DETERMINATION OF AMINOGLYCOSIDE, β-LACTAM, MACROLIDES, QUINOLONES, SULFONAMIDES, AND TETRACYCLINE (VETERINARY ANTIBIOTICS) IN BROILER CHICKEN MANURE AND LAND APPLIED MANURE

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INTRODUCTION

A variety of drugs and feed additives are approved for use in food-animal agriculture. There are four ways in which substances exhibiting antimicrobial activity are used in animals: therapy, metaphylaxis, prophylaxis, and growth promotion (Schwarz et al., 2001). Many antibiotics used in the animal food-producing industry are poorly adsorbed in the gut of the animal, resulting in as much as 30–90% of the parent compound being excreted. In addition, antibiotic metabolites can also be bioactive and can be transformed back to the parent compound after excretion (Sarmah et al., 2006).

It has been reported that in some cases, as much as 80% of the antibiotics administered orally to livestock, pass through the animal unchanged into bacteria-rich waste lagoons and is then spread on agricultural field as a source of fertilizer (USEPA, 2000). Thus, a significant percentage of the administered antibiotics may be excreted into the environment in active forms. After excretion, microbes can rapidly degrade the sugars, thereby allowing the compounds back to their bioactive forms. As most of the antibiotics are water-soluble, as much as 90% of one dose can be excreted in urine and up to 75% in animal feces (Sarmah et al., 2006).

The target analytes studied belonged to 6 different antibacterial groups: aminoglycoside (neomycin), β-lactam (amoxicillin), macrolides (erythromycin, tilmicosin and tylosin), quinolones (enrofloxacin, flumequine and norfloxacin), sulfonamides (sulfachloropyrazine, sulfadiazine and trimethoprim) and tetracycline (doxycycline). There is no registered veterinary medicine in National Pharmaceutical Control Bureau (BPFK) in Malaysia up to date. These 6 antibiotic classes were selected as target compounds in the present study due to their extensive use in veterinary medicine. These chemicals adminstered to farm animals largely end up in manure due to their low metabolization in the body (Kemper, 2008).

PROBLEM STATEMENT AND SIGNIFICANCE OF STUDY

Residues of antibiotics used for animal husbandry enter the environment either directly by spreading of manure or after collection and storage in form of sludge. Applied to farmlands the active ingredients reach the upper soil layer, where they either may accumulate or may be rinsed off into surface waters or may leach to groundwater where

they can impact both human and environmental health (Boxall et al., 2003). When manures are applied to land, there will be some movement of the pathogens through the soil matrix. The degree of mobility will affect the likelihood of pathogens reaching aquifers or surface waters. If these waters are subsequently used for irrigation of produce or for consumption by livestock, there are implications for food safety (Venglovsky et al., 2009).

Currently, there is no legislation in the European Union for limits of antibiotics in soils (Martı´nez-Carballo et al., 2007) and there is no registered veterinary medicine in National Pharmaceutical Control Bureau (BPFK) in Malaysia up to date. The present study is the first study on the simultaneous determination 6 different antibacterial groups: aminoglycoside, β-lactam, macrolides, quinolones, sulfonamides and tetracycline in chicken manure and land applied manure in the world.

RESEARCH OBJECTIVES

I. To determine the concentrations of aminoglycoside, β-lactam, macrolides, quinolones, sulfonamides, and tetracycline in broiler manure samples collected from different agricultural fields located in West Peninsular Malaysia with a single pre-treatment process.
II. To determine the presence of amino glycoside, β-lactam, macrolides, quinolones, sulfonamides, and tetracycline residues in manure-amended soil samples collected from different agricultural fields located in West Peninsular Malaysia.
III. To evaluate the efficiency of composting for reducing target veterinary antibiotics in broiler manure in a laboratory scale study.

