

## Use and Contamination of Veterinary Antibiotics in Two Swine Farming Systems in Phitsanulok Province, Thailand

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### Abstract

The objective of this study was to investigate the contamination of selected veterinary antibiotics in two swine farming systems in Phitsanulok province, Thailand. The samples including feeds, water supply, flush water, effluent, sediment, feces, and sludge were collected from typical and commercial swine farms. Soil samples were collected from agricultural field near the farms. The liquid samples were extracted with SPE, while the solid samples were extracted with ultrasonic-assisted coupled with SPE. The target antibiotics were analyzed by RRLC-MS/MS. The results showed that 7 antibiotics were found in feeds, aqueous and suspended solids of water supply at maximum concentrations of  $11,695.81 \pm 16.38$   $\mu\text{g}/\text{kg}$  (lincomycin),  $11,575.57 \pm 0.81$   $\text{ng}/\text{L}$  (ciprofloxacin) and  $461,942.13 \pm 12.40$   $\mu\text{g}/\text{kg}$  (lincomycin), respectively. Six antibiotics were found in aqueous and suspended solids of flush water and fresh feces at maximum concentrations of  $598.34 \pm 17.27$   $\text{ng}/\text{L}$  (sulfamethazine),  $62,918.29 \pm 8.96$   $\mu\text{g}/\text{kg}$  (lincomycin) and  $40,229.15 \pm 19.71$   $\mu\text{g}/\text{kg}$  (lincomycin), respectively. Erythromycin was found in aqueous, suspended solids and sediment of effluent at maximum concentrations of  $9,614.56 \pm 1.46$   $\text{ng}/\text{L}$ ,  $154,500.08 \pm 12.05$   $\mu\text{g}/\text{kg}$  and  $71,123.61 \pm 23.28$   $\mu\text{g}/\text{kg}$ , respectively. Six antibiotics were found in dried feces, dried sludge and agricultural field soil at maximum concentrations of  $26,614.38 \pm 21.47$   $\mu\text{g}/\text{kg}$  (lincomycin),  $14,353.39 \pm 1.55$   $\mu\text{g}/\text{kg}$  (ciprofloxacin) and  $28,909.29 \pm 2.73$   $\mu\text{g}/\text{kg}$  (trimethoprim). Veterinary antibiotics using in two swine farming systems resulted in the contamination of veterinary antibiotics in waste, treated waste and utilization applying to agricultural field. Consequently, to reducing contamination of antibiotics from swine farms in the environment should be paid attention.

**Keywords:** Contamination; Antibiotics; Swine farm

## 1. Introduction

Antibiotics are widely used in veterinary medicine to treat and prevent health problem from infectious disease in animals. In addition, in many countries they are often added to animal feeds as antibiotic growth promoters in order to increase productivity (Page and Gautier, 2012). During the year 1953s, The United states Food and Drug Administration (FDA) endorsed chlortetracycline and oxytetracycline as animal feed additives (Swartz, 2002) then they are widely accepted around the world. However, most antibiotics are poorly absorbed by animals (Zhu *et al.*, 2013) and subsequently excreted with the animal wastes, resulting in as much as 30-90% of the parent compound or its metabolites being excreted in feces, urine (Sarmah *et al.*, 2006) and ending up in manure storage tanks or lagoons (Lee *et al.*, 2007). Antibiotics can therefore either leave the wastewater treatment plant in treated water entering rivers, stream (Zhou *et al.*, 2013) or become part of the sewage sludge. These compounds may be transported into the environment via surface runoff, leaching, application of manure onto agricultural fields as fertilizer (Kümmerer, 2009), and plant uptake (Boxall *et al.*, 2006).

Thailand is one of ASEAN country which is a major source of swine production in the world after China, EU and U.S. For Thailand, modern intensive swine production began in 1973 with the importation of breeding stock from the United Kingdom and the United States (Beeghly, 1989). Commercial development of this sector is fostered by a small number of feed mill companies which provide piglets, feeds, drugs, veterinary services and farm management expertise to contracted pig producers. Therefore, this contract system plays an important role in development of Thai commercial swine industry. In parallel with this rapid development, antibiotics are increasingly used for both treatment and

growth promotion in Thailand's swine production. In addition, the typical swine farms are distributed in every region of the country. The treatment of swine disease has been not necessarily under veterinary control but the farmers have decision based on their experience and economic situation (Suriyasathaporn *et al.*, 2012) and most of these farms lacked of the good waste management. Therefore, both commercial and typical swine farms could be source of antibiotics contamination in the environment. The objective of the study was to investigate the contamination of selected veterinary antibiotics in feeds, feces, wastewater, water supply, and agricultural soil from different swine farming systems in Phitsanulok province, Thailand.

