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Occurrence of veterinary antibiotics in waste and environment of small-scale swine farms

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ABSTRACT: This research aimed to investigate the occurrence of selected veterinary antibiotics in waste and environmental samples from 3 types of small-scale swine farms with different waste management systems in Northern Thailand comprising farms without waste treatment system, farms with a pond system, and farms with a biogas system. Flush water, fresh and dried feces, effluents, sludge, and soils were collected for an antibiotic analysis. Solid-Phase Extraction (SPE) and ultrasonicated coupled with SPE were used for liquid and solid sample extraction. The target antibiotics were analyzed using Liquid Chromatography-Tandem Mass Spectrometry. In total, 19, 16, and 13 out of 21 target antibiotics belonging to 9 classes were found in flush water, fresh feces, and dried feces, respectively, in which the chlortetracycline was dominant in flush water (835.07–982.25 µg/l), fresh feces (354.46–498.38 µg/kg), and dried feces (95.05–154.94 µg/kg). There were 17 and 16 antibiotics in effluents from pond and biogas systems, respectively, of which 13 and 12 antibiotics were in the sludge of each system. Moreover, chlortetracycline showed the highest concentrations in effluents (179.83–245.08 µg/l) and sludge (80.84–104.83 µg/kg) from both treatment systems. For soil receiving wastewater, chlortetracycline and enrofloxacin were detected while 11 and 12 antibiotics were found in soil amended with sludge from biogas and pond systems, respectively, with the highest concentrations of chlortetracycline. This research demonstrated that waste management and utilization in small-scale swine farms are important sources of antibiotic distribution and contamination in the environment. Therefore, appropriate waste management systems for small-scale swine farms should be considered.

KEYWORDS: biogas treatment system, pond treatment system, small-scale swine farm, veterinary antibiotics, waste management

INTRODUCTION

Swine farm was considered one of the most important sources of incomes for households in Thailand. From 2008 to 2020, the number of swine had increased from around 7.7 to 12.10 million. In Northern Thailand, from 2008 to 2019, the growth rate of swine farms had increased by approximately 58.80% which was the highest rate compared to other regions of Thailand [1]. Small-scale swine farms were mostly found in the lower region of Northern Thailand which increased from 6,166 households (276,041 swine) to 17,829 households (411,072 swine) during 2010–2017 [1]. According to Suriyasathaporn et al [2], most of these farm owners lack knowledge on veterinary antibiotic usage for disease prevention and therapeutics for swine. The use of antibiotics was generally based on the experience and economic situations of farm owners. Therefore, antibiotic usage in these farms was improper.

Veterinary antibiotics play a vital role in the prevention and treatment of infectious diseases in swine. Nowadays, the use of antibiotics in livestock is increasing by more than half of all antibiotics used in other aspects. Swine usually obtain antibiotics via drug injection, drinking water, and feed additives. Lekagul et al [3] reported that antibiotics such as colistin, oxytetracycline, tylosin, lincomycin, halquinol, and tiamulin were found in swine feed. Meanwhile, approximately 30–90% of antibiotic compounds are excreted through swine urine and feces [4]. They end up in the waste and wastewater of the swine farms [5]. Generally, swine wastewater is treated in wastewater treatment systems before being released into the environment, and the biogas or pond treatment system is mostly used in small-scale swine farms.

These treatment systems are typically used for the removal of organic matter, nutrients, and solids in wastewater. For antibiotics, the pond system could remove only 40–50% of lincomycin and tetracycline in the swine wastewater [5]. The biogas system was able to remove 14.97–67.97% of tetracycline [6]. It is possible that the effluent from these treatment systems is still contaminated with antibiotics. Thus, these residual antibiotic compounds in manure and effluent from swine farms could contaminate both terrestrial and aquatic environments through runoff or leaching into surface water or groundwater [7]. Besides, antibiotics used in swine farms can spread in the environment through swine manure and can reach soil and groundwater [8]. Moreover, antibiotics found in the soil, surface water, and groundwater could still contribute to the development of antibiotic resistance by exercising selective pressure on the microbial cells in the environment, which causes a further effect on the environment and human health [9].

As a result, the waste from the small-scale swine farms may still be a source of antibiotic distribution and contamination in the environment. As there are limited data on veterinary antibiotic contamination in waste from small-scale swine farms and in nearby environments, further study is needed. The objective of this research was to investigate antibiotics in farm waste and the surrounding environment of small-scale swine farms with various waste management systems. The finding will serve as a baseline for the proper management of swine waste in order to reduce the unfavorable consequences of antibiotic use in livestock.

MATERIALS AND METHODS

Study site

In this research, 9 small-scale swine farms with 5–50 swine located in Phitsanulok, Sukhothai, and Uttaradit Provinces in Lower Northern Thailand were selected for investigation (Fig. S1) Based on wastewater treatment systems, representatives of the small-scale swine farms for this study were classified into a farm without wastewater treatment system (farm A); a farm with a retention pond (farm B), and a farm with a biogas system (farm C). Waste and environmental samples of these farms were collected for antibiotic analysis.

