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Systematic analysis of occurrence, density and ecological risks of 45 veterinary antibiotics: Focused on family livestock farms in Erhai Lake basin, Yunnan, China☆

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A B S T R A C T

Antibiotic pollution from family animal farms is often neglected, but the waste from these farms usually caused more harm to the surroundings because arbitrary discharge without effective disposal. The pollution status and ecological risks of 45 veterinary antibiotics on 33 family animal farms in Dali city, Erhai Lake basin of China, were firstly delivered. The results showed that antibiotic contamination was prevalent in different environmental mediums (feed, manure, wastewater and soil) on these family farms. Manure had highest antibiotic levels among all the environmental mediums. Tetracyclines (TCs) usually had higher concentrations (ND-404.95 mg/kg) than the other classes, among which chlorotetracycline (CTC) was the dominant type. Among different animal species, target 13 pig farms had the highest antibiotic concentrations, the most total types and unique types of antibiotics, which were followed by target 11 chicken farms then target 9 cattle farms. The antibiotic densities of animal waste were calculated by per animal, which showed that pig waste presented high density; and family chicken farms were characterized by quinolone antibiotics (QAs) and macrolide antibiotics (MAs) pollution. For the antibiotic ecological risks in effluent water, oxytetracycline (OTC), CTC, ofloxacin (OFL), enrofloxacin (ENR), ciprofloxacin (CIP) and sulfamethoxazole (SMX2) exhibited much more toxic effects on algae. OTC and doxycycline (DXC) posed high risk for invertebrate; while no antibiotic caused high ecological risk for fish. Some antibiotics were quantitatively detected in the soil but no antibiotic posed obvious ecological risks on soils. However, the interaction of synergistic or antagonistic effects between different antibiotics should be brought to the forefront. This study gave some information of antibiotic pollution on family livestock farms, which indicated that animal waste from family farms was indeed an important pollution source of antibiotics for the environment.

1. Introduction

Antibiotics, as one of emerging contaminants, have received increasing attention in recent years. Especially in China, a large amount of antibiotics are used every year, for example, in 2013, about 162,000 t of antibiotics was used, among which more than half (about 52%) was consumed for animal producing, to treat diseases or as feed additive (Zhang et al., 2015). Due to the incomplete metabolism by animal body, a large percentage (from 30% to 90%) of the used antibiotics might be excreted with urine and feces (Zhi et al., 2018; Chen et al., 2017). Moreover, China is one of the biggest producers of livestock, and about 51.6% of global pig population was produced in China in 2013 (Zhou et al., 2013a; Wei et al., 2011). Then a huge amount of animal waste is produced every year. If not being properly treated, these antibiotics in animal waste could further contaminate the soil (Wei et al., 2019), water (Kovalakova et al., 2020), food (He et al., 2016), and even develop antibiotic resistance genes (Zhang et al., 2019). Recently, with the increasing of safety awareness, many alternatives for antibiotics have been developed, but it is hard to replace the efficacy of antibiotics in the short term (Zhang et al., 2018; Suresh et al., 2018). Therefore, without an outright ban on the use of antibiotics as feed

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additives, veterinary antibiotics are still very attractive to farmers and are still widely used in livestock and poultry breeding industry in many countries.

Up to now, researchers have paid great attention to the antibiotic contamination on livestock farms, which usually focused on the large-scale and intensive farms. Researchers have provided many results about the antibiotics levels in animal wastes (manure and wastewater) on intensive farms. For example, Zhi et al. (2018) reported the high prevalence of antibiotics on intensive pig farm and dairy farm wastewater, which showed that chlorotetracycline (CTC, 130.67 μg/L), oxytetracycline (OTC, 82.59 μg/L) and doxycycline (DXC, 89.46 μg/L) were the dominant on pig farms while OTC (60.15 μg/L) and lincomycin (LIN, 34.82 μg/L) were the dominant antibiotics on dairy farm. Zhao et al. (2010) gave some results about veterinary antibiotic residues in manures in eight provinces of China, OTC were commonly detected in pig and cow dung, respectively; enrofloxacin (ENR) and norfloxacin (NOR) were dominant in chicken dung. Moreover, a rising number of researches focused on the potential risk of antibiotics from livestock waste, which was regarded as an important pollution source of antibiotics to the ambient mediums. Zhang et al. (2018) investigated two full-scale swine farms in South China, and the results showed that OTC and LIN were high levels in swine waste. In addition, the sludge and manure from these farms could pose potential risk for antibiotics spread. Mahmoud and Abdel-Mohsein (2019) implied that tetracycline antibiotics in intensive poultry farms could cause great risk to agricultural land if using broiler litter as fertilizer. Wei et al. (2019) studied antibiotic pollution in vegetable farm soil which was fertilized by livestock manure, which indicated that some antibiotics (OTC, CIP, etc.) indeed caused high risks for the land soils. More seriously, some human infections caused by zoonoses bacteria have been reported (Fey et al., 2000). Now, some new type of diseases (like novel coronavirus infection) gives us a wake-up call. Antibiotics, especially some sharing between animal and human, really need widespread attention.

From the above, the studies related to veterinary antibiotics contamination has become hotspot for researchers. However, the previous studies have mainly focused on the large-scale and intensive farms. What is the current pollution status of veterinary antibiotics on family livestock farms? What are the ecological risks of family farms to the surrounding environment? Which livestock species causes more pollution? All such questions are still unclear. It was reported that family mode farms are the common mode that can't be ignored for rural area in China (Gu et al., 2020; Cai et al., 2020). This kind of farms usually had small animal numbers (e.g. <500 pigs), but occupied a high percentage (58.2%) of the total farm numbers in the Chinese rural area (Gu et al., 2020; Veeck and Shaohua, 2000). Moreover, such large number of family livestock farms usually scattered all over the rural area, which brought misery to the waste collection. Importantly, these family farms might use antibiotic additives in animal diet (not forbidden at sampling period in China) and no effective disposal facilities for waste (Cai et al., 2020). Therefore, livestock waste from these family farms may be carrying high antibiotic concentrations, which is discharged arbitrarily without sufficient treatment. This absolutely brings greater harm to ecological environment than intensive farms with relatively effective processing facilities. Therefore, it is of great significance to provide the antibiotic pollution status and assess potential ecological risks of family livestock waste. Moreover, the antibiotics types studied in previous studies were relatively limited. For example, Mahmoud and Abdel-Mohsein (2020) just selected 4 types of TCs to assess ecological risk of animal waste on fish in Egypt. Wei et al. (2019) investigated 17 veterinary antibiotic residues in land soil for vegetable farm. The present study would give a comprehensive study about the pollution status of 45 antibiotics on 33 family farms.

To our best knowledge, the pollution pattern of veterinary antibiotic in Erhai Lake basin of Dali City hasn't been reported yet, which is characterized by family breeding farms. Therefore, we targeted at 45 antibiotics (5 classes), including tetracycline antibiotics (TCs), sulfonamides antibiotics (SAs), quinolones antibiotics (QAs), macrolides antibiotics (MAs) and β-lactams antibiotics (LAs). 33 family animal farms, including 7 dairy farms and 2 beef farms, 3 broiler farms, 8 layer farms and 13 pig farms, were selected from all over the Dali city. Therefore, the purposes of this study are: (1) to give a comprehensive study of antibiotics on family animal farms, including the occurrence and distribution and so on; (2) to trace the distribution of antibiotics in different environmental mediums (feed-waste-soil/effluent); (3) to compare the effects of different livestock species on antibiotic type and antibiotic density by per animal; (4) to assess the ecological risks of antibiotics on farmland soils and effluent wastewater surrounding these family livestock farms.

2. Materials and methods

2.1. Materials and instruments

This study selected 45 target antibiotics belonged to 5 classes (5 TCs, 17 SAs, 15 QAs, 6 MAs and 2 LAs). The full names, their abbreviations, manufacturer and grades were shown in the S1 in the supplementary materials. Other materials contained Acetonitrile (ACN), formic acid, methanol (MeOH), and disodium ethylenediaminetetraacetate (Na_2-EDTA). The manufacturer and grades were shown in the S1 in the supplementary materials. The instruments included N-EVAP 112 nitrogen evaporator and rotary evaporator. The standard stock solutions and standard working solutions were prepared according to the existing study (Zhi et al., 2018).

2.2. Sampling sites and sample collection

Dali City of Yunnan province is characterized by family breeding farms which usually have small number of animals and consistent operation mode. 33 representative family animal farms were selected in Dali city, which belongs to Erhai plain on the Yunnan-Guizhou plateau and is one of the famous historical cultural cities with tourist attraction. The area is about 1468 km^2, 70% of which is mountain area and water area accounting for 15% (Erhai Lake). It has about 652,000 people in the city. All the samples sites were scattered throughout the whole city, as shown in Fig. 1. The detail information of these family farms was shown in supplementary materials (Table S1), including location, animal number and so on.