LITERATURE REVIEW

The pathway of veterinary pharmaceuticals to waterway is different from human pharmaceuticals. While human pharmaceuticals discharge into the environment mainly through sewage treatment plants, veterinary pharmaceuticals could enter the environment not only through direct application in aquaculture and wash off from topical treatments; but also from livestock treatment plants. The runoff from manure-treated farmlands is also one of the major sources of veterinary pharmaceuticals to the environement. As such, veterinary medicines are considered as non-point source pollutants, and their environmental concentrations might be affected largely by precipitation. Once released into the environment, pharmaceuticals and their metabolites may run into surface waters or leach to groundwater where they amy affect the ecosystem as well as human health (Figure 1; Kim et al., 2008).

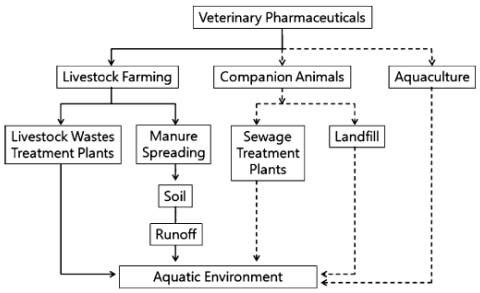

Fig. 1. Routes of veterinary pharmaceuticals entering the aquatic environment. Bold line indicates major contribution pathways, while dotted line represents relatively minor contribution pathways into the environment (Kim et al., 2008).

The findings obtained in the previos studies demonstrated that veterinary antimicrobial compounds can lead to the contamination of agricultural soils via fertilization with animal manure. (Liguoro et al., 2003; Blackwell et al., 2004; Martı´nez-Carballo et al., 2007; Aust et al., 2008 and Karcı and Balcıoğlu, 2009). Other findings were demonstrated that widespread of veterinary antibiotics to environmental water samples in Taiwan and Vientnam respectively (Managaki et al., 2007 and Lin et al., 2008). In Malaysia, simultaneous determination of eight sulfoamides in selected swine wastewater was developed by using high performance liquid chromatography (Malintan and Mohd, 2006). The present study is the first study on the simultaneous determination 6 different antibacterial groups: aminoglycoside, β-lactam, macrolides, quinolones, sulfonamides and tetracycline in chicken manure and land applied manure.

RESEARCH METHODOLOGY

Sample collection

In the present study, three states (Selangor, Negeri Sembilan and Melaka) located at west peninsular Malaysia were selected as research area. The manure samples will be collected from chicken farm and the agricultural soil samples will be collected at a depth of 10cm below the surface layer of soils and manure heaps. Discrete subsamples will be collected depending on the size of agricultural fields.

Characterization of manure and soil samples

Manure and soil samples will be characterized by using different parameters to obtain a comprehensive data about the samples and to establish a relationship between the recoveries and sample characteristics. These characteristics include pH, total organic carbon (TOC), Cr, Cu, Pb, Ni, Zn, Fe,Cd, K, Na, Ca, and Mg content.

Composting experimental design

The manure composting experiment was performed using 12 identical lab vessels. The wall of lab vessels had many small pores for aeration of manure composting. Different lab vessel containers contained 1 kg (dry weight basis) of manure material. All manures used in composting were exposed to darkness and humidification in an incubator with the capability to adjust temperature.

In the composting experiment of broiler manure at different temperature, the temperature was controlled at 15 □ C, 25 □ C, 35 □ C and 45 □ C. At the beginning of the composting process, the moisture content of the manure in each container was kept at 50–60% for optimum composting. To prevent moisture loss, the experimental vessels were covered with paddy straw. Manure moisture was controlled at 1- d intervals by adding enough water to obtain a constant moisture content throughout the composting period. The manure was turned at 1-d intervals during the first 28 d of composting and at 4-d intervals thereafter until day 42 in order to ensure an oxygen supply to maintain aerobic conditions of the materials. Samples for analysis were taken from each manure

composting test at days 0, 1, 3, 5, 7, 10, 14, 22, 28, 35 and 42 of composting for determination of veterinary antibiotics concentrations and moisture content of the composting samples.

Temperature was measured daily by a digital thermometer in the centre of the material. In order to avoid heat loss or external heating, the temperature of the incubator was adjusted in order to assure that the temperature of the manure was 1–2 □ C lower than the incubator temperature every day (Bao et al., 2009).