Sampling methods and sample analysis

Sampling methods

Three replications of each representative of small-scale swine farms (farm A, B, and C) were selected from 3 provinces of the study sites. Liquid and solid samples of waste and environmental samples were collected for analysis of physical and chemical properties as well as antibiotic concentrations. The liquid samples included flush water and effluents of the swine farms while the solid samples included feces, sludge, soil receiving wastewater, and soil amended with sludge. Fig. 1 shows the layout of sample types collected from each representative of small-scale swine farms.

For all farm types, grab samples of flush water from swine houses were collected at the beginning, during, and end of the cleaning periods and mixed to generate composite samples of the flush water. For farms B and C, 3 effluent samples were collected from wastewater treatment systems. These liquid samples were preserved at 4 °C prior to an analysis of physicochemical properties and at −18 °C for an antibiotic analysis [10, 11].

Five grab samples of fresh feces were randomly collected before swine pen cleaning and mixed to generate a composite sample. The composite samples of 5 grab samples of dried feces were taken at the depth of 0–20 cm of the manure heaps of stockpile. These manures are generally applied in the agricultural field and become a source of antibiotics.

Sludge samples were grabbed from the sludge stockpile of farms B and C. For soil treated with sludge from farms B and C, 3 samples of the soil in agricultural fields were collected at 0–20 cm below ground surface [11]. Then, they were mixed to be a composite soil sample of agricultural fields as antibiotics were mainly accumulated and distributed at this level [12].

For farm A, composite samples of the soil receiving wastewater were also taken. All solid samples were preserved at 4 °C for a physicochemical properties analysis and at −18 °C for an antibiotic analysis [10].

In this study, all samples from all 9 smallholder swine farms were collected in a dry period during January–March 2021 in which all types of samples were collected in the same day for each farm.

Sample analysis

Physicochemical analysis of samples

All liquid samples were analyzed according to the standard methods for the examination of water and wastewater [13]. pH, temperature, and EC were measured on-site. COD, BOD, TSS, and TKN were analyzed in the laboratory using the closed reflux method, Azide modification method, gravimetric method, and the Kjeldahl method, respectively.

All solid samples were analyzed for pH, organic matter (OM) using the Walkley and Black method [14], cation exchange capacity (CEC) using the ammonium method, N using the Kjeldahl method, P using Bray II method [15], and K using ammonium acetate method [16].

Antibiotic analysis of samples

The liquid samples were filtered through glass fiber filter (0.7 µm, Whatman GF/F). Then, they were adjusted to pH 3 using 4 M H_2SO_4 prior to spiking with 100 µl of Internal Standards (IS) and adding 0.2 g Na_2EDTA for antibiotic extraction. After preconditioning the SPE cartridges with 10 ml methanol and 10 ml distilled water, the samples were loaded at a flow rate of 5–10 ml/min. Then, the cartridges were rinsed with 10 ml of milliQ water, and excess water was removed under vacuum for 2 h. Antibiotics retained on the cartridge were eluted with 12 ml of 5% (v/v) methanol and then reduced to dryness under nitrogen flows. Finally, the residues were diluted by adding 1 ml methanol and filtered through 0.22 µm membrane. The final extract was transferred to a 2 ml amber vial and stored at −18 °C until Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis. Before performing LC-MS/MS analysis, 100 µl aliquot of each extracted sample was evaporated and reconstituted in a mixed solvent of methanol: 0.2% formic acid and 2 mM ammonium acetate, 30:70 (v/v) [10].

The solid samples of 0.5 g of freeze-dried feces and sludge and 1 g of freeze-dried soil sample were added