In total, we collected 179 samples from the target 33 family animal farms, containing 38 feed samples, 49 manure samples, 34 wastewater samples and 58 soil samples. 38 feed samples and 49 manure samples were obtained according to the different livestock types and different pig ages. 34 wastewater samples contained 17 influent samples and 17 effluent samples, where the influent and effluent mean the wastewater directly from piggery or cowshed and after simple storage pool, respectively (chicken farms have no wastewater). 58 soil samples included 29 reference soil (R-) samples and 29 fertilized soil (F-) samples by livestock waste. It should be noted that, because of the terrain, some districts have few farms for samples.

The specific procedures of sample collection are conducted according to our previous report (Zhi et al., 2018) and are shown in Section S2 in supplementary materials.

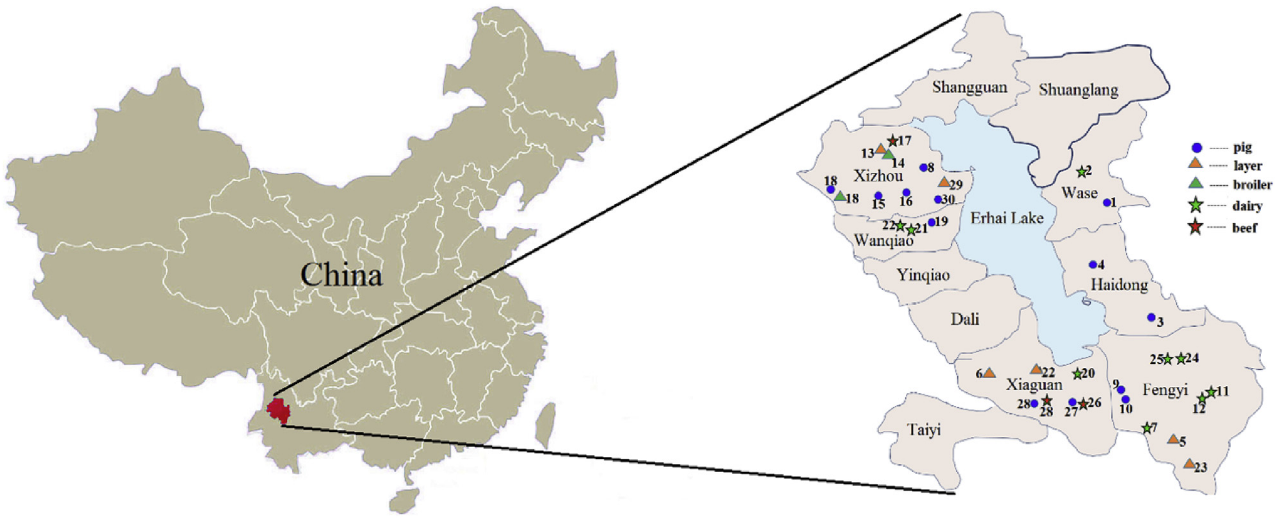

Fig. 1. Sample location for different farms (the number means sampling sequence).

2.3. Sample preparation

For water samples, the preparation was conducted according to Zhi et al. (2018). Briefly, the volume for most of the wastewater samples was 50 mL, and a few effluent samples which were relative clean had 100 mL volume. To weaken the binding effects between some antibiotics and cations, adding 0.1 g $Na_2EDTA \cdot 2H_2O$ into wastewater samples was adopted. Then formic acid solution was used to adjust the pH of water samples to around 3.0. For the solid samples, they were firstly freeze-dried, followed by being grinded evenly. Then the solid samples (1.0 g for manure, 5.0 g for soil and feed) were extracted by a mixed liquor of MeOH: ACN: citrate buffer ratio = 1:1:2 for 2 times.

Then all the water samples or the extracts for solid samples would go through the solid phase extraction (SPE) procedure with Oasis HLB cartridges, cleaning, elution, N_2 blowing and re-dissolving. The obtained liquids were filtered and stored in the refrigerator until analysis. Specific program parameters were shown in Section S3.

2.4. Sample analysis

All the target veterinary antibiotics were analyzed by high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), and the conditions were conducted according to the previous report (Zhi et al., 2018).

2.5. Ecological risk

Risk quotient (*RQ*) was usually calculated for different environmental mediums to evaluate the ecological risks of the detected antibiotics. As reported, there are 4 levels of ecological risks, according to the *RQ* values: $RQ \geq 1$ (high risk), $0.1 \leq RQ < 1$ (median risk); $0.1 \leq RQ < 0.01$ (low risk) and $RQ \leq 0.01$ (no risk) (Wei et al., 2019). In this study, the *RQ* values were calculated for effluent water and soil fertilizered by manure, according to the methods in the previous publications (Yang et al., 2016; Xie et al., 2019; Xu et al., 2013).

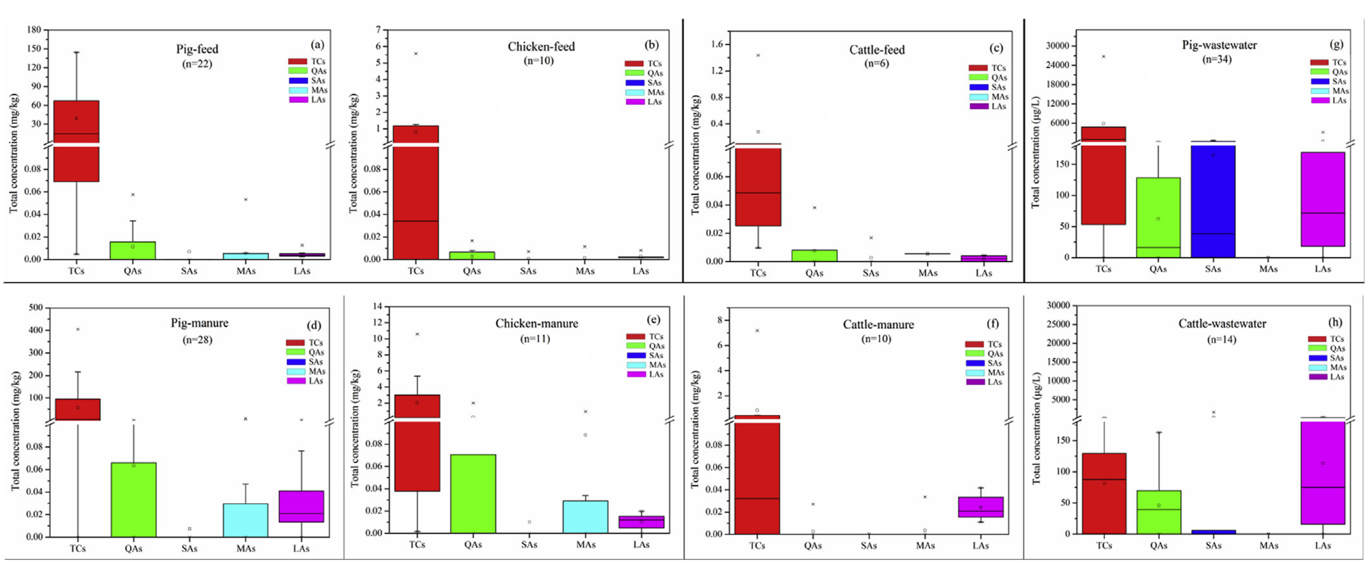

Fig. 2. Total concentration of antibiotics for different animal species.

3. Results

3.1. Total antibiotics on different livestock farms

So far there is no antibiotic pollution data on family farms, thus this study has provided a comprehensive investigation on pollution status of 45 antibiotics on 33 farms of Erhai Lake basin. It was shown that veterinary antibiotics were high prevalent on these family livestock farms. Fig. 2 shows the total concentration of antibiotics for different livestock species. We can see that family pig farms had highest concentration of antibiotics, followed by family chicken farms and then cattle farms. For example, in feed, the TCs concentrations were 0.0–144.61 mg/kg for pig farms (Fig. 2 (a)), which was higher than those of chicken farms (ND-5.57 mg/kg) (Fig. 2 (b)) and cattle farms (ND-1.44 mg/kg) (Fig. 2 (c)). In manure, TCs concentrations were 0.0–404.95 mg/kg for pig farms (Fig. 2 (d)), but they were ND-10.60 mg/kg and ND-7.18 mg/kg for chicken (Fig. 2 (e)) and cattle farms (Fig. 2 (f)), respectively. In wastewater, they were 0.0–21930.43 μg/L for pig farms and ND-188.62 μg/L (Fig. 2 (g)) for cattle farms (Fig. 2 (h)). QAs, SAs and MAs also had higher concentrations in the pig farm, followed by chicken farms, as a whole. However, cattle farms usually had higher levels of LAs residue: LAs presented higher levels on cattle farms than those on chicken farms in feed and manure; and especially higher level in cattle farm wastewater (Fig. 2 (h)) than those in pig farm wastewater (Fig. 2 (g)).

