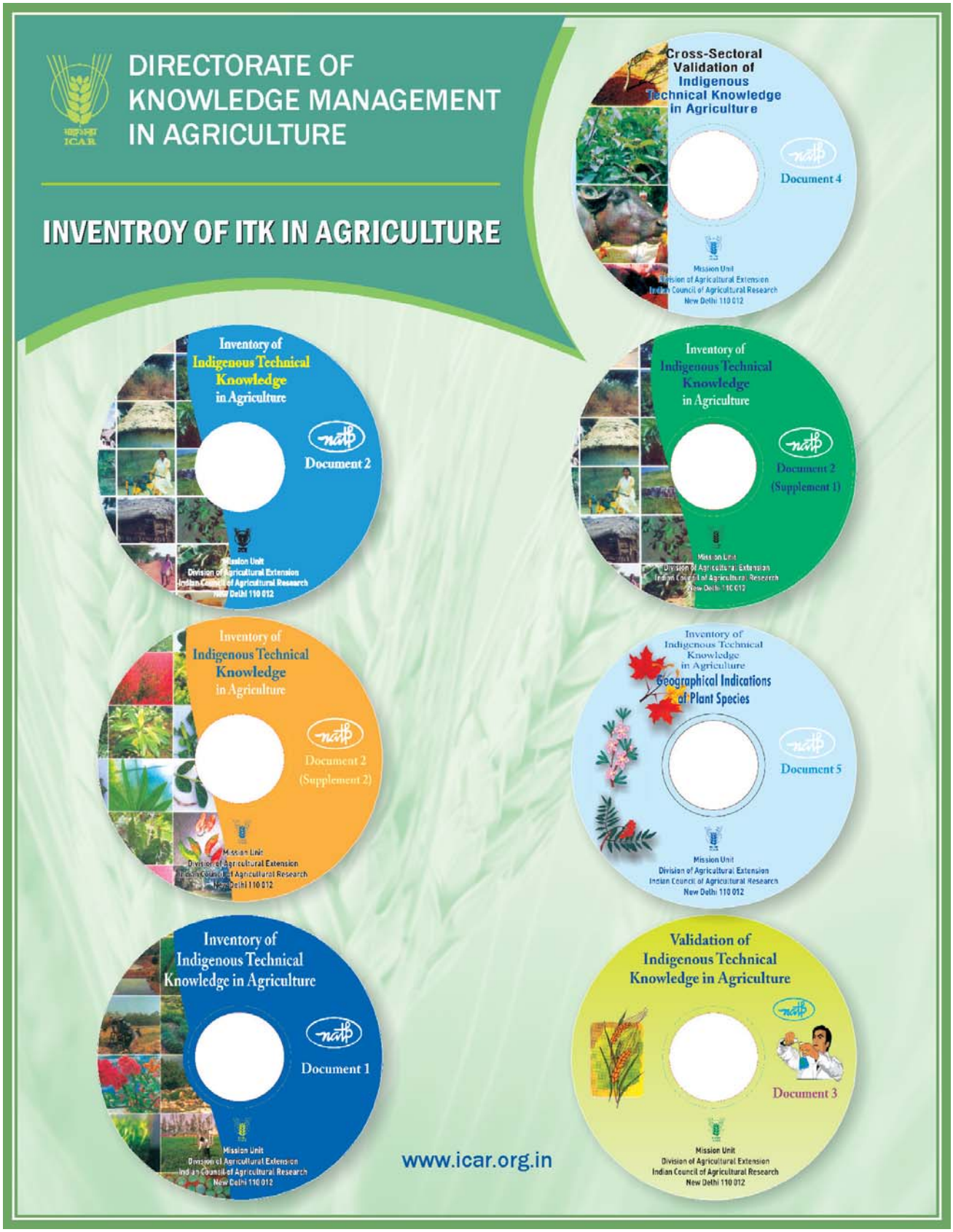
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