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Beta-agonist residues in cattle, chicken and swine livers at the wet market and the environmental impacts of wastewater from livestock farms in Selangor State, Malaysia

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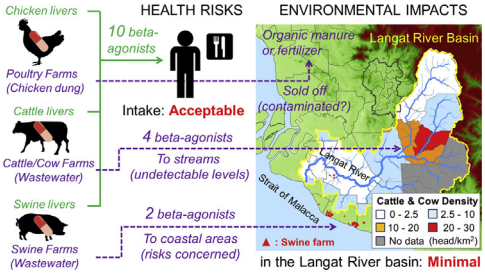
HIGHLIGHTS

- Ten beta-agonists were detected in liver specimens purchased at 14 wet markets.
- Ractopamine showed a highest residual concentration (21.6 μg/kg) in swine liver.
- The health risk of ractopamine and clenbuterol residues was assessed to be minimal.
- Five beta-agonists were detected in wastewater samples collected at 4 livestock farms.
- The Environmental impact of ractopamine (30.1 μg/L) from swine farms was evaluated.

GRAPHICAL ABSTRACT



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ABSTRACT

Fourteen beta-agonists were quantitatively analyzed in cattle, chicken and swine liver specimens purchased at 14 wet markets in Selangor State, Malaysia, by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The health risks of ractopamine and clenbuterol residues in the Malaysian population were assessed based on quantitative data and meat consumption statistics in Malaysia. Wastewater samples collected at swine farms (n = 2) and cattle/cow farms (n = 2) in the Kuala Langat district were analyzed for the presence for the 14 compounds. Wastewater in chicken farms was not collected because there was negligible discharge during the breeding period. The environmental impacts caused by beta-agonists discharged from livestock farms were spatially assessed in the Langat River basin using a geographic information system (GIS). As a result, 10 compounds were detected in the liver specimens. Ractopamine, which is a permitted compound for swine in Malaysia, was frequently detected in swine livers; also, 9 other compounds that are prohibited compounds could be illegally abused among livestock farms. The health risks of ractopamine and clenbuterol were assessed to be minimal as their hazard quotients were no more than 7.82×10^{-4} and 2.71×10^{-3}, respectively. Five beta-agonists were detected in the wastewater samples, and ractopamine in the swine farm resulted in the highest contamination (30.1 μg/L). The environmental impacts of the beta-agonists in the Langat River basin were generally concluded to be minimal, but the ractopamine contamination released from swine farms

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was localized in coastal areas near the estuary of the Langat River basin because most swine farms were located in that region.

1. Introduction

The contamination of veterinary drugs in the environment has been an important issue because their residues could result in the increase of drug resistance bacteria and antibiotic-resistance infection (Lombardo-Agüí et al., 2015). In Malaysia, sulfonamides have been detected in effluents from swine farms (Malintan and Mohd, 2006), and the residues have been detected in chicken meat products (Cheong et al., 2010). Additionally, broiler manure and manure-amended agricultural soils have been contaminated by veterinary antibiotics and progesterone (Ho et al., 2014). These facts indicate that veterinary drugs are commonly used in Malaysia, and drug resistance bacteria or antibiotic-resistance infection could become a serious environmental issue.

Beta-agonists are one of the most common veterinary drugs to treat cardiogenic shock, acute heart failure, bradyarrhythmias, asthma and chronic obstructive pulmonary disease for livestock as well as to treat diseases and infections for humans (Yu et al., 2011). However, they have been abused as a growth promoter for livestock, which could lead to a considerable muscle mass increase as well as a decrease in fat accumulation (Mersmann, 1998). In Malaysia, swine farms had been alerted to the possibility of abusing beta-agonists for producing leaner meat by the Ministry of Health Malaysia (Ponniah et al., 2004). Beta-agonist residues produced via improper use could lead to adverse effects as a long-term treatment of beta-agonists in patients with obstructive airway disease, including an increased risk for adverse cardiovascular events (Salpeter et al., 2004).