3.2. Single antibiotic concentration on different livestock farms

This section will focus on the residual levels of single type/kind of antibiotics on different livestock farms. The concentrations of specific types of antibiotics on different livestock farms were shown in Tables 1–4 (feed, manure, wastewater and soil). Overall, TCs were the dominant types on all these farms, with high concentrations and high frequency of occurrence. Table 1 shows OTC had highest concentration in feed (ND-143.98 mg/kg), followed by CTC (ND-90.95 mg/kg). As shown in Table 2, DXC had highest concentration in manure (ND-370.34 mg/kg), followed by CTC (ND-

Table 1
Single antibiotic concentration in different animal feed.

Antibiotics		Antibiotics concentration in feed (mg/kg)								
		Pig (n = 22)			Cattle (n = 6)			Chicken (n = 10)		
Class	type	min	max	mean	min	max	mean	min	max	mean
TCs	OTC	–	143.98	19.38	–	0.02	0.00	–	0.09	0.02
	DXC	–	0.88	0.09	–	0.10	0.03	–	10.49	1.28
	CTC	–	90.95	17.12	–	1.41	0.24	–	5.22	0.76
	DMC	–	0.12	0.02	–	0.01	0.00	–	0.01	0.00
	TC	–	4.75	0.86	–	–	–	–	0.14	0.02
QAs	OFL	–	0.01	0.00	–	–	–	–	0.02	0.00
	ENR	–	–	–		–	–	–	–	–
	DIF	–	–	–	–	–	–	–	–	–
	SPA	–	–	–	–	–	–	–	–	–
	NAL	–	–	–	–	–	–	–	–	–
	FLE	–	0.01	0.00	–	–	–	–	–	–
	LOM	–	–	–	–	–	–	–	–	–
	SAR	–	–	–	–	–	–	–	–	–
	CIP	–	0.01	0.00	–	–	–	–	0.01	0.00
	ENO	–	0.02	0.00	–	–	–	–	–	–
	FLU	–	–	–	–	–	–	–	–	–
	CIN	–	–	–	–	–	–	–	–	–
	ORB	–	0.00	0.00	–	0.03	0.00	–	–	–
	NOR	–	0.01	0.00	–	–	–	–	–	–
	OXO	–	–	–	–	–	–	–	–	–
SAs	SC	–	–	–	–	–	–	–	–	–
	SIM	–	–	–	–	–	–	–	–	–
	SDZ	–	–	–	–	–	–	–	–	–
	STZ	–	–	–	–	–	–	–	–	–
	SMX1	–	–	–	–	–	–	–	–	–
	SPD	–	–	–	–	–	–	–	–	–
	SMR	–	0.11	0.00	–	0.02	0.00	–	0.00	0.00
	SMM	–	0.00	0.00	–	–	–	–	–	–
	SDMD	–	0.00	0.00	–	–	–	–	–	–
	SMT	–	–	–	–	–	–	–	–	–
	SDX	–	–	–	–	–	–	–	–	–
	SMX2	–	–	–	–	–	–	–	–	–
	SIX	–	–	–	–	–	–	–	–	–
	SB	–	–	–	–	–	–	–	–	–
	SDM	–	–	–	–	–	–	–	–	–
	SQX	–	–	–	–	–	–	–	–	–
	SME	–	0.00	0.00	–	–	–	–	–	–
MAs	RTM	–	–	–	–	–	–	–	–	–
	CLA	–	–	–	–	–	–	–	–	–
	AZI	–	0.01	0.00	–	–	–	–	–	–
	SPI	–	–	–	–	–	–	–	–	–
	TIL	–	0.05	0.00	–	0.00	0.00	–	0.01	0.00
	LIN	–	–	–	–	–	–	–	–	–
LAs	OXA	–	–	–	–	–	–	–	–	–
	PENG	–	24.35	1.11	–	0.01	0.00	–	0.00	0.00

206.25 mg/kg); while in wastewater, CTC was highest (ND-25008.78 μg/L), followed by DXC (ND-3203.85 μg/L). Soil samples had relative low antibiotic concentrations, with highest concentration (305.56 μg/L) of CTC. Among QAs, orbifloxacin (ORB, ND-0.03 mg/kg) was highest in cattle feed (Table 1); while CIP (ND-2.01 mg/kg) and ofloxacin (OFL, ND-1259.15 μg/L) were highest in chicken manure (Table 2) and pig wastewater (Table 3), respectively. For SAs, some types of antibiotics were also detected: sulfamerazine (SMR) in pig feed, sulfaquinoxaline (SQX) in chicken manure and soil, and sulfamonomethoxine (SMM) in cattle wastewater. Among LAs, penicillin G (PENG) had high frequency of occurrence with the highest concentration of 3145.18 μg/L in pig wastewater. Among MAs, only tilmicosin (TIL) and azithromycin (AZI) were quantitatively detected in feed and manure (none detected in wastewater and soil). For different livestock species, pig farms usually had higher antibiotic concentrations as a whole, which was in accord with the conclusion in section 3.1.

3.3. Total antibiotic concentrations and detection rates in different environmental mediums

Although antibiotic residues have been compared among different animal species, it is also necessary to compare the residual characteristics of different environmental mediums, including soil samples. The total antibiotic concentrations for 5 classes in feed, manure, wastewater and soil were shown in Fig. S2 (a)–(d), respectively; the detection rates of different antibiotic types were shown in Fig. 3. For different environmental mediums, the total concentrations of TCs, QAs and MAs were in the order of manure samples>feed samples>water samples>soil samples. Total TCs concentrations were ND-144.61 mg/kg, ND-404.95 mg/kg, ND-21930.43 μg/L and ND-781.34 μg/kg for feed, manure, wastewater and soil, respectively. For all these 167 samples, TCs had the highest residual levels in different environmental mediums. The detection rates of TCs were also the highest, and had an order of manure (0–94.0%)>wastewater (0–85.0%)≈feed (0–84.0%)>soil (0–9.0%)

Table 2
Single antibiotic concentration in different animal manure.

Antibiotics		Antibiotics concentration in manure (mg/kg)								
		Pig (n = 28)			Cattle (n = 10)			Chicken (n = 11)		
class	type	min	max	mean	min	max	mean	min	max	mean
TCs	OTC	–	120.50	12.80	–	0.44	0.05	–	2.64	0.26
	DXC	–	370.34	14.48	–	0.44	0.08	–	10.49	1.28
	CTC	–	206.25	29.53	–	6.64	0.67	–	4.99	0.47
	DMC	–	0.19	0.03	–	0.01	0.00	–	0.01	0.00
	TC	–	5.61	0.66	–	0.20	0.02	–	0.14	0.02
QAs	OFL	–	0.04	0.00	–	–	–	–	–	–
	ENR	–	0.13	0.01		–	–	–	0.14	0.01
	DIF	–	–	–	–	–	–	–	–	–
	SPA	–	0.02	0.00	–	–	–	–	0.02	0.00
	NAL	–	–	–	–	–	–	–	–	–
	FLE	–	0.07	0.01	–	–	–	–	–	–
	LOM	–	–	–	–	–	–	–	–	–
	SAR	–	0.04	0.00	–	–	–	–	0.13	0.01
	CIP	–	0.55	0.02	–	–	–	–	2.01	0.19
	ENO	–	0.08	0.00	–	–	–	–	–	–
	FLU	–	–	–	–	–	–	–	–	–
	CIN	–	0.02	0.00	–	–	–	–	–	–
	ORB	–	0.03	0.00	–	0.03	0.00	–	–	–
	NOR	–	0.05	0.00	–	–	–	–	–	–
	OXO	–	–	–	–	–	–	–	–	–
SAs	SC	–	–	–	–	–	–	–	–	–
	SIM	–	–	–	–	–	–	–	–	–
	SDZ	–	–	–	–	–	–	–	–	–
	STZ	–	–	–	–	–	–	–	–	–
	SMX1	–	–	–	–	–	–	–	–	–
	SPD	–	–	–	–	–	–	–	–	–
	SMR	–	–	–	–	–	–	–	–	–
	SMM	–	0.01	0.00	–	–	–	–	–	–
	SDMD	–	0.10	0.00	–	–	–	–	–	–
	SMT	–	–	–	–	–	–	–	–	–
	SDX	–	–	–	–	–	–	–	–	–
	SMX2	–	–	–	–	–	–	–	–	–
	SIX	–	0.02	0.00	–	–	–	–	–	–
	SB	–	–	–	–	–	–	–	–	–
	SDM	–	–	–	–	–	–	–	–	–
	SQX	–	–	–	–	–	–	–	0.12	0.01
	SME	–	0.00	0.00	–	–	–	–	–	–
MAs	RTM	–	–	–	–	–	–	–	–	–
	CLA	–	–	–	–	–	–	–	–	–
	AZI	–	0.10	0.01	–	–	–	–	0.03	0.00
	SPI	–	–	–	–	–	–	–	0.97	0.09
	TIL	–	8.46	0.37	–	0.03	0.00	–	0.03	0.00
	LIN	–	–	–	–	–	–	–	–	–
LAs	OXA	–	–	–	–	–	–	–	–	–
	PENG	–	1.85	0.10	–	0.04	0.02	–	0.02	0.01