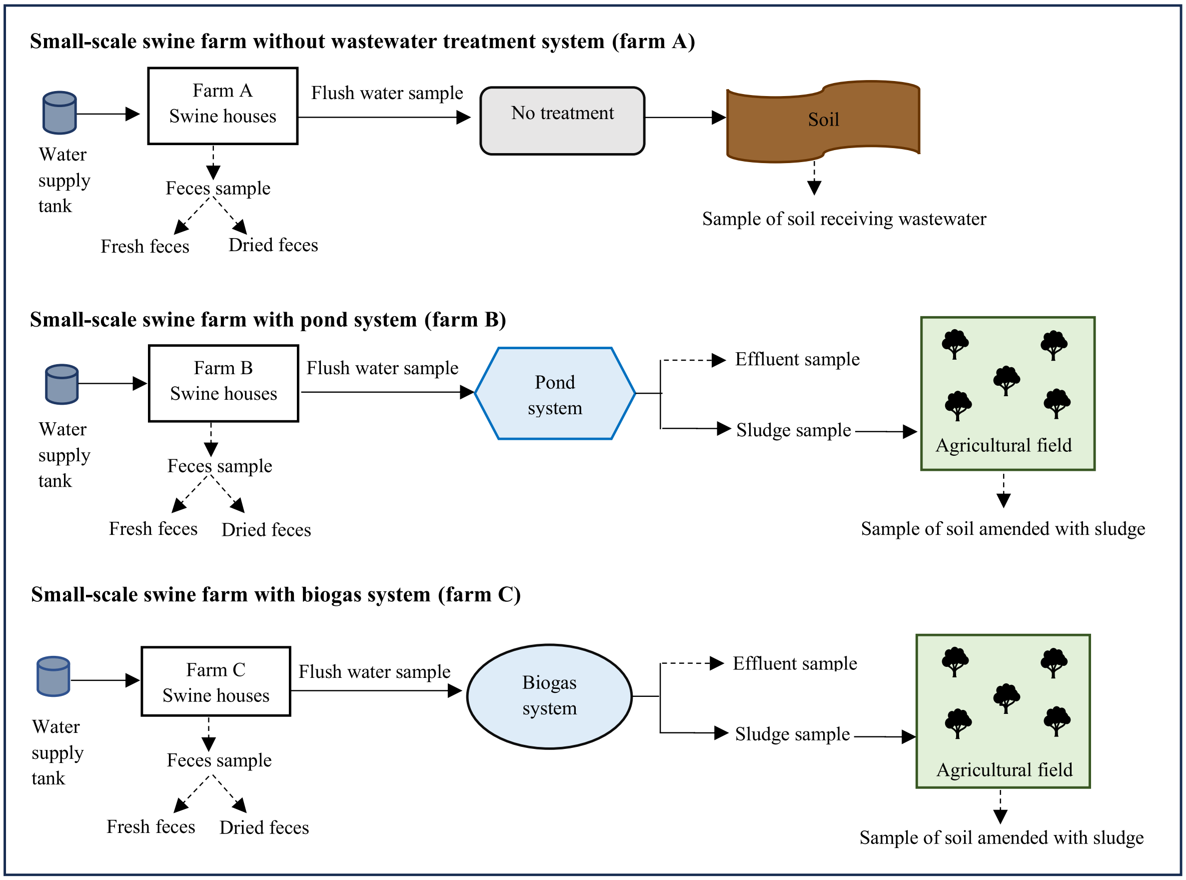

Fig. 1 The layout of sample types collected from each representative of small-scale swine farm.

with IS (100 µl each). Then, the samples were mixed and placed in a refrigerator at 4 °C overnight before extraction. The process started by adding 15 ml of phosphate buffer (pH 2) and 20 ml of acetonitrile into the samples, followed by vortex mixing for 1 min, ultrasonication for 5 min, and centrifugation at 3,000 rpm for 30 min. Then, a supernatant was transferred to a 250 ml round-bottom flask. The extraction process was repeated 3 times, and the supernatants from all extractions were combined in the round-bottom flask. The extracts from solid samples were evaporated at 50 °C to remove remaining organic solvents and diluted to 200 ml with milliQ water.

For removing negatively charged humic and fulvic acids of organic matter in the solid samples, strong anion exchange (SAX) cartridges and hydrophilic-lipophilic balance (HLB) cartridges were set up in tandem. Before the SPE cleanup, 0.5 g of Na_2EDTA was added into each aqueous extract in order to chelate with metal cations. Each tandem cartridge was preconditioned with methanol and milliQ water, 10 ml each. The diluted extract was passed through the cartridge at a flow rate of 5–10 ml/min. Then, the SAX cartridge was removed, and the HLB cartridge was rinsed with 10 ml milliQ water to remove weakly bound impurities and Na_2EDTA. The antibiotic retained on the HLB cartridge was eluted, reconstituted, and analyzed the same as those described in liquid sample analysis [10].

In this study, the 21 target antibiotics were selected based on a preliminary survey in the study area and the report of common veterinary antibiotics used in swine production in Thailand [17]. These antibiotics belonging to 9 different classes of compounds based on their usage in swine production which were 4 tetracyclines consisting of chlortetracycline (CTC), tetracycline (TC), oxytetracycline (OTC), and doxycycline (DCX); 6 fluoroquinolones comprising enrofloxacin (ENR), ceftriaxone (CTO), ciprofloxacin (CIP), nalidixic acid (NOR); ofloxacin (OFC), and norfloxacin (NFC); 2 β-lactamases comprising amoxicillin (AMX) and penicillin G (PENG); 2 macrolides containing erythromycin (ETM) and tilmicosin (TIL); 2 sulfonamide antibiotics consisting of sulfadiazine (SDZ) and sulfamethazine (SMZ); trimethoprim (TMP) in diaminopyrimidines; lincomycin (LIN) in lincosamide; tiamulin (TML) in pleuromutilins; and 2 polymyxin comprising colistin-A (CT-A) and colistin-B (CT-B).