Sample Pretreatment and Solid Phase Extraction (SPE)

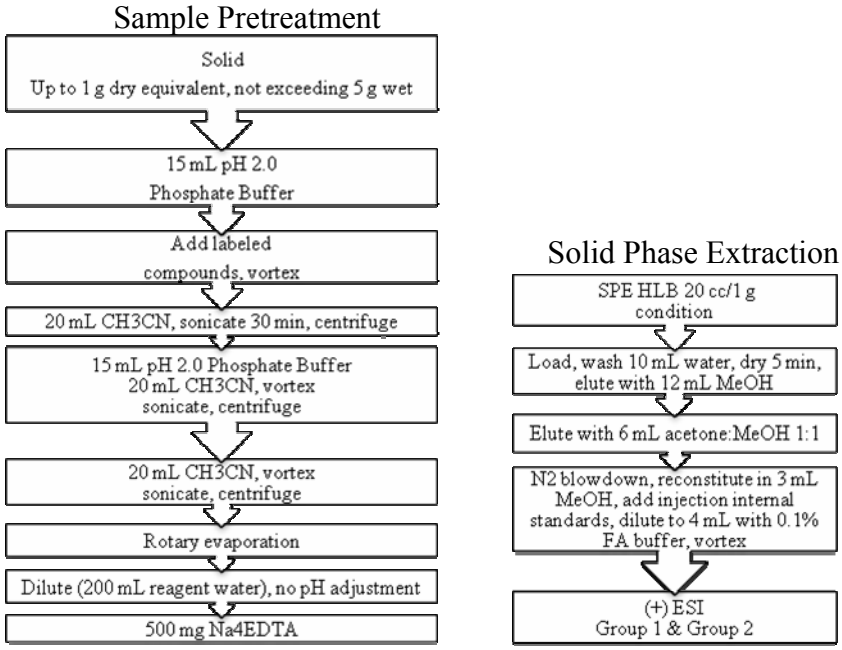

Figure 2. Flow chart for determination of pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS (USEPA Method 1694, 2007)

LC–MS/MS analysis

The analyses of veterinary antibiotics were performed using LC–MS/MS as described (EPA Method 1694, 2007). The LC instrument was a TSQ Quantum Ultra (Thermo Scientific) separations module with an Xterra MS C18 column (150mm×2.1mm i.d., 3.5µm) (Waters Corp., Milford, MA) at 40 □ C; the flow rate was 0.15-0.30 ml/min and injection volume was 15µl. A mobile-phase gradient was used to separate the Group 1 compounds. The solvent compositions were: A, 0.3% Formic Acid and 0.1% Ammonium Formate in HPLC water; B, 1:1 Acetonitrile:Methanol. The solvents were mixed as follows: 0–4 min 95% A, 5% B; 4–22.5 min 95% A, 5% B; 22.5–23 min 12% A, 88% B; 23–26 min 100% B; 26–26.5 min 100% B; 26.5-33 min 95% A, 5% B. The total run timewas 33 min. Another mobile-phase gradient was used to separate the Group 2 compounds at the flow rate of 0.20-0.23 ml/min. The solvent compositions were: A, 1:1 acetonitrile: methanol, B, HPLC H_2O, with 5mM Oxalic acid. The solvents were mixed as follows: 0–1 min 10% A, 90% B; 1–18min 10% A, 90% B; 18–20 min 40% A, 60% B; 20–24 min 90% A, 10% B; 24–24.3 min 90% A, 10% B; 24.3-28min 90% A, 10% B. The total run time was 28 min. Atmospheric pressure ionization-tandem mass

spectrometry was performed on a benchtop triple quadrupole mass spectrometer operated in electrospray ionization mode. Acquisition was done in the multiple-reaction monitoring mode (MRM) in electro spray negative (ESI +). Analyte concentrations were calculated by the internal standard method using $^{13}C_3$-Antrazine as an internal standard.

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