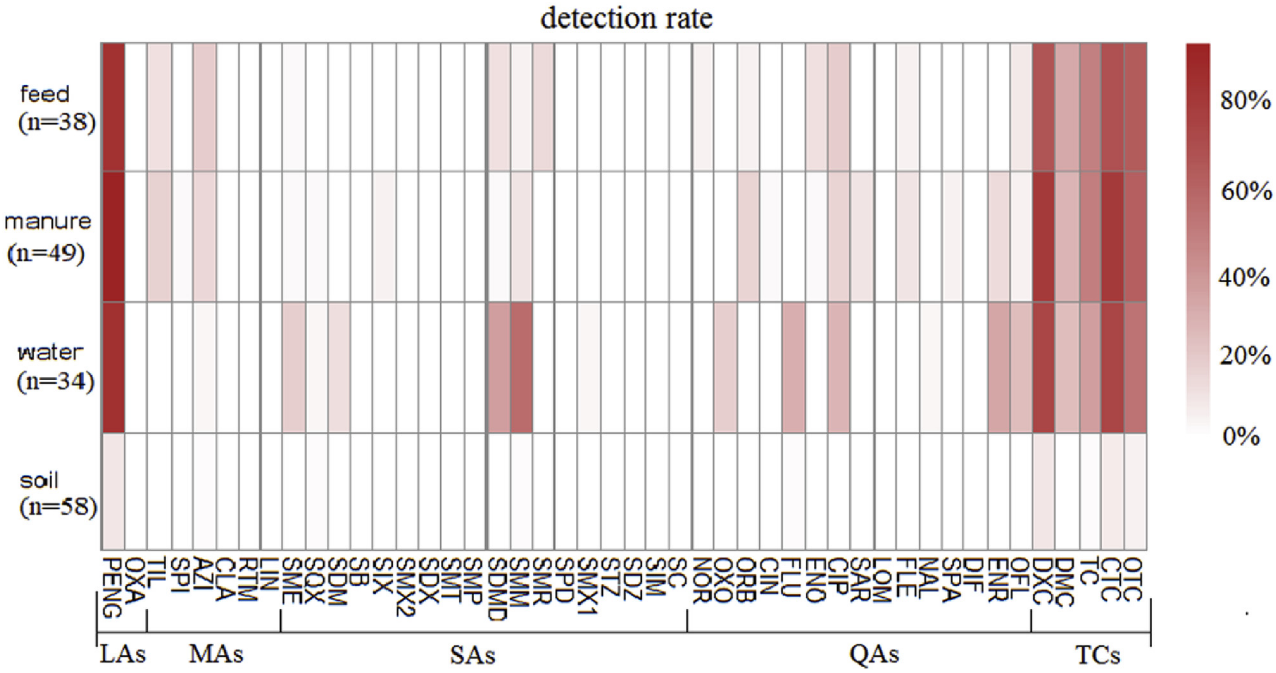

Fig. 3. Detection rates of antibiotics in different environmental mediums.

(Fig. 3). However, SAs had obvious higher concentrations (ND-1131.80 μg/L) and detection rates (0–55.9%) in wastewater than those in the other environmental mediums. However, soil samples had lowest antibiotics concentrations and detection rates. Take TCs as example, the concentration was ND-404.95 mg/kg in manure, but ND-781.34 μg/kg in soil. The detection rate was 0–94.0% for manure, but 0–9.0% for soil. It is obvious that PENG had relative high detection rates in different environmental mediums, such as feed (84.2%), manure (93.9%) and wastewater (85.3%), except in soil (0–8.6%).

3.4. Antibiotic density for different livestock species

For most studies, antibiotic residual levels were usually presented to show the pollution status. However, the concentrations did not fully represent the current pollution situation on the study area. Therefore, we use antibiotic density to asses which kind of livestock caused more pollution, which was calculated as follow:

$$I = \frac{C \times \eta \times 1000}{N}$$

where, *I* means the antibiotic density in manure or wastewater by per real livestock on different family farms (μg/kg/(per animal) or ng/L/(per animal)); *N* mean the total animal number; *C* means the detected concentrations on each farm (mg/kg or μg/L); η means the detection rate of each farm (%); 1000 is for unit conversion.

Fig. 4 shows the antibiotic densities by per animal in manure (a) and wastewater (b). Fig. 4 (a) shows the antibiotic densities of manure on different farms. We can see that, for most of the detected antibiotics, pig farms caused high antibiotic densities. Nearly a half (48.8%) of the antibiotics caused high antibiotic densities on pig farms; while it was 4.6% on chicken farms and no high densities on cattle farms. Moreover, for family chicken farms, SQX and spiramycin (SPI) had high densities. For antibiotic densities of wastewater on different farms (Fig. 4 (b)), pig wastewater caused more pollution by per animal, especially for TCs, but QAs (flumequine (FLU), nalidixic acid (NAL), and CIP) caused high densities in cattle wastewater.

3.5. Ecological risks of antibiotics in wastewater and soil

Up to now, little information about the ecological risks has been obtained for family livestock farms. For family livestock farms, a main kind of waste is the effluent of animal wastewater, which is only simply treated or no treated water, and is usually discharged directly into the outside environment (soil or river). Therefore, we calculated different *RQ* values of effluent from different family farms for algae, invertebrate and fish, as shown in Fig. 5 (because of the growth and breeding characteristics, no poultry wastewater was collected). As we can see, some antibiotics really caused high ecological risk. OTC, CTC, OFL, ENR, CIP and sulfamethoxazole (SMX2) exhibited much more toxic effects on algae and caused high risk on some family farms. OTC and DXC posed high risk on invertebrate; while no antibiotic caused high risk for fish. For different livestock species, wastewater from pig farms was more likely to have high risk. For OTC, 40.0% of family pig farms caused high risk on algae, while 14.3% of cattle farms caused high risk on algae. For CTC, 20.0% of family pig farms caused high risk on algae, while none of cattle farms caused high risk on algae. The reason is due to that higher antibiotic concentrations were usually detected in pig wastewater than in cattle wastewater.

Besides, manure as another kind of livestock waste, was mainly applied in the farmland as fertilizer after simply stacking and rotting. Therefore, antibiotics can enter into the soil and cause contamination. So the ecological risks of antibiotics for soil have also been calculated (shown in Fig. S2). It was shown that all the antibiotics had no toxic effects on algae, invertebrate or fish.

4. Discussion

4.1. Residual characteristics of antibiotics among different livestock species

The present study has attempted to analyze and evaluate pollution status of 45 veterinary antibiotics on 33 family farms in

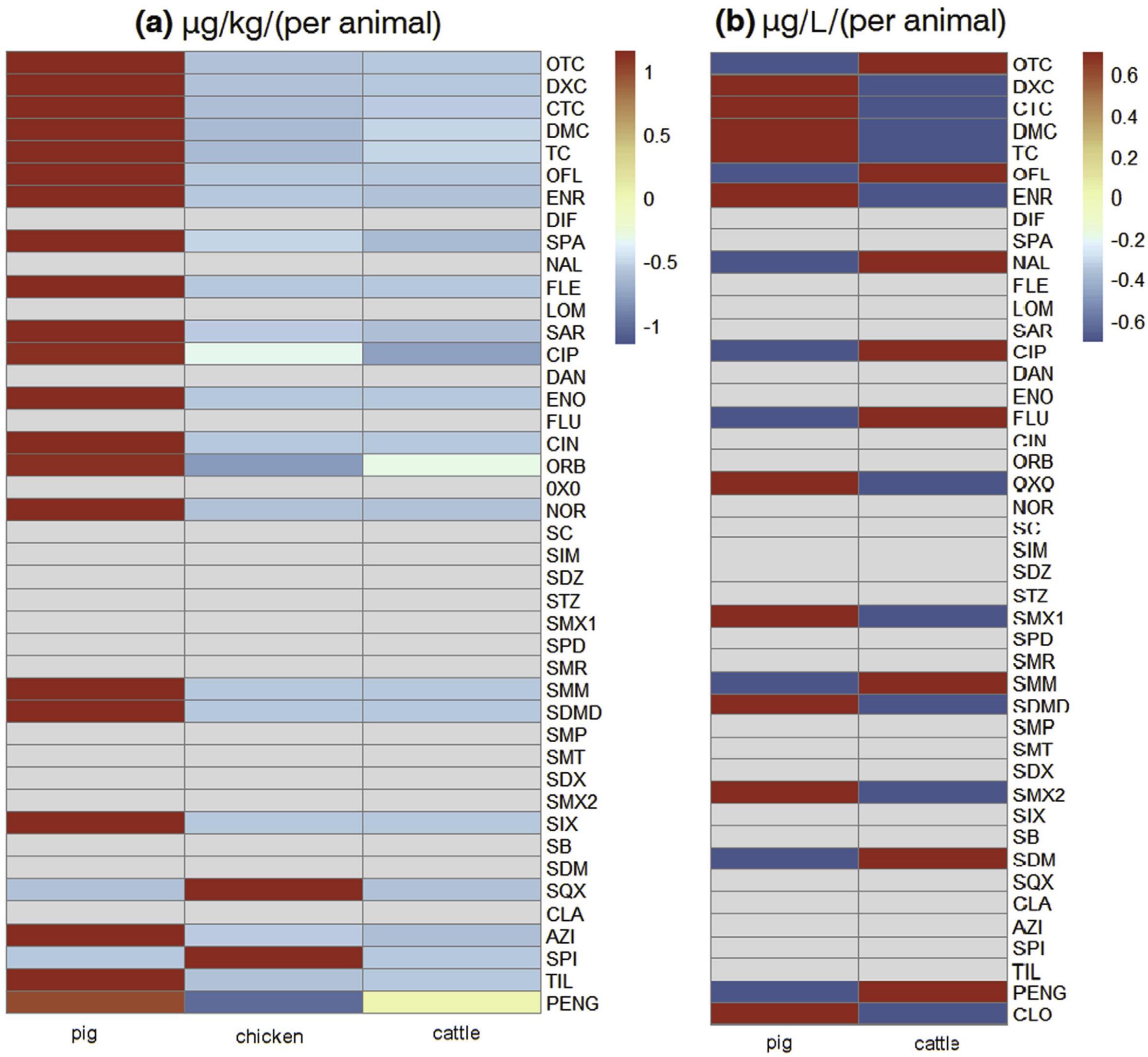

Fig. 4. Antibiotic densities by per animal in manure (a) and wastewater (b).