All drugs approved for use in food-producing animals have a withdrawal time to prevent residues in food of animal origin that are potentially harmful to consumers (Gehringa et al., 2004). The withdrawal time is defined as the time that should be elapsed after the administration of a pharmacologically active substance to the time when treated animals can be slaughtered for the production of safe food stuff (Damte et al., 2012). In other words, a residual concentration in a tissue must be below a given maximum residue limit (MRL) when the animals are slaughtered and sold on the market. Beta-agonists, except ractopamine for swine, are listed as prohibited drugs for food in Malaysia (Din et al., 2015); however, beta-agonist residues (terbutaline, salbutamol and clenbuterol) have been detected in swine liver specimens (Ponniah et al., 2004). Regular inspections of veterinary drug residues in animal products are conducted in certified poultry processing plants and slaughter houses by the Department of Veterinary Services Malaysia, whereas those in wet markets are under jurisdiction of the Ministry of Health Malaysia (personal communication). Therefore, it is a concern that beta-agonists and other veterinary drugs could be abused among some livestock farms.

Given the illegal use of beta-agonists among livestock farms, their wastewater could be contaminated by the abused drugs and discharged from the farms. There is no specific regulation or federal law for livestock effluent standards except for the regulations that are enforced through various state enactments and by-laws (Sakai et al., 2016). Wastewater treatment depends on livestock farms, except for swine farms that have been obliged to attach at least 3 retention ponds. There are a number of livestock farms in the Langat River basin; this basin has a major role in supplying tap water to the capital region, whereas the contamination of beta-agonists in their wastewater has not been elucidated. To scrutinize a potential for their environmental impacts, their occurrence and spatial distribution in the Langat River basin need to be clarified.

In the present study, cattle, chicken and swine liver specimens were purchased at 14 wet markets in Kuala Lumpur and Selangor state, and 14 beta-agonists were analyzed to identify their residues and elucidate the health risks caused by them in local consumers. Four wastewater samples were collected at swine and cattle/cow farms, and the 14 compounds were analyzed to spatially assess their environmental impacts in the Langat River basin using a geographic information system (GIS). The main objective of this study is to holistically elucidate the environmental and human health risks caused by the use of beta-agonists.

2. Materials and methods

2.1. Standards and reagents

Fourteen beta-agonist standards (cimaterol, terbutaline, salbutamol, zilpaterol, cimbuterol, ractopamine, clenbuterol, brombuterol, tulobuterol, mabuterol, hydroxymethyl clenbuterol, clenpenterol, isoxsuprine and mapenterol) as well as an internal standard (ractopamine-d_3) and a surrogate standard (clenbuterol-d_9) were provided by the Department of Veterinary Services Malaysia. A working solution mixed with the 14 beta-agonists (1 mg/L), an internal standard solution (1 mg/L) and a surrogate standard solution (0.5 mg/L) were prepared with methanol. A beta-glucuronidase from Helix pomatia type HP-2 was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample collections and water quality monitoring

The location of 14 wet markets and the numbers of cattle, chicken and swine liver specimens are shown in Fig. 1 and Table 1. A total of 30 specimens of cattle and swine livers and 36 specimens of chicken liver were randomly purchased from the 14 wet markets. The numbers of specimens in each wet market depended on a number of stalls; a single specimen was purchased at a stall to avoid redundant specimens from a single animal. The specimens were brought back to a laboratory and frozen at −20 °C prior to the sample pretreatment. Four wastewater samples were collected from cattle/cow farms ($n = 2$) and swine farms ($n = 2$) in the Kuala Langat district in January 2014. One liter of wastewater was collected using a stainless steel container and put into a polypropylene bottle. The collected samples were kept in a cooler box and brought to the laboratory. The samples were stored at 4 °C until the sample pretreatment was conducted.

In the wastewater samples, pH and electrical conductivity (EC), salinity, ion concentrations of calcium (Ca^{2+}), sodium (Na^{+}), potassium (K^{+}) and nitrate (NO_3^{-}) were measured by LAQUAtwin (HORIBA, Japan), and dissolved oxygen (DO) was measured by Accumet AP84 (Fisher Scientific, Malaysia) during the sampling. The chemical oxygen demand (COD_{Mn}), biochemical oxygen demand (BOD) and suspended solids (SS) were analyzed by standard methods in the laboratory.