## 2. Materials and methods

### 2.1 Site and system description

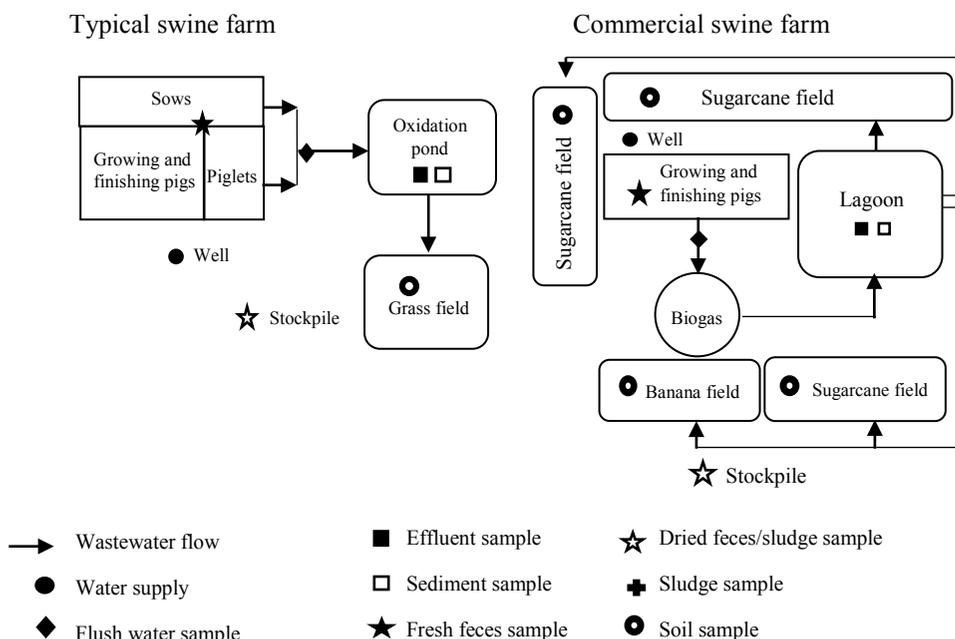
One typical and one commercial farms with different wastewater management systems were selected for this study. The two swine farms, representing typical swine feeding operations in Phitsanulok province, are located in Mueang and Bang Rakam district. The typical farm consists of several buildings for piglets, growing and finishing pigs and sows. This farm accommodated 150-pigs small scale, including 40 piglets, 100 growing and finishing and 10 sows. The swine houses were flushed daily with water supply and the mixed flush water was directly discharged into an oxidation pond. Wastewater in the pond was partially applied onto grass field nearby the farm. For commercial farm, it was designed for 750-pigs medium scale with evaporative cooling system. The swine houses were flushed daily with water supply and the flush water was treated in a biogas system followed by a lagoon. The lagoon wastewater was partially applied onto the sugarcane and banana fields nearby the farm.

## 2.2 Samples and sampling methods

Various samples were collected in July, 2016 from the two swine farms. On the two farms, the collected samples included water supply from storage tank, fresh feces and flush water from swine houses, effluent and sediment from the oxidation pond and lagoon, dried feces or dried sludge from stockpiles, and soil from agricultural fields. Fresh feces samples from typical farm were taken by randomly collecting from different swine houses and then combining into one composite sample. For commercial farm, fresh feces were composited from 5 to 6 grab samples and then combining into one composite sample. The flush waters were sampled at washing time, composited from 5 to 6 grab samples and then combining into one composite sample. The effluent samples were composited from 5 to 6 grab samples. Dried feces and dried sludge were collected from stockpile and soil samples were collected at a depth of 20 cm below the surface soil.

Ten discrete subsamples were collected, and composite samples were prepared by mixing equal quantities of subsamples and selected by the quadripartite method. The swine layout of the two swine farms and the sampling site were shown in Figure 1.

1,000 mL of water supply, 200 mL of flush water, and 500 mL of effluent were collected using the brown amber bottles which were rinsed with sample water before collection. All the water samples collected were adjusted to pH 3 using 4 M H<sub>2</sub>SO<sub>4</sub> added with methanol (5% v/v) to inhibit microbial activity and then transported to the laboratory in a cooler. 500 g of feed, feces, sludge, sediment, and soil samples were collected and stored in 1 L brown glass bottles and preserved by adding with 2 g of sodium azide. Upon arrival at the laboratory, the samples were immediately stored at 4 °C. Before being analyzed, the solid samples were freeze-dried, sieved through a 0.5 mm pore size and then kept at -18 °C in the dark until extraction (Zhou *et al.*, 2012).