Erhai Lake basin of China. The results of antibiotic residues among different livestock species (Fig. 2) showed that family pig farms had highest concentration of antibiotics, followed by family chicken farms and then cattle farms; but cattle farms usually presented higher levels of LAs residues. Since the 1940s, antibiotics contributed much to animal breeding industry (Forman and Burch, 1947). Antibiotics can increase the efficiency of animal growth, by improving the structure of intestinal flora and digestibility of nutrients (Dibner and Richards, 2005), preventing and controlling diseases (Zhi et al., 2018) and improving the environment hygiene (Kobayashi, 2010). However, for different livestock species, the antibiotic types and usage are different, due to the different physiological property, growth period, conditions and infected germs of different animals (Zhi et al., 2018; Wei et al., 2011). In China, pork production is the main pillar industry of livestock husbandry, and more than 463 million pigs were produced annually, accounting for 51.6% of global pig population (Zhou et al., 2013a). Therefore, pig breeding was driven largely by people's demand and more antibiotics were used to ensure economic interest. This is why more antibiotics have been used in pig breeding industry. In addition, compared with cattle, disease types and incidence for pigs are relatively more and higher, including intestinal respiratory and contagion diseases, etc. Therefore, for pig farms, antibiotics are used to be high to improve feed efficiency, prevent disease and ensure a fast growth rate (Holt et al., 2011). For poultry, it is also a fast-growing and easily sick animal species, which also need antibiotics to promote growth and prevent disease. For cattle, the diseases are in relatively low frequent. Especially for dairy cattle, they usually are in milk production period for a long time and can not use antibiotics. The disease types are usually mastitis and gynecological diseases in a certain period. This is why cattle farms usually presented low levels of antibiotic residues. In a report on 36 antibiotics usage for different animals in China, about 52.2% of the total amount of antibiotics is for pig, 19.6% for chicken and 12.5% for other animals.

In addition to residual concentration mentioned above, antibiotic residual species represent the diversity of antibiotic use among different livestock species. Some animals were used to use these drugs, but others animals were likely to use other drugs. Some animals used more types of antibiotics, and some just used very few. So we tried to use Venn map to analyze the unique antibiotics types among different livestock species, shown in Fig. 6. The overlapped numbers are shared kinds of antibiotics by different animals, and the non-overlapped numbers are unique kinds for certain animals. It can be seen that family pig farms had the most total residual kinds and unique residual kinds of antibiotics, which

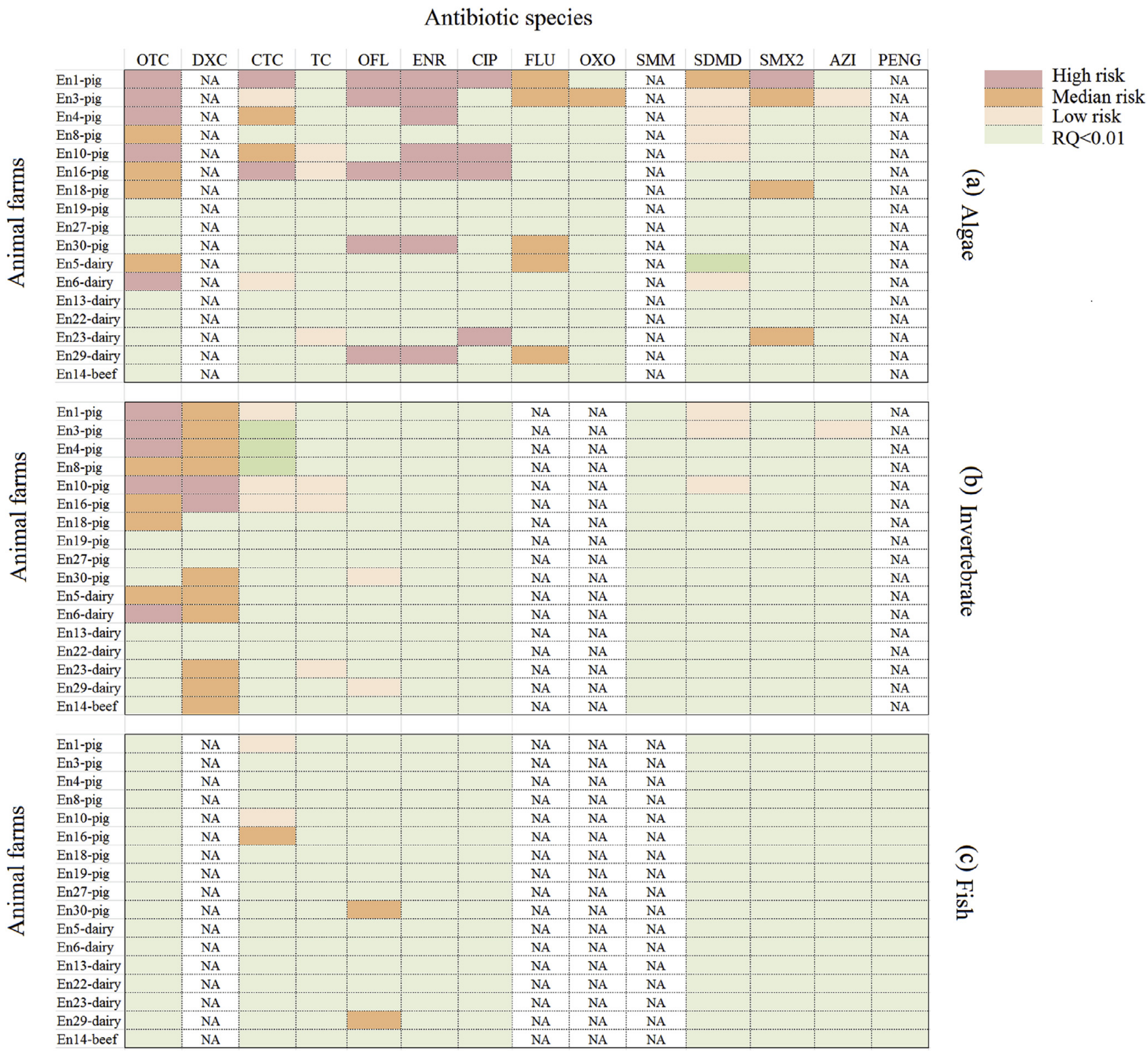

Fig. 5. Risk quotients of the detected antibiotics in water effluent to (a) algae, (b) invertebrate and (c) fish.

was followed by chicken then cattle. The unique antibiotics numbers for pig farm are 7, 9, 4 and 2 in feed (Fig. 6 (a)), manure (Fig. 6 (b)), wastewater (Fig. 6 (c)) and soil (Fig. 6 (d)), respectively; while family chicken farms had 0, 2 and 3 unique antibiotics types in feed (Fig. 6 (a)), manure (Fig. 6 (b)) and soil (Fig. 6 (d)), respectively. For family cattle farms, they had less antibiotic kinds used than the other two livestock species. In addition, there were more types of antibiotics in manure than those in feed for pig and chicken. For example, total number are 22 (Fig. 6 (b)) in manure but 18 (Fig. 6 (a)) in feed for pigs. This may be due to the fact that some antibiotics were introduced by injection therapy rather than by addition in feed.