The LC-MS/MS with an ultimate 3000 HPLC liquid phase system combined with TSQ Endura triple quadrupole mass spectrometer with an electrospray ionization source (Thermo Fisher Scientific, San Jose, CA USA) was used in this research. For quality assurance and quality control of this study, the coefficient (R^2) of determination of all calibration curves of 21 standard antibiotics indicated good linearity with R^2 greater than 0.99. The spiking recovery rates of the 21 antibiotics were tested in all sample types which ranged from 60 to 110%, while their relative standard deviations (RSDs) for 3 replicates ranged from 2.6 to 10.2%. Furthermore, the limit of detection (LOD) was calculated as the concentrations produced peak with signal-to-noise ratio (S/N) values of 3, and the results revealed the LOD of 21 antibiotics in the range of 0.01–20 µg/l.

Statistical analysis

Physicochemical characteristics and antibiotic concentrations of samples were analyzed using descriptive statistics. Antibiotic concentrations in each sample type were compared between farm types using non-parametric statistics (Kruskal-Wallis H test) at a significance level of 0.05.

RESULTS

Physicochemical properties of samples

The physicochemical properties of liquid samples are shown in Table S1. In this study, the flush water of farm A was considered as the effluent because it was directly discharged into the environment without treatment. COD, BOD, TSS, and TKN in farm A flush water were higher than those in the effluent standard of Thailand for a small-scale swine farm. For effluents of farm B and farm C, BOD and COD were higher than those in the effluent standard. This was according to Klomjek [18] and Sreesai et al [19] that found COD and BOD in the effluent of pond systems receiving swine wastewater were higher than those in the swine effluent standard. Furthermore, COD and TSS in the effluent of the biogas and pond system were lower, but BOD and TKN were greater, compared to those in the effluent of conventional wastewater treatment systems for swine wastewater in Taiwan [20].

The physicochemical properties of solid samples as fresh and dried feces, sludge, and soil samples are shown in Table S2. The range of pH, OM, and CEC of these solid samples were 6.1–6.7, 19.0–31.8%, and 3.9–5.5 cmol/kg, respectively. All soil samples were classified as sandy clay loam.

Antibiotic concentration in liquid samples

Antibiotics in flush water

In the flush water of all farm types, 19 out of the 21 target veterinary antibiotics were detected at varying concentrations of 0.35 to 892.25 µg/l (Table 1). The antibiotics found in the flush water may be influenced by the quantity and frequency of antibiotic usage in farms [11]. Among all target antibiotics in flush water, CTC, TML, and MY in tetracycline, pleuromutilin, and lincosamide classes were found at high concentrations of 826.79–900.59, 305.07–422.14, and 118.36–145.10 µg/l, respectively. These results are supported by findings of previous works from Chan et al [5] that found tetracycline, pleuromutilin, and β-lactam classes in flush water of swine farms. Similarly, Jarat et al [11] reported contamination of lincosamide, sulfonamide, and macrolide classes in flush water of swine farm, while Zhou et al [4] and Wei et al [21] found antibiotics in a class of tetracyclines in flush water of swine farm.

The results showed that the concentration of almost all antibiotics detected in the flush water of the 3 types of small-scale swine farms with different waste management systems was similar, especially the concentration of the main antibiotic used in the farm such as tetracycline, pleuromutilin, and lincosamide classes. This was due to these swine farms holding identical farm sizes, the same patterns of drug-using process, quantity, and frequency of antibiotic usage. Moreover, these small-scale swine farms also have the same way of urine and fresh feces handling.

Antibiotics are mainly used for the prevention and treatment of swine common diseases at each stage of swine production such as sows, piglets, growing, and finishing swine. Moreover, antibiotics are added to swine feed as a growth promoter. However, they cannot be completely metabolized and absorbed into the swine digestive system. Then, the metabolites of these compounds were excreted via urine and found in flush water. This is supported by the report from Cheng et al [22] that 70–90% of antibiotics were excreted through the urines and feces of swine.

In flush water, antibiotic concentration in the TC Class (CTC > DXC > TC > OTC) was the highest when compared to the other 8 classes. This was similar to the reports from Lekagul et al [3], Chan et al [5], and Wei et al [21] which detected high levels of antibiotics in TC class in the flush water of swine pen. While Zhou et al [23, 24] reported that TCs were often added to swine feed for all swine ages to improve growth and prevent swine diseases due to their low costs and a broad range of activities.

Antibiotics in effluent samples

There were 16 and 17 residual antibiotics detected in the effluents of the biogas and pond treatment systems, respectively. The average antibiotic concentrations in the effluent samples were different in the range of 0.01–245.08 µg/l (Table 1). CTC, MY, and TC were found to be dominant residual antibiotics in the effluent. However, the concentration of CTC, DXC, NOR, SDZ, SMZ, MY, and TML in the effluent showed statistical differences ($p < 0.05$) between 2

Table 1 Antibiotic concentrations in flush water samples and effluent samples.

Class	Antibiotic	Flush water sample (µg/l)			Effluent sample (µg/l)	
		Farm A (N=3)	Farm B (N=3)	Farm C (N=3)	Farm B (N=3)	Farm C (N=3)
Tetracycline	CTC	892.25±11.45	854.17±27.13	835.07±9.54	245.08±5.12[a]	179.83±0.72[b]
	TC	89.53±5.81	85.46±2.45	83.59±2.97	46.92±2.68	45.86±3.92
	OTC	17.92±3.42	23.98±1.38	25.16±3.49	8.20±0.19	5.25±0.52
	DXC	120.01±6.87	110.15±13.22	99.73±17.09	40.33±0.29[a]	29.43±0.51[b]
Fluoroquinolone	ENR	67.98±8.41	66.64±14.18	64.64±8.53	23.68±0.01	37.31±0.01
	CTO	90.01±11.69	99.68±19.06	90.01±12.38	5.84±0.02	2.31±0.02
	CIP	7.41±1.12	8.58±0.18	8.19±0.09	2.88±0.01	0.86±0.02
	NOR	0.61±0.24[a]	0.35±0.62[ab]	0.17±0.45[b]	0.13±0.05[b]	0.16±0.05[a]
	OFC	ND	ND	ND	ND	ND
	NFC	ND	ND	ND	ND	ND
β-lactam	AMK	57.06±10.81	62.21±5.43	67.65±8.17	28.78±7.29	22.97±7.29
	PCN	0.66±0.24	0.72±0.28	0.86±0.12	0.01±0.01	ND
Macrolide	ETM	2.69±0.33	2.70±0.57	2.09±0.57	1.45±0.39	0.26±0.27
	TIL	104.36±14.32	32.63±5.74	97.63±9.55	0.53±0.72	0.22±0.19
Sulfonamide	SDZ	6.65±0.70	7.62±0.60	6.91±0.32	3.66±0.90[a]	1.41±0.10[b]
	SMZ	4.10±0.85[b]	8.76±0.61[a]	7.33±0.40[ab]	4.58±0.16[a]	1.31±0.03[b]
Diaminopyrimidine	TMP	3.23±0.93	3.00±0.95	3.12±0.99	0.23±0.19	1.25±0.13
Lincosamide	MY	129.61±13.87	129.61±13.86	129.61±13.87	51.83±6.64[a]	39.50±0.50[b]
Pleuromutilin	TML	402.29±16.17	398.09±16.24	360.52±58.78	15.70±17.85[a]	10.19±8.89[b]
Polymyxin	CT-A	5.92±7.97	5.65±7.64	4.13±5.32	ND	ND
	CT-B	1.47±0.25	ND	ND	ND	ND

The data show Mean±SD; ND = Not Detected. ND was not included in the statistical comparison, and values followed by the same letter are not significantly different at a p-value of 0.05.

wastewater treatment systems as all of these antibiotics in the effluent of biogas treatment systems were found at high concentration except NOR. This was due to not only the operational parameters such as biomass concentration, retention time, pH, and temperature that affect antibiotic removal efficiency of each wastewater treatment system but also the physicochemical properties of antibiotics particularly the K_{ow} (octanol-water coefficient) and an antibiotic adsorption process in the treatment systems [22, 25]. The results of this study showed that the concentrations of antibiotics in the effluent were lower than those in the flush water. This indicated that antibiotics in wastewater were removed by the wastewater treatment systems of the swine farms. However, some compounds remained in the effluents. These results were in line with earlier studies that found the same classes of antibiotics in the effluents of the swine wastewater treatment system [5, 21, 26–28].

Antibiotics in solid samples

Antibiotics in feces and sludge

There were 16 and 13 target antibiotics found in fresh and dried feces at different average concentrations in the range of 0.01–498.38 and 0.01–122.72 µg/kg, respectively (Table 2). Relatively high concentrations of CTC, TML, and MY were found in the fresh and dried feces. There were 12 and 13 target antibiotics in sludge from biogas and pond systems, respectively (Table 2). CTC, TML, and MY were the dominant residual antibiotics in the sludge of both systems. This result was in line with the report from Chan et al [5] that found antibiotics in the class of tetracycline (520 µg/kg of DCX; 81 µg/kg of CTC; 13 µg/kg of TC; and 7 µg/kg of OTC), in the class of pleuromutilin (160 µg/kg of TIL), and in the class of fluoroquinolones (3 µg/kg of ENR) in the sludge of pond system. Zhang et al [29] also reported antibiotics in the class of tetracycline (21.9–23.6 µg/g of CTC and 28.6–31.1 µg/g of OTC) in the sludge of pond system. While Jarat et al [11] found antibiotics in the lincosamide class (4,090 µg/kg of LIN) in the sludge of biogas system.

Solid waste was considered a source of antibiotic distribution as it was applied to soil for nutrient supplements. Thus, this study compared antibiotic concentration in dried feces and sludge. Results revealed that most of the target antibiotics showed significantly higher concentration levels in the dried feces than in the sludge from both treatment systems. It can be explained by biodegradation processes in the treatment systems whereby most of the antibiotics were decomposed by microorganisms [30] and left at low concentration levels in the sludge. The biodegradation process was reported as the most important mechanism for removing antibiotics in swine wastewater [27]. This process occurred under both aerobic and anaerobic conditions [27] as some groups of microorganisms

Table 2 Antibiotic concentrations (µg/kg) in solid samples.

Antibiotic	Fresh feces sample			Dried feces and Sludge sample			Soil sample		
	Farm A (N=3)	Farm B (N=3)	Farm C (N=3)	Farm A, B, and C (N=9)	Farm B (N=3)	Farm C (N=3)	Farm A (N=3)	Farm B† (N=3)	Farm C† (N=3)
CTC	498.38±7.36^{a}	444.62±36.55^{ab}	354.46±27.54^{b}	122.72±26.95^{a}	104.83±7.49^{ab}	80.84±9.63^{b}	98.60±0.56^{a}	45.64±0.56^{ab}	35.50±0.50^{b}
TC	41.53±3.06^{a}	32.34±4.97^{ab}	11.16±1.60^{b}	19.32±3.62^{a}	18.66±1.39^{ab}	4.70±0.57^{b}	ND	9.33±0.29	1.87±0.03
OTC	11.29±4.06	12.14±3.85	12.06±1.30	8.73±1.91	8.64±3.34	6.66±1.89	ND	10.80±2.63	2.67±0.18
DXC	50.50±0.50^{a}	38.17±0.29^{ab}	31.33±0.58^{b}	24.00±0.50^{a}	22.83±0.29^{ab}	19.33±0.29^{b}	ND	12.22±1.97	ND
ENR	28.35±1.68^{a}	23.35±4.40^{ab}	10.81±0.72^{b}	9.19±1.94	9.17±2.16	8.35±2.86	0.27±0.23	2.37±0.15	0.36±0.22
CTO	37.00±0.00^{a}	35.92±0.14^{ab}	33.17±0.29^{b}	25.23±1.84	23.93±0.06	23.27±0.46	ND	5.23±4.40	2.88±2.52
CIP	2.48±0.03^{a}	2.28±0.03^{ab}	2.18±0.03^{b}	1.79±0.10^{a}	1.74±0.05^{ab}	1.67±0.00^{b}	ND	0.27±0.00	0.11±0.05
NOR	0.07±0.02^{a}	0.02±0.01^{ab}	0.01±0.01^{b}	0.01±0.01	0.01±0.01	ND	ND	ND	ND
OFC	ND	ND	ND	ND	ND	ND	ND	ND	ND
NFC	ND	ND	ND	ND	ND	ND	ND	ND	ND
AMK	16.14±3.14	28.80±3.51	31.34±6.80	ND	ND	ND	ND	ND	ND
PCN	ND	ND	ND	ND	ND	ND	ND	ND	ND
ETM	1.36±0.12	0.60±0.27	0.48±0.22	ND	ND	ND	ND	ND	ND
TIL	15.28±4.63	10.68±0.28	10.17±0.29	ND	ND	ND	ND	ND	ND
SDZ	2.54±1.16	3.68±1.02	2.96±0.88	1.07±0.09^{a}	1.07±0.13^{ab}	0.91±0.12^{b}	ND	0.77±0.06	0.37±0.06
SMZ	3.21±0.12	4.54±0.71	2.97±0.46	1.22±0.08^{a}	1.17±0.03^{ab}	0.99±0.01^{b}	ND	0.83±0.06	0.67±0.06
TMP	1.25±0.22	1.45±0.37	1.24±0.98	0.09±0.07^{a}	0.06±0.00^{ab}	0.04±0.00^{b}	ND	1.09±0.99	0.71±0.58
MY	56.67±1.53^{a}	53.33±0.58^{ab}	50.17±0.29^{b}	27.71±5.26	24.00±0.54	25.48±4.72	ND	14.67±0.29	18.33±0.29
TML	217.81±38.39	173.48±6.89	118.23±11.24	111.35±15.73^{a}	107.74±13.51^{ab}	69.00±1.00^{b}	ND	35.42±0.52	28.17±0.29
CT-A	ND	ND	ND	ND	ND	ND	ND	ND	ND
CT-B	ND	ND	ND	ND	ND	ND	ND	ND	ND

The data show Mean±SD; ND = Not Detected. ND was not included in the statistical comparison, and values followed by the same letter are not significantly different at a p-value of 0.05.
† Soil amended with sludge.

could develop resistance to the antibiotics and be able to metabolize them [31]. In addition, Kihampa [32] reported that high antibiotic removal efficiencies were observed during the pond treatment process related to its fast degradation through hydrolytic cleavage and finally mineralized to CO_2 and water. However, high adsorption of antibiotics onto sediment or sludge could delay their degradation through the activities of microorganisms [33] and let them in the sludge. A drying process under daylight of the sludge from the pond and biogas systems also induces the degradation and evaporation of antibiotics and reduces their antibiotic concentration [34]. Therefore, the solid waste in the form of sludge showed less antibiotic concentration than that in the form of dried feces in which their antibiotics were only removed by the drying process.

Antibiotics in soil samples

Only 2 target antibiotics (CTC and ENR) were detected in the soil receiving wastewater of farm A, while soil amended with sludge from farms B and C contained 11 and 12 target antibiotics, respectively, with concentrations ranging from 0.11 to 45.64 µg/kg (Table 2). The result showed the antibiotics in classes of tetracycline, fluoroquinolone, sulfonamide, diaminopyrimidines, lincosamide, and pleuromutilins were found in the soil amended with sludge from the biogas and pond treatment systems. This was similar to that reported by Zhang et al [29] on the contamination of CTC, TC, and OTC in vineyard soil applied with biogas slurry, and in line with previous report demonstrating that agricultural soil treated with manure was contaminated with antibiotics [26]. It could thus be assumed that antibiotics in sludge are released into the soil. The distribution of antibiotics in the soil can be affected by many factors such as types of antibiotics, frequency of antibiotic usage, and environmental factors [35].

The antibiotics in soils can be degraded or inactivated through abiotic or biotic processes including transformation and degradation [35, 36] and sorption and desorption onto soil components [37]. For most antibiotics, the sorption and desorption processes are not only the functions of their polarity and water solubility, but also controlled by pH, speciation of antibiotic compounds, soil properties, organic matter contents, and types of divalent cations [29, 35]. These processes also affect the leaching and transportation of antibiotics into groundwater or surface water [38]. Antibiotic degradation, hydrolysis, and photolysis are considered the most important pathways for the abiotic degradation of antibiotics as Braschi et al [39] and Mitchell et al [40] reported that antibiotics in the β-lactam class are especially susceptible to hydrolytic degradation, whereas antibiotics in quinolone and tetracycline classes were degraded through a photolysis process when they were spread on the soil surface during an application of manure and slurry to agricultural sites [34]. The results of this research indicated that the application of swine solid waste contaminated with antibiotics can cause antibiotic distribution in the environment with different antibiotic concentrations depending on the waste management. This should be a concern because antibiotic residuals may encourage antibiotic resistance of microorganisms in soil and environment.

CONCLUSION

The occurrence of antibiotics in various types of waste and treated waste from small-scale swine farms was investigated. The concentration of target antibiotics in flush water was a statistical difference between farm types. Most of the target antibiotics were found remaining in the effluent from pond and biogas wastewater treatment systems. Antibiotics contaminated in dried feces were found at higher concentrations compared to those in sludge from both treatment systems. Meanwhile, sludge and soil amended with sludge from pond system contained higher antibiotic concentration than those from biogas system. CTC, TML, and MY were the dominant antibiotics in most samples. It can be concluded that swine waste is a major source of veterinary antibiotic contamination in the environment through swine manure and urine. Pond and biogas wastewater treatment systems could reduce some antibiotics in wastewater. Besides, all types of contaminated solid waste should be treated properly to prevent antibiotic distribution and contamination of the environment. The standard criteria for antibiotic concentration in effluent and sludge from swine farms should be established.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.051.

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Appendix A. Supplementary data

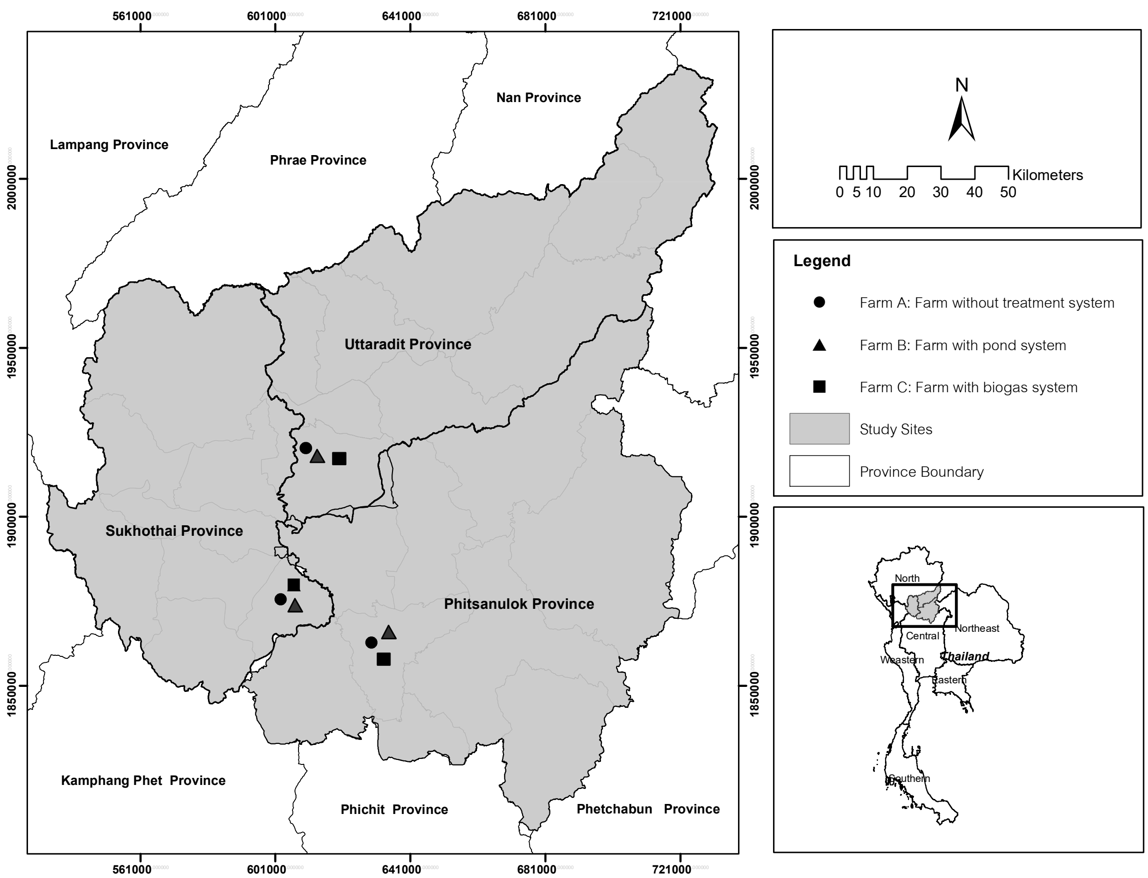

Fig. S1 Map of the study sites in Lower Northern, Thailand.

Table S1 Physicochemical properties of liquid samples.

Liquid sample	pH	Temp. (°C)	EC (ms/cm)	COD (mg/l)	BOD (mg/l)	TSS (mg/l)	TKN (mg/l)
Flush water							
Farm A	6.78±0.08	28.17±0.29	5.12±0.50	1,418.78±158.43	358.71±114.63	154.22±22.93	216.84±59.69
Farm B	7.12±0.39	29.33±0.29	4.40±0.16	1,201.22±270.17	361.89±32.52	156.11±20.02	155.75±22.44
Farm C	7.49±0.35	30.00±0.00	4.97±0.28	2,130.53±694.67	492.24±79.19	319.78±33.05	234.87±25.21
Effluent							
Farm A	6.78±0.08	28.17±0.29	5.12±0.50	1,418.78±158.43	358.71±114.63	154.22±22.93	216.84±59.69
Farm B	7.55±0.05	31.00±0.50	2.32±0.22	380.17±0.29	116.32±1.42	49.17±0.76	199.83±1.26
Farm C	7.75±0.05	32.33±0.29	3.78±0.21	351.83±1.04	84.67±0.29	40.56±0.41	96.67±2.89
Standard†	5.50–9.00	–	–	350.00	80.00	200.00	200.00

The data show Mean ± SD; Number of the sample (N) = 3. The effluent standard in this table is determined for swine farm type B (500–5,000 pigs) and type C (50–500 pigs).

† Source: Thailand's effluent standard for swine farm, Ministry of Natural Resources and Environment, Thailand [41].

Table S2 Physicochemical properties of solid samples.

Solid sample	pH	OM (%)	CEC (cmol/kg)	TN (mg/kg)	P (mg/kg)	K (mg/kg)	Soil texture
Feces							
Fresh feces	6.69±0.09	31.81±0.73	3.91±0.11	2.02±0.30	5.25±0.15	1.40±0.12	–
Dries feces	6.26±0.16	23.35±1.96	4.33±0.42	1.82±0.30	5.03±0.12	1.05±0.40	–
Sludge							
Farm B	6.36±0.36	23.12±0.84	4.59±0.27	1.71±0.09	4.53±0.11	1.01±0.09	–
Farm C	6.32±0.12	25.03±0.45	5.21±0.06	1.95±0.05	4.86±0.07	1.29±0.04	–
Soil							
Farm A	6.20±0.65	19.04±0.32	4.09±0.09	0.82±0.15	3.44±0.16	0.26±0.08	Sandy clay loam
Farm B[†]	6.05±0.09	23.13±0.42	4.87±0.19	1.51±0.09	4.16±0.14	0.96±0.08	Sandy clay loam
Farm C[†]	6.23±0.09	24.85±0.38	5.52±0.11	1.80±0.05	4.29±0.08	1.27±0.07	Sandy clay loam

The data show Mean ± SD; Number of the sample (N) = 3.
[†] Soil amended with sludge.