**Figure 1.** Layout and sampling sites of the two swine farms

## 2.3 Sample extraction

### 2.3.1 Water samples

The collected liquid samples (1,000 mL of water supply, 200 mL of flush water, and 500 mL of effluent) were extracted by solid phase extraction (SPE). The liquid samples were filtered through glass fiber filters to remove suspended solids (SS) and then filtered liquid samples were spiked with 100  $\mu$ L of the internal standards (IS) for chemical analysis. The meclocycline, lincomycin-D3, sulfamerazine-D4, sulfamethazine-13C6, sulfamethoxazole-D4, ciprofloxacin-D8, trimethoprim-D3, erythromycin-13C-D3 and thiabendazole-D4 were used as the IS. The liquid samples were passed through Oasis HLB cartridges (6 mL, 500 mg) under vacuum at a flow rate of 5-10 mL/min. The target compounds were eluted with 12 mL methanol and then the eluates were evaporated to near dryness under a gentle stream of nitrogen and redissolved in 1 mL of methanol. After filtration through a 0.22  $\mu$ m membrane to remove particles, the final extract was transferred to a 2 mL amber vial and stored at -18 °C until RRLC-MS/MS analysis. Just prior to the RRLC-MS/MS analysis, 100  $\mu$ L aliquot of each sample extract was evaporated and reconstituted in a mixed solvent (methanol: 0.2% formic acid and 2 mM ammonium acetate, 30:70, v/v) (Zhou *et al.*, 2012).

### 2.3.2. Solid samples

The solid samples (0.5 g of freeze-dried feces, sludge, 2 g of freeze-dried sediment, soil, feed, and all of each SS) were extracted by ultrasonication. The solid samples were weighted into a 30 mL glass tube, followed by addition of 100  $\mu$ L of the IS for chemical analysis. Then the samples were mixed and placed in a refrigerator

at 4 °C overnight. The samples were extracted with 10 mL acetonitrile and 10 mL citric acid was added into glass tube followed by mixing on a vortex mixer for 1 min, ultrasonicated for 15 min and centrifuged at 3,500 rpm for 10 min. The supernatant was piped into a 200 mL round-bottom flask. The extraction process was repeated twice and the supernatants from the three extractions were combined. The extract in the round-bottom flask was evaporated at 50 °C, and diluted to 200 mL with MilliQ water. The extracts were purified by passing through tandem SAX cartridges (6 mL, 500 mg) and HLB cartridges (6 mL, 200 mg) under vacuum at a flow rate of 5-10 mL/min. The elution and reconstitution conditions were the same as those described in Section 2.3.1.

## 2.4 Chemical and quantification analysis

The chemicals in this study included 14 antibiotics belonging to six groups of widely used in swine production of Thailand, comprising Lincosamides: lincomycin (LIN), Sulfonamides: sulfamerazine (SMR), sulfameter (SM), sulfamethazine (SMZ), sulfamonomethoxine (SMM), Fluoroquinolones: ciprofloxacin (CFX), marbofloxacin (MAR), Diaminopyrimidines: trimethoprim (TMP), Macrolides: erythromycin (ETM), tylosin (TYL), Tetracyclines: chlortetracycline (CTC), methacycline (MC), oxytetracycline (OTC), tetracycline (TC) being selected as the target analytes.

The target antibiotics were analyzed using RRLC-MS/MS, Agilent Liquid Chromatography 1200 series RRLC system coupled to an Agilent 6460 triple quadrupole MS equipped with an electrospray ionization (ESI) source (Agilent, Palo Alto, CA, USA) in multiple-reaction monitoring (MRM) mode. Nitrogen gas was

used as the drying and collision gas. LC and MS parameters were measured using an Agilent Eclipse Plus-C18 (100 mm × 2.1 mm, 1.8 μm) column with its corresponding pre-column filter (2.1 mm, 0.2 μm). The column temperature was set at 40 °C. Gas temperature and gas flow were set at 325 °C and 6 L/min, respectively. Sheath gas flow and sheath gas temperature were set at 11 L/min and 350 °C. The injection volume for each sample was 5 μL. The chemical and instrument were supported from state key laboratory of organic geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Science, Guangzhou China.

Quantification of the target compounds used the IS method. Calibration lines of six concentration points (1, 5, 10, 50, 100, and 200 μg/L in methanol containing 0.1% formic acid, v/v), were used for quantification of individual antibiotic. The linearity of the calibration curve in this range was confirmed with a high linear correlation coefficient ( $R^2 > 0.99$ ). The limit of detection (LOD) and limit of quantitation (LOQ) for the target compound were calculated based on the signal-to-noise ratio (SNR) near the target peak. The analytes were identified by comparing the retention times (within 2%) and the ion ratios (within 20%). Data acquisition was performed under Agilent Mass Hunter, Quantitative Analysis version B 03.01/Build 3.1.170.0 software.

### 3. Results and Discussion

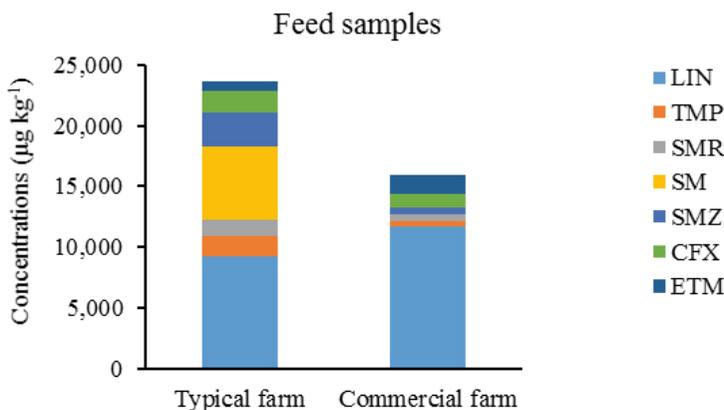
#### 3.1 Antibiotics in the swine feed samples

Seven antibiotics including, lincomycin, sulfamerazine, sulfamethazine, sulfameter, ciprofloxacin, erythromycin, and trimethoprim were found in feed from typical farm with mean

concentrations of  $9,191.72 \pm 1.15$ ,  $1,369.18 \pm 1.60$ ,  $5,970.40 \pm 2.21$ ,  $1,802.84 \pm 3.31$ ,  $2,782.72 \pm 0.01$ ,  $825.44 \pm 0.05$ , and  $1,712.14 \pm 1.55$  μg/kg, respectively. Six antibiotics lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in feed from commercial farm with mean concentrations of  $11,695.81 \pm 16.38$ ,  $502.73 \pm 0.09$ ,  $535.64 \pm 0.05$ ,  $1,102.21 \pm 0.07$ ,  $1,570.48 \pm 0.03$ , and  $474.63 \pm 0.91$  μg/kg, respectively (Figure 2).

Based on interview with farmers, these antibiotics were commonly used in feed for growth promotion and disease prevention. In fact, all the antibiotics were detected in feed which were mixed on the typical farm by farmer under the experience and decision. For commercial farm, antibiotics were used and mixed in feed on the farm under the control and supervision of farm veterinarians that were conducted on Good Agricultural Practices for pig farm in Thailand. Many antibiotics are not completely absorbed in the gut, resulting in the excretion of the parent compound and its breakdown metabolites (Boxall *et al.*, 2004). Most antibiotics concentrations in feed samples from typical farm were higher than those from commercial farm. These were due to pigs in typical farm found in different growth stages of swine, including piglets, growing and finishing, and sows; especially, newly weaned piglets, were often fed with various antibiotics with high dosage to prevent and treat diseases.

Lincomycin was found at highest concentrations in feed samples from the two farms. It is commonly used for growth promotion enhanced pig productivity (Pollmann *et al.*, 1980) as well as disease treatment and control (Rajić *et al.*, 2006). It is effective in reducing the



**Figure 2.** Concentrations of antibiotics in the feed samples

*Clostridium* spp. infection (diarrheal disease) in all ages of pigs (Silva *et al.*, 2015). Besides, the other antibiotics, including sulfonamides groups, ciprofloxacin, trimethoprim and erythromycin were found in feed samples that are often detected in swine feeds as Zhao *et al.* (2013) and Chen *et al.* (2012) reported. Furthermore, FDA (2015) reported lincomycin, sulfamerazine, sulfamethazine and erythromycin are approved for use in food-producing animals.

### 3.2 Antibiotics in fresh feces and flush water samples

Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in fresh feces from typical farm with mean concentrations of  $40,229.15 \pm 19.71$ ,  $3,158.36 \pm 0.19$ ,  $11,803.98 \pm 1.20$ ,  $24,594.8 \pm 5.65$ , and  $4,833.13 \pm 0.87$  µg/kg, respectively. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in fresh feces with mean concentrations of  $22,524.32 \pm 1.78$ ,  $3,242.96 \pm 0.66$ ,  $2,349.33 \pm 0.44$ ,  $11,575.57 \pm 0.81$ ,  $1,328.08 \pm 0.36$ , and  $1,911.87 \pm 0.03$  µg/kg, respectively (Figure 3).

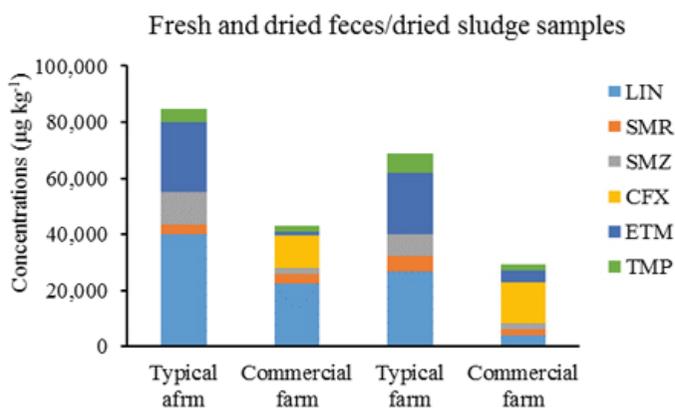
Lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, and trimethoprim were found in aqueous of the flush water from typical farm with mean concentrations of  $74.22 \pm 11.02$ ,  $4.42 \pm 0.01$ ,  $51.03 \pm 0.60$ ,  $21.90 \pm 0.23$ ,  $54.94 \pm 2.72$ , and  $2.44 \pm 0.42$  ng/L, respectively. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of the flush water with mean concentrations of  $62,918.29 \pm 8.96$ ,  $5,556.01 \pm 0.13$ ,  $9,296.18 \pm 0.85$ ,  $17,472.79 \pm 0.69$ ,  $3,602.91 \pm 0.84$ , and  $4,620.62 \pm 0.12$  µg/kg, respectively. For commercial farms, lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in aqueous of the flush water with mean concentrations of  $351.24 \pm 40.56$ ,  $0.92 \pm 0.04$ ,  $598.34 \pm 17.27$ ,  $64.25 \pm 1.04$ , and  $286.34 \pm 0.53$  ng/L, respectively. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of the flush water with mean concentrations of  $9,395.90 \pm 16.67$ ,  $788.32 \pm 0.05$ ,  $865.03 \pm 0.73$ ,  $3,334.30 \pm 0.95$ ,  $5,452.01 \pm 1.61$ , and  $1,061.89 \pm 0.52$  µg/kg, respectively (Figure 4).

All the antibiotics were detected in fresh feces and flush water which were reflected the dosage and frequency of antibiotics used in farms. These data demonstrated that swine farms are considered as an important pollution source of various antibiotics to the receiving environments (Qiao *et al.*, 2012). Most antibiotics concentrations in aqueous phase of flush water from commercial farm were higher those from typical farm, while antibiotics concentrations in SS from typical farm were higher than those from commercial farm. This may due to pigs in commercial farm were found older age and more number of pigs than typical farm. Therefore, pigs in commercial farm consume and excrete more than typical farm. Thus, the antibiotics and their metabolites were excreted via feces and urine and contaminated in flush water. Animals consume antibiotics as much as 30 to 90% that is released into the manure and urine (Sarmah *et al.*, 2006). Moreover, typical farm was operated with open system; the floor was easy to be dirty from slurry, dust and soil around the swine houses and it was not separated between dry and wet area. Thus, the swine houses were flushed with water supply that was contaminated with high antibiotics which may cause of antibiotic increasing in the flush water.

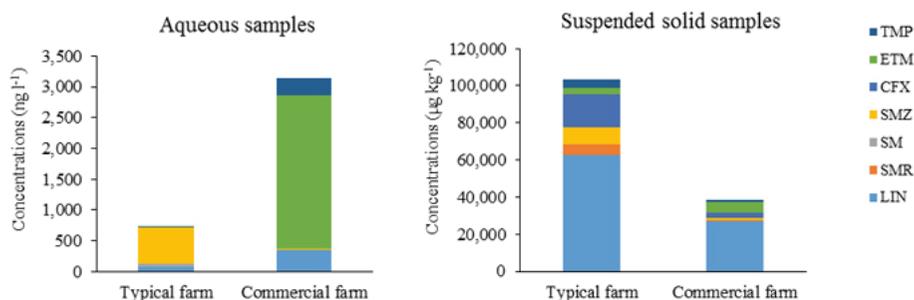
### 3.3 Antibiotics in dried feces and dried sludge samples

Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in dried feces from typical farm with mean concentrations of  $26,614.38 \pm 21.47$ ,  $5,858.58 \pm 2.41$ ,  $7,658.73 \pm 0.61$ ,  $21,911.02 \pm 4.80$ , and  $6,586.56 \pm 2.67$   $\mu\text{g}/\text{kg}$ , respectively. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in dried sludge which was treated by a biogas system with mean concentrations of  $4,090.42 \pm 1.94$ ,  $1,987.7 \pm 0.12$ ,  $2,292.66 \pm 0.31$ ,  $14,353.39 \pm 1.55$ ,  $4,522.49 \pm 0.76$ , and  $1,887.45 \pm 0.33$   $\mu\text{g}/\text{kg}$ , respectively (Figure 3).

The concentrations of lincomycin, sulfamethazine and erythromycin were lower in dried feces than in fresh feces. Lincomycin, sulfamerazine, sulfamethazine and trimethoprim were also lower in dried sludge than in fresh feces. Sulfamethazine was found in dried feces and dried sludge reported by Zhang *et al.*, 2015. These suggest that these antibiotics might be degraded or evaporated during the drying process under sunlight and biogas system. Thus, the drying process may be a better way to degrade excessive antibiotics in feces.



**Figure 3.** Concentrations of antibiotics in the fresh and dried feces/sludge samples



**Figure 4.** Concentrations of antibiotics in the flush water samples

### 3.4 Antibiotics in the effluent samples

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, erythromycin, and trimethoprim were found in aqueous phase of effluent from typical farm with mean concentrations of  $120.03 \pm 0.05$ ,  $1.79 \pm 0.25$ ,  $51.13 \pm 0.03$ ,  $773.12 \pm 1.82$ ,  $9,614.56 \pm 1.46$ , and  $1.47 \pm 0.05$  ng/L, respectively. Sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS with mean concentrations of  $7,594.17 \pm 0.06$ ,  $31,972.81 \pm 0.49$ ,  $102,747.26 \pm 0.77$ ,  $24,553.76 \pm 0.56$ ,  $154,500.08 \pm 12.05$ , and  $8,128.14 \pm 0.34$  µg/kg, respectively. Lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in sediment with mean concentrations of  $29,624.04 \pm 3.12$ ,  $518.79 \pm 0.12$ ,  $3,001.58 \pm 0.50$ ,  $24,562.79 \pm 1.65$ ,  $14,641.29 \pm 4.19$ ,  $71,123.61 \pm 23.28$ , and  $514.69 \pm 0.06$  µg/kg, respectively. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, and erythromycin were found in aqueous phase of effluent with mean concentrations of  $734.46 \pm 4.35$ ,  $7.26 \pm 3.42$ ,  $3.72 \pm 0.02$ , and  $3.07 \pm 0.01$  ng/L, respectively. Lincomycin, sulfamerazine, sulfamethazine, erythromycin, and trimethoprim were found in SS with mean concentrations of  $17,275.33 \pm 0.20$ ,  $1,462.53 \pm 0.01$ ,  $36,986.96 \pm 0.36$ ,  $2,997.80 \pm 2.53$ ,

and  $1,540.20 \pm 0.36$  µg/kg, respectively. In addition, lincomycin, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in lagoon sediment with mean concentrations of  $11,751.66 \pm 0.05$ ,  $595.48 \pm 1.83$ ,  $2,350.70 \pm 1.57$ ,  $1,677.83 \pm 0.13$ , and  $634.66 \pm 0.05$  µg/kg, respectively (Figure 5).

Most antibiotic concentrations in wastewater from typical farm were higher than those from commercial farm. These results suggest that different antibiotic removal efficiencies from wastewater depend on wastewater treatment process corresponding to Gulkowska *et al.*, 2008. The results from the present study demonstrated that sulfamerazine, trimethoprim in aqueous and lincomycin in SS were decreased from flush water by an oxidation pond. In addition, sulfamethazine, erythromycin, trimethoprim in aqueous, ciprofloxacin and erythromycin in SS were decreased from flush water by a biogas system.

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in sediment from oxidation pond. Erythromycin was found at highest concentrations and trimethoprim was found at lowest concentrations in sediment samples from typical farm. For commercial

farm, lincomycin, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in lagoon sediment. Lincomycin was found at highest concentrations and sulfamethazine was found at lowest concentrations. Most antibiotic concentrations in aqueous phase, SS and sediment from typical farm were higher than those from commercial farm. These indicated that antibiotic concentrations in wastewater from commercial farm were decreased by the biogas system corresponding to Zhao *et al.*, (2013). These may depend on wastewater treatment methods. In fact, swine wastewater from typical farm was stored in an oxidation pond and drained onto agricultural field, while wastewater from commercial farm was already treated with a biogas system before it was drained onto agricultural field. Thus, antibiotic

in the effluent of typical farm were higher than those in commercial farm. However, the antibiotics could not be treated by these wastewater treatment methods. In addition, most antibiotic concentrations in wastewater from the two farms were found in SS higher than sediments and aqueous phase. These suggest that most antibiotics were transferred into the solid phase via sorption as well as eliminated from liquid phase by photodegradation. Such high concentrations in SS would have negative impacts on soil if wastewater and sludge are applied on agricultural field such as effects on soil microbial diversity (Chander *et al.*, 2005). Thus, sorption of antibiotics in solid phase can reduce their mobility, reactivity, and bioavailability for microbial degradation (Hatzinger and Alexander, 1997).

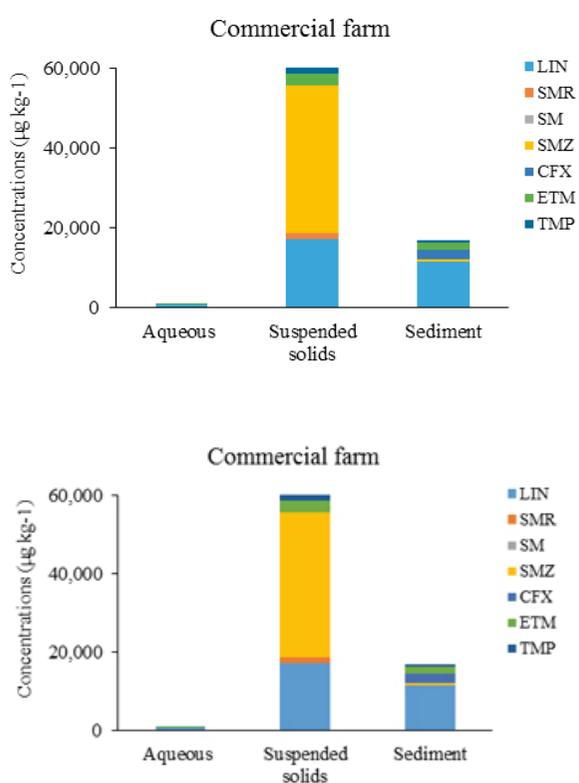


Figure 5. Concentrations of antibiotics in the effluent samples

### 3.5 Antibiotics in the water supply samples

Lincomycin, sulfamerazine, sulfameter, sulfamethazine, and trimethoprim were found in aqueous phase of water supply from typical farm with mean concentrations of  $113.54 \pm 2.75$ ,  $0.98 \pm 0.01$ ,  $175.67 \pm 7.67$ ,  $3,060.88 \pm 158.90$ , and  $0.76 \pm 0.05$  ng/L, respectively. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of water supply from typical farm with mean concentrations of  $461,942.13 \pm 12.40$ ,  $6,780.73 \pm 0.11$ ,  $7,093.44 \pm 0.01$ ,  $15,250.27 \pm 0.03$ ,  $6,028.59 \pm 0.05$ , and  $6,727.57 \pm 0.22$   $\mu\text{g}/\text{kg}$ ,

respectively. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in aqueous phase of water supply with mean concentrations of  $59.96 \pm 31.03$ ,  $46.99 \pm 1.90$ ,  $100.45 \pm 23.34$ ,  $11,575.57 \pm 0.81$ ,  $693.60 \pm 665.44$ , and  $72.11 \pm 19.43$  ng/L, respectively. Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in SS of water supply with mean concentrations of  $235,535.60 \pm 6.93$ ,  $43,275 \pm 0.03$ ,  $94,853.61 \pm 4.93$ ,  $36,706.82 \pm 0.04$ ,  $3,112.59 \pm 0.23$ , and  $30,239.23 \pm 1.24$   $\mu\text{g}/\text{kg}$ , respectively (Figure 6).

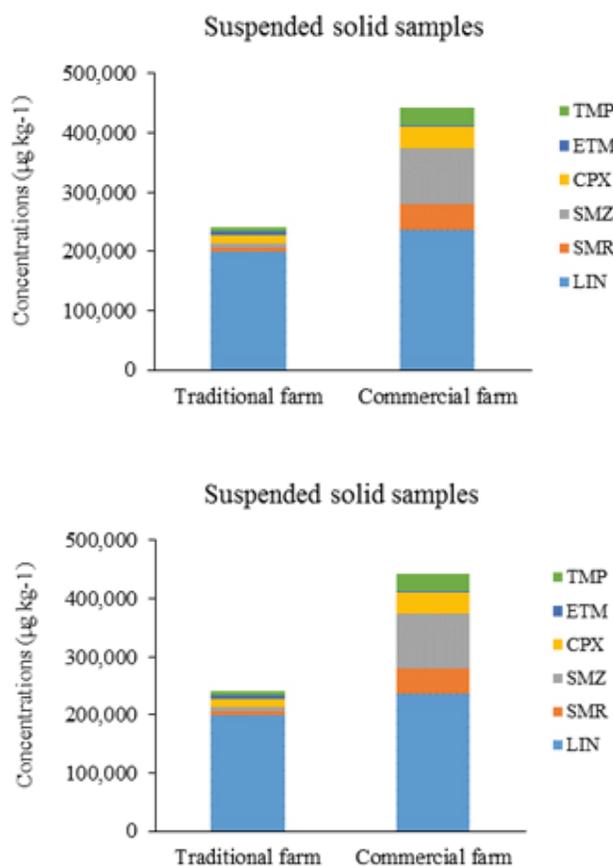


Figure 6. Concentrations of antibiotics in the water supply samples

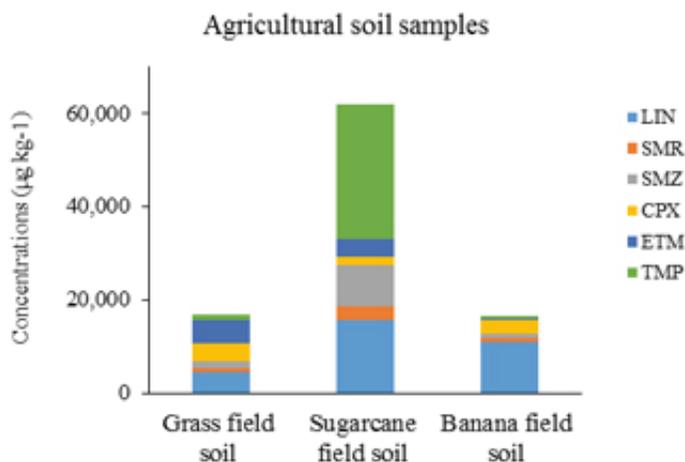
These antibiotics were found in water supply corresponding with Zhao *et al.* (2013) and Yao *et al.* (2017). Base on the farms survey and interview with the farmers, water from the shallow wells farms was pumped and kept in the storage tanks in each farm as water supplies. Water supplies were used for watering pigs and flush manure from swine houses. From this study, antibiotics were found in the effluent samples which were drained on soil in these farms. These suggest that antibiotics might be reach the shallow wells by different pathways (Carvalho and Santos, 2016). The contamination of antibiotics in the subsoil depends on the frequency of wastewater discharge, physico-chemical properties and processes of each compound such as solubility, sorption, degradation as well as soil properties (Boyroura *et al.*, 2018).

### 3.6 Antibiotics in the agricultural soil samples

Lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in grass field soil from typical farm with mean concentrations of  $4,466.82 \pm 2.19$ ,  $751.76 \pm 0.31$ ,  $1,665.75 \pm 1.22$ ,  $3,593.42 \pm 0.63$ ,  $5,245.68 \pm 0.03$ , and  $1,100.09 \pm 1.07$   $\mu\text{g}/\text{kg}$ , respectively. For commercial farm, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin, and trimethoprim were found in sugarcane field soil with mean concentrations of  $15,674.54 \pm 21.24$ ,  $2,975.03 \pm 6.96$ ,  $8,863.72 \pm 17.67$ ,  $1,743.70 \pm 0.95$ ,  $3,716.27 \pm 0.97$ , and

$28,909.29 \pm 2.73$   $\mu\text{g}/\text{kg}$ , respectively. In addition, lincomycin, sulfamerazine, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in banana field soil with mean concentrations of  $10,809.47 \pm 24.44$ ,  $885.26 \pm 0.97$ ,  $870.4 \pm 0.99$ ,  $2,961.25 \pm 0.45$ ,  $405.87 \pm 0.48$ ,  $693.86 \pm 0.31$   $\mu\text{g}/\text{kg}$ , respectively. Most antibiotic concentrations in sugarcane field soil were higher than those the other soil samples (Figure 7).

The present study also showed that the soil nearby swine farms was contaminated with various antibiotics. Ciprofloxacin and erythromycin were found at high concentrations in grass soil of the typical farm which directly received the effluent from oxidation pond. On the other hand, lincomycin, sulfamerazine, and sulfamethazine were found at high concentrations in agricultural soil from commercial farm. The antibiotic residue in soils was reported in many studies (Boxall, 2004; Hamscher *et al.*, 2005; Martinez-Carballo *et al.*, 2007). Ciprofloxacin, sulfonamides, and tetracyclines could persist in soils a long time (Zuccato *et al.* 2000), and only a moderate degradation of various tetracyclines occurred within 180 days (Hamscher *et al.* 2002), while soil without antibiotics used find them due to a habitat of indigenous antibiotics produced by soil microorganisms (Gottlieb, 1976). Thus, soil nearby the swine farms risked for antibiotics accumulated higher than soil without waste from swine farms. However, the occurrence of veterinary antibiotics in the environment matrices from the swine farms depend on breeding, pig age, farm size and farm management.



**Figure 7.** Concentrations of antibiotics in the agricultural soil samples

#### 4. Conclusion

The study of occurrence of selected veterinary antibiotics from typical and commercial swine farms revealed that lincomycin, sulfamerazine, sulfamer, sulfamethazine, ciprofloxacin, erythromycin and trimethoprim were found in all samples (feeds, flush water, wastewater, water supply, fresh feces, dried feces, dried sludge, and agricultural soil), except sulfamer being not found in all samples from commercial farm. The present study also indicated that antibiotics from swine farms could enter the environment with direct leaching of swine wastewater and waste utilization as fertilizer applying to agricultural field. As a result of different farm managements, especially wastewater treatment process, antibiotic concentrations were differently found in the samples. Consequently, reducing contamination of antibiotics from swine farm to the environment should be paid more attention.

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