4.2. Residual characteristics among different antibiotics

4.2.1. Total antibiotic concentrations among different classes

This study aimed to show the pollution status of 5 different classes (TCs, QAs, SAs, MAs and LAs) antibiotics on family farms. The results from Fig. 2 showed that TCs had the highest residual levels and detection rates among the selected 5 classes. The results are consistent with many other studies. For example, Wang et al. (2016) indicated that the total TCs concentrations could be high to 166.7 mg/kg in pig manure and 388.7 μg/L in wastewater. Zhi et al. (2018) showed that, in wastewater of three large-scale farms (1 pig farm and 2 dairy farms), TCs presented in higher levels than other classes of veterinary antibiotics. Besides, high concentrations of TCs have also been reported on livestock farms worldwide (Karcı and Balcıoglu, 2009). A statistic about veterinary antibiotics in the United States in 2017 showed that the total amount of TCs was highest among different classes of antibiotic in animal waste (Administration, 2017). From the above, TCs were usually detected with high residual levels on family farms. The reason may be that TCs have a long history for curing animal bacterial infections (Wei et al., 2011), for their low price, quick effect and broad-spectrum

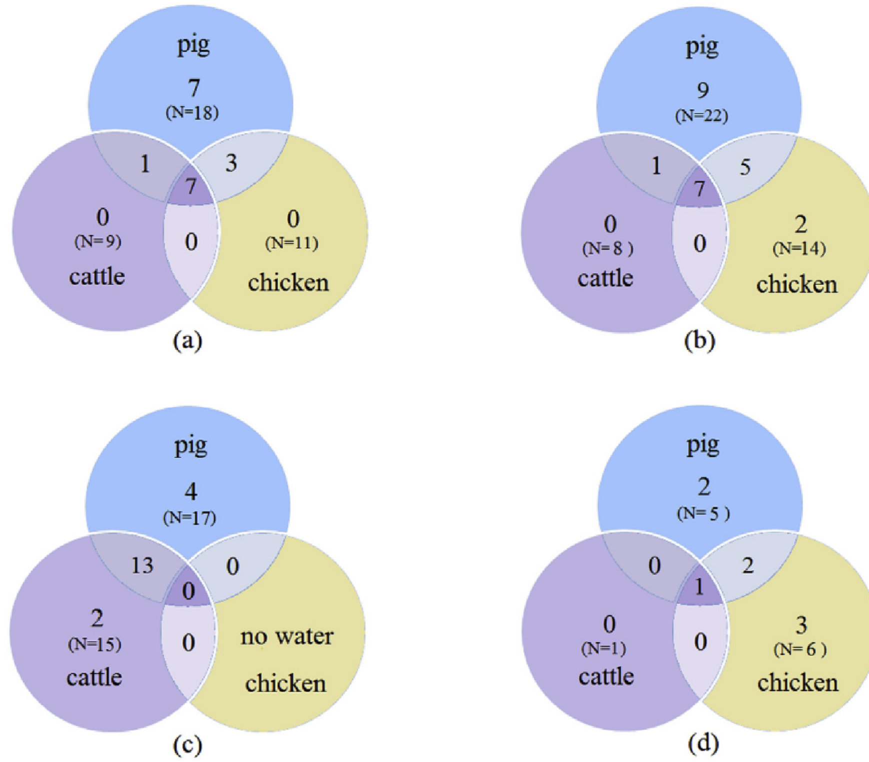

Fig. 6. Venn map of the antibiotic number for different animal species (a: feed; b: manure; c: wastewater; d: soil; N: total detected number).

antibacterial. Then TCs were usually added in feed to improve animal growth, or to prevent some diseases (Zhou et al., 2013b) such as curing respiratory and alimentary tract infections.

Moreover, other classes of antibiotics were also detected. This implied that antibiotics of different classes were commonly used on these family farms, not less than intensive livestock farms (Zhou et al., 2013a; Zhi et al., 2018). QAs are ubiquitous for different livestock and had the second highest concentration, which may be due to the wide applicability for different livestock. SAs are usually used to cure certain diseases for some livestock species (Wei et al., 2011) and they are more biodegradable and soluble. This is why SAs had low levels for all the samples, but relatively higher in wastewater. MAs have played a more and more important role in the animal breeding industry nowadays, but their residual concentrations were scarcely reported (Zhi et al., 2018). In the present study, MAs were obviously detected in pig farms and chicken farms, but almost not detected on cattle farms. It seemed to be that LAs (especially PENG) had relative high detection rates (Fig. 3). This may be due to PENG was widely used to treat infectious diseases, like cow mastitis. Oliver et al. (2020) pointed that TCs and LAs were usually used to manage bacterial disease in cows. And it was reported that, in the USA in 2015, tetracyclines and penicillins accounted for 71% and 10% of total antibiotic usage, respectively (Food and Agriculture Organisation of the United Nations, 2015).

4.2.2. Single antibiotic concentrations for different classes

Although total concentrations of different classes gave some results, the analysis of single antibiotic concentrations was also very meaningful (Tables 1–4). Among TCs, OTC, CTC and DXC were the dominant types in all the mediums of these family farms. Many studies have shown the similar results. Zhao et al. (2010) showed that the maximum level of CTC (17.68 mg/kg) was higher than OTC (10.56 mg/kg) in chicken dung. But Hu et al. (2010) investigated the representative antibiotics in four livestock farms of northern China and showed the highest types was OTC, up to 183.5 mg/kg in manure samples, which was lower than the maximum concentration for manure in this study (206.2 mg/kg). This indicated that family livestock farms indeed caused high levels of antibiotic residues in the waste. TCs were usually reported having higher concentration than other classes antibiotics (Zhi et al., 2018), because they were usually added in feed for livestock to accelerate growth and cure diseases (Zhou et al., 2013b). Among SAs, SMR was the highest type in feed; while SMM, sulfadimidine (SDMD) and sulfisoxazole (SIX) were the dominant types in manure and wastewater samples. The difference may be due to the different physicochemical properties for these antibiotics. It was reported that SMM was dominant in the effluent water, but not in the influent water (Zhang et al., 2018). Chen et al. (2017) indicated that sulfadimethoxine (SDM) and SMM were the dominant antibiotic species in animal wastewater (Zhou et al., 2013b). Among QAs, the detected types of antibiotic are relatively diversified, but the residual levels are not high. OFL, ORB, enoxacin (ENO), CIP and NOR were the dominant types in feed. ENR and CIP were relatively high in manure samples; OFL, ENR and CIP were the dominant species in wastewater. Most of the QAs were not detected in soils, except FLU. QAs were usually added in feed in swine farm, especially ENO (Zhi et al., 2018), therefore, they were commonly detected in livestock waste. Zhao et al. (2010) showed that ENO had high detection rate of 64.3% and high residual concentration (1420.76 mg/kg) in animal waste. But this study did not detect such high levels for QAs. Among MAs, TIL and AZI could be quantitatively detected in feed and manure; while only AZI could be detected in wastewater; no MAs residual could be detected in soil samples. Very little information could be gained for MAs, because they were rapidly degraded and susceptibility to light and pH (Ho et al., 2014; Schlüsener and Bester, 2006). AZI has become an emerging contaminant for the public (Vermillion Maier and Tjeerdema, 2018). Some studies showed the high detection rate of MAs (23%–52%) and high

Table 3
Single antibiotic concentration in different animal wastewater.

Antibiotics		Antibiotics concentration in wastewater (μg/L)					
		Pig (n = 20)			Cattle (n = 12)		
class	type	min	max	mean	min	max	mean
TCs	OTC	–	274.58	109.01	–	99.10	14.16
	DXC	–	3203.85	608.92	–	129.55	49.00
	CTC	–	25008.78	5034.49	–	–	–
	DMC	–	100.22	36.30	–	–	–
	TC	–	545.70	103.87	–	124.65	17.81
QAs	OFL	–	1259.15	76.92	–	136.55	22.72
	ENR	–	110.32	25.07		49.70	3.82
	DIF	–	–	–	–	–	–
	SPA	–	–	–	–	–	–
	NAL	–	–	–	–	40.60	5.80
	FLE	–	–	–	–	–	–
	LOM	–	–	–	–	–	–
	SAR	–	–	–	–	–	–
	CIP	–	135.20	23.02	–	109.35	14.07
	ENO	–	–	–	–	–	–
	FLU	–	33.02	8.64	–	29.25	7.95
	CIN	–	–	–	–	–	–
	ORB	–	–	–	–	–	–
	NOR	–	–	–	–	–	–
	OXO	–	20.45	8.80	–	–	–
SAs	SC	–	–	–	–	–	–
	SIM	–	–	–	–	–	–
	SDZ	–	–	–	–	–	–
	STZ	–	–	–	–	–	–
	SMX1	–	5.32	0.30	–	–	–
	SPD	–	–	–	–	–	–
	SMR	–	–	–	–	–	–
	SMM	–	1131.80	172.45	–	1701.80	150.49
	SDMD	–	200.55	29.87	–	25.77	2.74
	SMT	–	–	–	–	–	–
	SDX	–	–	–	–	–	–
	SMX2	–	49.82	4.10	–	55.55	0.43
	SIX	–	–	–	–	–	–
	SB	–	–	–	–	–	–
	SDM	–	6.70	1.00	–	2.92	0.41
	SQX	–	–	–	–	3.85	0.37
	SME	–	5.37	0.37	–	1.95	0.16
MAs	RTM	–	–	–	–	–	–
	CLA	–	–	–	–	–	–
	AZI	–	–	–	–	–	–
	SPI	–	–	–	–	–	–
	TIL	–	–	–	–	–	–
	LIN	–	–	–	–	–	–
LAs	OXA	–	–	–	–	–	–
	PENG	–	3145.18	492.45	–	337.23	91.71

residual concentrations in soils (83.04 mg/kg of AZI, 3.10 mg/kg of TIL and 2.46 mg/kg of TYL) (Wei et al., 2019). The results were in higher levels than those in this study. For LAs, just PENG could be detected in all the mediums. Although some reports once showed the high consumption of LAs (Junker et al., 2006), they were not always detected. This might be on account of the unique structure of LAs, whose β-lactam ring was liable to hydrolytic cleavage (Zhou et al., 2013c).

4.3. Difference of antibiotic residues between environmental mediums

4.3.1. Difference of antibiotics in different mediums

For different environmental mediums, manure samples were detected with higher concentrations than others. It is easily to understand that livestock body could accumulate antibiotics after uptake feed containing antibiotics (Zhang et al., 2019). The discharge ratio of antibiotics from animal body varied from 30% to 90% (Zhi et al., 2018; Chen et al., 2017). It was reported that most of the antibiotics detected were enriched in manure than in feed; and OTC could be enriched by 33.9 times (Zhang et al., 2019). This is why manure samples had higher antibiotic levels than feed samples. Then antibiotics could enter into wastewater with livestock's urine and washing water. However, some antibiotics are more easily adsorbed to the particles, for example QAs (Zhou et al., 2013c), while SAs are more easily soluble in water and biodegraded (Xu et al., 2011). This is why SAs were relatively higher than QAs in wastewater, but much lower than QAs in manure. It is precisely because of these different properties of antibiotics, antibiotics in wastewater may have different dominant types from those in feed and manure. Modifying soil by manure would result in some antibiotics migrating into soil. Soil usually had similar residual order to that of manure, because it was directly modified by manure. But soil was often detected with much less antibiotics than manure, because of biodegradation, photo-degradation and other process (Xu et al., 2011; Zhou et al., 2013c).

4.3.2. Source of antibiotics in livestock waste

In order to understand whether the source of antibiotics in animal waste is from feed addition, we compared the antibiotic

Table 4
Single antibiotic concentration in soils around different animal farms.

Antibiotics		Antibiotics concentration in soil (μg/kg)								
		Pig (n = 22)			Cattle (n = 18)			Chicken (n = 18)		
class	type	min	max	mean	min	max	mean	min	max	mean
TCs	OTC	–	74.66	4.84	–	–	–	–	–	–
	DXC	–	14.90	1.20	–	9.8	0.5	–	772.58	44.20
	CTC	–	305.56	17.66	–	–	–	–	100.35	6.06
	DMC	–	–	–	–	–	–	–	–	–
	TC	–	11.57	0.52	–	–	–	–	–	–
QAs	OFL	–	–	–	–	–	–	–	–	–
	ENR	–	–	–		–	–	–	–	–
	DIF	–	–	–	–	–	–	–	–	–
	SPA	–	–	–	–	–	–	–	–	–
	NAL	–	–	–	–	–	–	–	–	–
	FLE	–	–	–	–	–	–	–	–	–
	LOM	–	–	–	–	–	–	–	–	–
	SAR	–	–	–	–	–	–	–	–	–
	CIP	–	–	–	–	–	–	–	–	–
	ENO	–	–	–	–	–	–	–	–	–
	FLU	–	–	–	–	–	–	–	5.13	0.28
	CIN	–	–	–	–	–	–	–	–	–
	ORB	–	–	–	–	–	–	–	–	–
	NOR	–	–	–	–	–	–	–	–	–
	OXO	–	–	–	–	–	–	–	–	–
SAs	SC	–	–	–	–	–	–	–	–	–
	SIM	–	–	–	–	–	–	–	–	–
	SDZ	–	–	–	–	–	–	–	–	–
	STZ	–	–	–	–	–	–	–	–	–
	SMX1	–	–	–	–	–	–	–	–	–
	SPD	–	–	–	–	–	–	–	–	–
	SMR	–	–	–	–	–	–	–	–	–
	SMM	–	–	–	–	–	–	–	0.32	0.02
	SDMD	–	–	–	–	–	–	–	–	–
	SMT	–	–	–	–	–	–	–	–	–
	SDX	–	–	–	–	–	–	–	–	–
	SMX2	–	–	–	–	–	–	–	–	–
	SIX	–	–	–	–	–	–	–	–	–
	SB	–	–	–	–	–	–	–	–	–
	SDM	–	–	–	–	–	–	–	–	–
	SQX	–	–	–	–	–	–	–	0.36	0.02
	SME	–	–	–	–	–	–	–	–	–
MAs	RTM	–	–	–	–	–	–	–	–	–
	CLA	–	–	–	–	–	–	–	–	–
	AZI	–	–	–	–	–	–	–	–	–
	SPI	–	–	–	–	–	–	–	–	–
	TIL	–	–	–	–	–	–	–	–	–
	LIN	–	–	–	–	–	–	–	–	–
LAs	OXA	–	–	–	–	–	–	–	–	–
	PENG	–	3.67	0.26	–	–	–	–	4.07	0.52

species in feed and antibiotics residuals in animal manure (Fig. 7) and wastewater (Fig. S3). From Fig. 7, it is obvious that most of the antibiotics had similar trend in manure (Fig. 7(b)) to that in feed (Fig. 7(a)). This showed that higher antibiotic levels in feed seemed to cause higher antibiotic residual levels in manure. For example, OTC presented in high concentration in feed and also high in manure in pig farms of P1, P9, P16 and P28. Pig farm 1 (P1) had high TCs in feed which corresponded to high TCs in manure. There are also some other similar rules which have been marked in black border in Fig. 7. In addition, we have compared the antibiotics residues in wastewater and in feed (Fig. S3). Some similar trends were also observed. In conclusion, the addition of antibiotics in feed contributed a lot to the antibiotic residues in animal waste. It was reported that antibiotic pollution in China was more serious than that in developed countries (Zhang et al., 2015). After used for animal, antibiotics can be discharged with animal waste, and then enter into agricultural land with animal waste. The environmental and public health problems caused by antibiotics have attracted global attention. (Zhou et al., 2013a). Therefore, antibiotic as growth promoters in animal feed has been banned by Sweden in 1986 and then by the European Union in 2006 (Hamid et al., 2019). The situation in China began in 2015 and from July of 2020, enterprises will stop producing commercial feeds containing antibiotic growth promoters. Therefore, many studies focused on developing functional feed additives to replace antibiotics for improving performance of animals (Ma et al., 2019). And many feed additives have been developed, such as essential oil (Li et al., 2012), probiotic (Pan et al., 2017) and chito-oligosaccharide (Liu et al., 2010), to reduce diarrhea rate and improve growth performance.

Another source of antibiotics in animal waste is therapeutic antibiotic use. But therapeutic use of antibiotics usually occurs during disease outbreaks, which may happen intermittently. There are hundreds of antibiotics in use today. SAs are usually used to treat digestive tract, respiratory tract and inflammatory diseases, chronic respiratory disease, and so on. LAs (PENG) and MAs (TIL etc.) can also be used for respiratory diseases, intestinal and urinary tract infections. For cattle, LAs (penicillin) is one of the most commonly used antibiotics, for respiratory infections and other

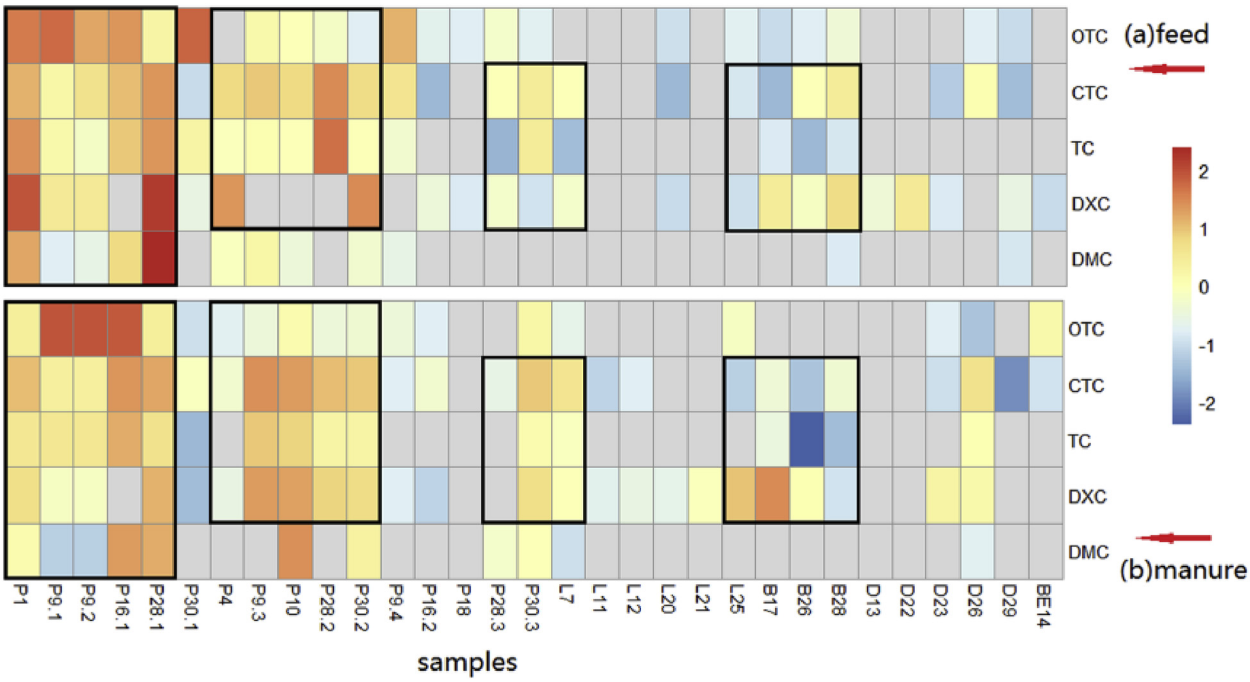

Fig. 7. Detection characteristics of antibiotics in feed (a) and manure (b).

bacterial infections (mammitis). This is why PENG concentrations were high in cattle waste. However, TCs were usually added in animal feed for disease prevention and promote growth, because of their low price and quick effect and broad-spectrum antibacterial. In general, antibiotic contamination caused by therapeutic usage does not occur consistently, which may degrade within hours or days after treatment. Therefore, controlled experiments are needed to further determine the regularity of such pollution.

4.4. Ecological risks of antibiotics to the surroundings

From the above, it can be seen that antibiotic contamination is prevalent on family farms. Therefore, we examined the ecological risks of antibiotics to the surrounding environment (effluent (Fig. 5) and soil (Fig. S2)). The present study showed that some antibiotics really caused high ecological risk for water, including OTC, CTC, OFL, ENR, CIP, SMX2 and DXC. Some studies also reported high risks of these antibiotics. For example, Xu et al. (2013) showed that OFL had high risk for algae during an investigation on seawater. Park and Choi (2008) reported that SMX exhibited much more toxic effects on algae than some other antibiotics, and SMX was identified as a high potential risk antibiotic to animals or plants in aquatic environment. The reason may be that these antibiotics could inhibit phagocytic activity even at very low concentrations. Moreover, in this study, algae are more sensitive to antibiotics, which followed by invertebrate and then fish, which is also accord to other reports. Xie et al. (2019) also reported that algae were the most sensitive biota to the target antibiotics. Lützhøft et al. (1999) showed that invertebrates were not affected as much as algae. Kümmerer (2009) also reported that antibiotics seemed unlikely to affect fish in aquatic environment. The similar results were also gained by a recent literature (Xie et al., 2019). But, considering a long-term bioaccumulation and combined toxicity of multiple antibiotics for invertebrate or fish, the risks of antibiotic pollution cannot be ignored.

For soils, all the antibiotics presented no toxic effects on algae, invertebrate or fish. This is associated with low concentrations of antibiotics in the soil. However, some papers reported that CTC, OTC, CIP and ENR had caused severe ecological risk for soil (Sun et al., 2017; Wei et al., 2019; Li et al., 2015). Although no obvious toxic effects were detected in the present study for soils, the ecological risk should be emphasized due to the possible interactions effects of different veterinary antibiotics (Wei et al., 2019).

5. Conclusion

The present study is the first to present the pollution situation and ecological risks of antibiotic on family animal farms in Dali city of China. It was found that family pig farms usually had highest antibiotic residual levels, which followed by chicken farms and then cattle farms. Antibiotic density could be calculated to assess antibiotic pollution for different livestock species. We also found that antibiotics were ubiquitous in livestock's feed, which means adding antibiotics usually occurred on family livestock farms. Livestock's manure and wastewater were obtained with higher antibiotic concentration than those of feed and soil, which implied that animal waste from family farm has become a non-negligible source of veterinary antibiotic pollution to the surroundings. Among different classes, TCs were usually detected with highest levels and highest detection rates. Moreover, the antibiotic residues in effluent and soil indicated that the antibiotic pollution could be spread by the application of livestock waste. Ecological risks of antibiotics were analyzed, which showed that antibiotics caused no obvious toxic effects on soils, but they posed high risks for algae and invertebrate in effluent water. This study delivered the severity of antibiotic pollution from family farms and also illustrated the importance of animal waste disposal on the small family farms, to relieve the transmission of antibiotics to the surroundings.

Credit author statement

Suli Zhi: Conceptualization, Writing- Original draft preparation, Funding acquisition; **Shizhou Shen:** Resources, Methodology; **Jing Zhou:** Investigation, Resources, Data curation; **Gongyao Ding:** Reviewing and Editing; **Keqiang Zhang:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115539.

References

Administration, F.D., 2017. Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals. Department of Health and Human Services, Maryland, 2017.
Cai, Y., Yu, J., Du, Z., 2020. Analysis on the treatment of livestock and poultry manure and its influencing factors in family farm and combination of planting and breeding farm. Ecol. Econ. 36, 178–185.
Chen, J., Liu, Y.-S., Zhang, J.-N., Yang, Y.-Q., Hu, L.-X., Yang, Y.-Y., Zhao, J.-L., Chen, F.-R., Ying, G.-G., 2017. Removal of antibiotics from piggery wastewater by biological aerated filter system: treatment efficiency and biodegradation kinetics. Bioresour. Technol. 238, 70–77.
Dibner, J.J., Richards, 2005. Antibiotic growth promoters in agriculture: history and mode of action. Poultry Sci. 84, 634–643.
Fey, P.D., Safranek, T.J., Rupp, M.E., Dunne, E.F., Ribot, E., Iwen, P.C., Bradford, P.A., Angulo, F.J., Hinrichs, S.H., 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. N. Engl. J. Med. 342, 1242–1249.
Food and Agriculture Organisation of the United Nations, 2015. Global Aquaculture Production 1950-2015. Available at: www.fao.org/fishery.
Forman, C., Burch, J., 1947. Use of sodium sulfonamides as single injection specific treatment in foot rot. J. Am. Vet. Med. Assoc. 111, 208–214.
Gu, Y., Shen, S., Han, B., Tian, X., Yang, F., Zhang, K., 2020. Family livestock waste: an ignored pollutant resource of antibiotic resistance genes. Ecotoxicol. Environ. Saf. 197, 110567.
Hamid, H., Zhao, L.H., Ma, G.Y., Li, W.X., Shi, H.Q., Zhang, J.Y., Ji, C., Ma, Q.G., 2019. Evaluation of the overall impact of antibiotics growth promoters on broiler health and productivity during the medication and withdrawal period. Poultry Sci. 98, 3685–3694.
He, L.Y., Ying, G.G., Liu, Y.S., Su, H.C., Chen, J., Liu, S.S., Zhao, J.L., 2016. Discharge of swine wastes risks water quality and food safety: antibiotics and antibiotic resistance genes from swine sources to the receiving environments. Environ. Int. 92–93, 210–219.
Ho, Y.B., Zakaria, M.P., Latif, P.A., Saari, N., 2014. Occurrence of veterinary antibiotics and progesterone in broiler manure and agricultural soil in Malaysia. Sci. Total Environ. 488–489, 261–267.
Holt, J.P., Heugten, E., Graves, A.K., See, M.T., Morrow, W.E.M., 2011. Growth performance and antibiotic tolerance patterns of nursery and finishing pigs fed growth-promoting levels of antibiotics. Livest. Sci. 136, 184–191.
Hu, X., Zhou, Q., Luo, Y., 2010. Occurrence and source analysis of typical veterinary antibiotics in manure, soil, vegetables and groundwater from organic vegetable bases, northern China. Environ. Pollut. 158, 2992–2998.
Junker, T., Alexy, R., Knacker, T., Kummerer, K., 2006. Biodegradability of C-14-labeled antibiotics in a modified laboratory scale sewage treatment plant at environmentally relevant concentrations. Environ. Sci. Technol. 40, 318–324.
Karcı, A., Balcıoglu, I.A., 2009. Investigation of the tetracycline, sulfonamide, and fluoroquinolone antimicrobial compounds in animal manure and agricultural soils in Turkey. Sci. Total Environ. 407, 4652–4664.
Kobayashi, Y., 2010. Abatement of methane production from ruminants: trends in the manipulation of rumen fermentation. Asian Aust. J. Anim. Sci. 23, 410–416.
Kovalakova, P., Cizmas, L., McDonald, T.J., Marsalek, B., Feng, M., Sharma, V.K., 2020. Occurrence and toxicity of antibiotics in the aquatic environment: a review. Chemosphere 251, 126351.
Kümmerer, K., 2009. Antibiotics in the aquatic environment-a review-part II. Chemosphere 75, 435–441.
Li, P., Piao, X., Ru, Y., Han, X., Xue, L., Zhang, H., 2012. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-Australas. J. Anim. Sci. 25, 1617–1626.
Li, C., