VETERINARY DRUG RESIDUES IN CHICKEN, PORK AND BEEF IN PENINSULAR MALAYSIA IN THE PERIOD 2010-2016

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ABSTRACT. Monitoring of veterinary drug residues in chicken, swine and cattle was conducted in Peninsular Malaysia from 2010 to 2016. Tissue samples were collected from slaughterhouses and processing plants. A total of six groups of veterinary drugs were analysed in 8,708 samples using bioassay or immunoassay and LCMS method. The average violation rate was 3.4%, 2.5%, 1.9%, 0.8%, 1.6% and 2.7% for year 2010, 2011 and 2012, 2013, 2014, 2015 and 2016, respectively.

Keywords: veterinary drug residues, food of animal origin, monitoring program

INTRODUCTION

Veterinary drugs are a group of substances belonging to different chemical classes and therapeutic areas, e.g. antibiotics, antiparasitics, non-steroidal anti-inflammatory drugs (NSAIDs), hormones and β-agonists. They are generally used to prevent or cure disease, to reduce potential for disease or as growth promoter to increase feed conversion (Dugane J.Q., 2000). They can be administered in feed, drinking water or by injection (Reig M and Toldrá F, 2008). If veterinary drugs are not used correctly, this could lead to the presence of veterinary drug residues. Ingesting residues of drugs or their metabolites in meat and other foods of animal origin may cause adverse effects which include; genotoxic, immunotoxic, carcinogenic or endocrine effects, constituting an important consumer's health risk (Croubels S. *et al.,* 2004). Antibiotic residues in food are suspected to be responsible for drug allergy (Al-Ghamdi *et al.,* 2000) and vehicle for evolution and dissemination of AMR (Bogiali and Corcia 2009).

The Department of Veterinary Services (DVS), Ministry of Agriculture and Agro-based Industry Malaysia as part of its mandate, monitor the presence of veterinary drug residues through the National Residue Programme. This programme aims for prevention of residue occurrence on the farm to ensure foods of animal origins are safe for human consumption. The purpose of the programme is to verify that the management of farm animal complies with Malaysian Good Agricultural Practices (MyGAP) and Veterinary Health Mark (VHM) for animal products.

Antibiotics that have maximum residue limits (MRLs); sulphonamides, tetracyclines, quinolones, macrolides, β-lactam and aminoglycoside, were selected for the residue monitoring due to the high frequency of violations reported worldwide, especially for sulphonamides, which record the highest violation rate in the USA (Dey B.P. *et al.,* 2003) and public health considerations

(Bates J. *et al.,* 1994; Boisseau J., 1993; Heitzman R.J., 1993). Some quinolones are increasingly involved in antibiotic resistance phenomena, characterising both animal and human isolates (Engberg J. *et al.,* 2001). Nitrofuran, β-agonist and chloramphenicol were monitored to investigate their usage in farm animal although this drug was banned from use in Malaysia since 1998 (Malaysia Food Act 1983 (Act 281) & Regulations).

The results of a study by Sakai *et al.* (2016) showed that 10 types of β-agonists were detected in cattle, chicken and swine liver specimens purchased at wet markets in Kuala Lumpur and Selangor state. Another study by Malintan and Mohd (2006) reported sulfonamides in effluents from swine farms. Thus, monitoring of residues in food meant for human consumption is essential.

The aim of this study is to present the findings from the Malaysian Residues Programme in locally produced chicken, duck, pork and beef from 2010 to 2016. The findings are useful to assess compliance for veterinary drug residues as stated in Malaysian Food Regulation with the goal of providing safer food supplies to the public and evaluating the effectiveness of the monitoring program.

MATERIALS AND METHOD

Sample Collection

The targeted sampling and sample collection by the DVS meat inspector covers 3 species; poultry, swine and bovine. The samples were taken at the slaughterhouses of swine and bovine, and certified poultry processing plants under the Veterinary Health Mark (VHM). The number of samples taken, adapted from Council Directive 96/23/EC (EU Commission, 1996), was between 0.03% and 0.15% of the production depending on the animal species. A total of 8,708 samples were collected over six years.

The monitoring programme was conducted in four zones comprising eleven states of Peninsular Malaysia: Perlis, Perak and Penang in northern zone, Kuala Lumpur, Selangor, Negeri Sembilan and Malacca in the central zone, Terengganu, Kelantan and Pahang in the eastern zone and Johor Bahru in the southern zone.

Chemicals and Reagents

Analytical standards were purchased from Sigma Aldrich (St. Louis, MO, USA), Witega Laboratorien, Berlin and Dr. Ehrenstorfer Gmbh, Germany. Analytical grade solvents were used for sample extraction and clean-up, while gradient grade solvents were used for residue re-dissolvation and mobile phase during LC-MS/MS analysis. Both analytical grade solvent and gradient grade solvent were purchased from Merck Millipore (M) Sdn Bhd and Fisher Scientific (M) Sdn Bhd. The water was purified in a Milli-Q® Intergral (Millipore, USA). Solid-phase extraction (SPE) cartridge used were Oasis HLB (Waters Corporation, US). The antibiotic discs were purchased from MAST Diagnostics, UK. Bacteria used in microbiological screening test are *B. cereus*, *B. subtilis*, *E. coli* and *K. rhizophila* obtained from Microbiologics, USA.

Residue Analysis

All the samples were first analysed using the microbiological inhibition test, Six Plate Test as described previously (Myllyniemi *et al.* 2001) for detection of tetracyclines, sulphonamides and quinolones. For 3 banned drugs in Malaysia, chloramphenicol, nitrofuran and β-agonist, screening by ELISA was applied. These screening tests was used to rapidly detect the samples suspected to be non-compliant. Samples giving a positive response at the screening stage were further analysed by liquid chromatography coupled to mass spectrometry (LC-MS) for confirmation and quantification of the molecule(s). Table 1 describes the outlines of residue analysis for veterinary drugs.

The samples were analysed at the Veterinary Public Health Laboratory in Salak Tinggi. Quality controls of the analyses were performed, and the proficiency tests were conducted for samples obtained from FAPAS in the United Kingdom or local PT provider.

RESULTS AND DISCUSSION

A total of 8,708 of tissue samples were collected and analysed in each year. Figure 1 shows the overall results of total violation rate in chicken and duck, pork and beef in Peninsular Malaysia from 2010 to 2016. 180 samples were detected with at least one type of drug (2.1%). The percentage of violation rate ranged was 0.8% (year 2014) to 3.1% (year 2010).

Figure 2 shows violation rates of veterinary drugs in chicken and duck, pork and beef. Violation rate for chicken and duck was 2.6% in 2010, decreased 1% in the following year and increased 1.3% in 2012. The rate decreased in 2013 and 2014 and slightly in 2015. However, the violation rate was highest in 2016.

Violation rates in pork were very high in 2010 and 2011. After that, it was reduced tremendously for three consecutive years until 2014 where the rate was 1.6% reduced 7.5% compared to year 2010. However, it was

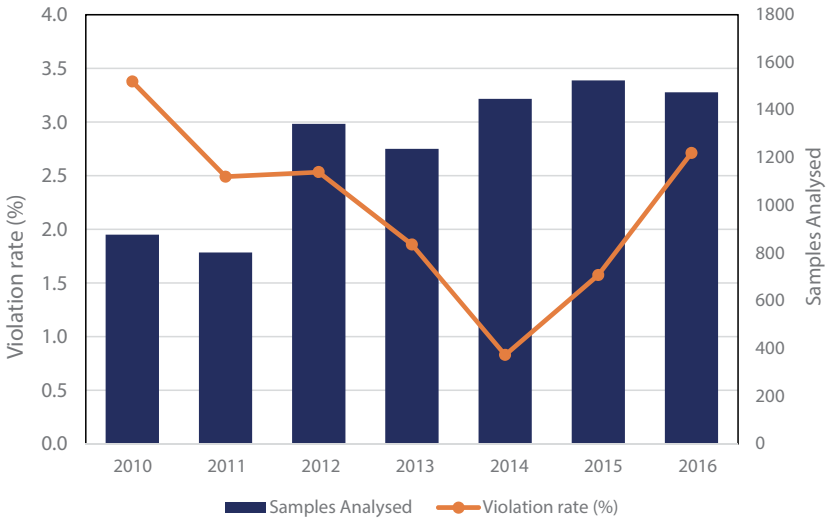

Figure 1. Total violation rates for veterinary drugs in chicken and duck, pork and beef from 2010 to 2016

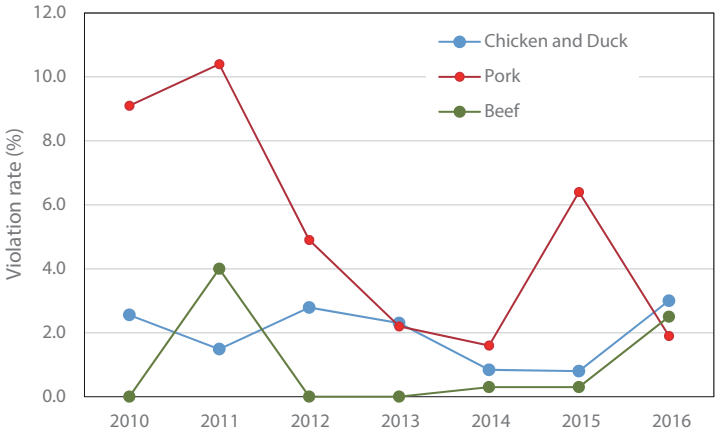

Figure 2. Violation rates of veterinary drugs in chicken and duck, pork and beef from 2010 - 2016

Table 1. Outlines of Veterinary Drug Residues Analysis for Confirmatory of Suspected Samples

Residue Class	Samples	Sample extraction & clean-up	Reference
Tetracyclines	Bovine, swine, poultry	(1) Add 7 ml McIlvaine/EDTA buffer to 2 g sample, mix and centrifuge (2) Mix supernatant with 1 ml 20% TCA and keep at -20°C for 15 min (3) Centrifuge for 15 min and filter (4) Clean up by Sep-Pak cartridge (5) Concentrate and reconstitute in 2% formic acid and 0.1mM oxalic acid. Filter through 0.2 µm PVDF	Oka H., Ito Y. and Matsumoto H. (2000), P02/13AN (Fougeres)
Sulphonamides	Bovine, swine, poultry	(1) Add 800 µl water to 2 g sample (2) Extract with 5 ml acetronitrile, mix and centrifuge (3) Evaporate supernatant to dryness (4) Residue reconstitution with 0.2% formic (5) Filter through a 0.2 µm PVDF	LMVUCM/P02-22, Fougeres
Quinolones	Bovine, swine, poultry	(1) Extract 1g sample with 10 ml Glycine/HCl (2) Centrifuge and filter supernatant (3) HLB SPE cartridge clean-up (4) Evaporate to dryness and reconstitute in 0.1% formic acid	Marni S. (2010)
Nitrofuran	Bovine, swine, poultry	(1) Add 1 ml water to 1 g sample (2) Clean extract with series of solvent extraction, methanol, ethanol and diethyl ether (3) Digestion with 1M HCl derivatize containing 2-nitrobenzaldehyde in acidic medium (4) Neutralize metabolites with 5 ml of 0.1 M K_2HPO_4 and 0.4 ml of 1 M NaOH (5) Extract metabolites with ethyl acetate (6) Evaporate to dryness and reconstitute with 50% methanol (6) Filter supernatant through a 0.2 µm PVDF	SOP BIO 221 V1
Chloramphenicol	Bovine, swine, poultry	(1) Add 800 µl water to 2 g sample (2) Extract with 6 ml ethyl acetate, mix and centrifuge (3) Evaporate supernatant to dryness (4) Reconstitute with 0.25 ml water and clean-up with 0.25 ml iso-octane (5) Filter aqueous phase with trough a 0.2 µm PVDF. For swine and bovine, deconjugation drug from tissue with β-glucuronidase and further clean-up with SPE is necessary	SOP CSD 301 v2, Technical Note (Fougeres)
β-agonist	Bovine, swine, poultry	(1) Extract 5 g sample with 20 ml phosphate buffer pH 5 and hydrolyse in acidic condition (2) De-conjugation overnight with β-glucuronidase (3) SPE clean-up with mixed mode SPE (4) Evaporate eluent to dryness (5) Reconstitute sample in ammonium formate and ACN (4) Filter through 0.2 µm PVDF	SOP CSD 306 v1

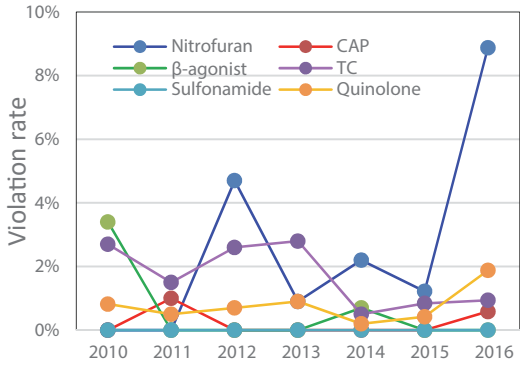

Figure 3. Violation rates of veterinary drugs in chicken and duck from 2010-2016

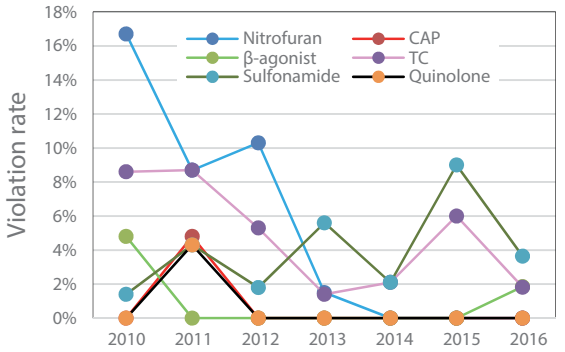

Figure 4. Violation rates of veterinary drugs in pork from 2010-2016

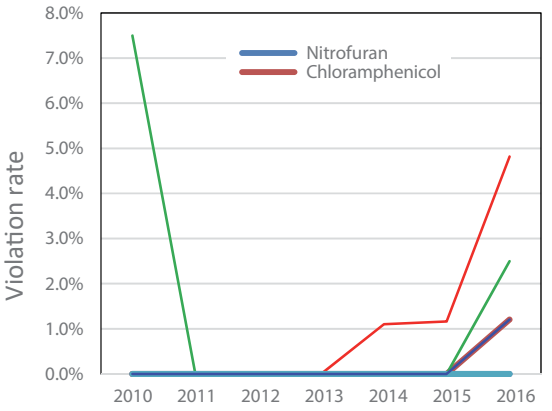

Figure 5. Violation rates of veterinary drugs in beef from 2010-2016

increased 4.8% in 2015 and reduced to 1.9% in 2016.

There was no incidence of veterinary drug residues in 2010, 2012 and 2013 for beef. In 2011, the violation rate was 4%, 0.3% in 2 years 2014 and 2015. However, its increased more than 2% in 2016.

The trend for violation in pork was decreasing except in 2015 even though pork had the highest violation rate in 2010 in comparison to chicken and duck and beef. The violation rate for beef showed increasing trend started from 2012 to 2016.

The use of veterinary drugs in developed countries from food samples is generally reported <1%. The proportion of non-compliant results in Europe between 1997 to 2013 for targeted samples was in the range of 0.25% to 0.34% and in 2014 was 0.37% from 425,232 targeted samples tested from 28 countries members of the European Union (EFSA 2013, 2014). According to European Food Safety Authority, EFSA for samples that originated from non-EU countries, the exceedance rates were 1.0% and 2.5% for 2014 and 2013, respectively (EFSA, 2013, 2014). Results from antibiotic residues monitoring program for 2008/2009 in Brazil showed only 0.03% of meat. Study by Rakotohyarinome *et al*, 2013 in Madagascar showed high incidence rate of drug residues where 37.2% of pork samples were contaminated with drug residues and were exceeded their legislation limit (Nonaka, et al, 2011).

The violation rates of veterinary drugs in Korea from different animal species from 2002 to 2011 were below 0.5% (Kim, MK *et*

al., 2013). Yamaguchi et al 2015 reported positive percentage for antibiotic residues monitoring program in Vietnam from 2012 to 2013 in chicken, pork and beef were 17.3, 8.8 and 7.4% respectively with an average of 11.9%. This study showed that the even though the violation rate in Malaysia for drug residues was higher than the developed countries, it was very much lowered in comparison to other developing countries.

Nevertheless, this monitoring study only focus on 6 groups of veterinary drugs and did not reflect the actual violation rate. With the use of more sensitive detection procedure and with the inclusion of all known veterinary drugs the detection of contaminated samples could be enhanced.

CONCLUSION

This study showed that there was increasing trend of violation rate in chicken/duck and beef samples containing veterinary drug residue. This is of concern because it may cause a potential hazard to public health and could contribute to increase the problem of drug resistance of pathogenic bacteria. Extension program should be enhanced to aware the farmers about the withdrawal period of the drugs as well as ill-effects of drug residues to human health because. Strengthen the inspection at the farm level including feedstuffs and processing establishment will help to decrease the violation rate. Drug residues issue should be shared responsibility of the government, industry, academia, veterinary. Further studies that provide information/evidence of link between inappropriate drug usage, drug residues and drug resistance in bacterial pathogen are needed to implement the appropriate control strategies professional, and animal producers.

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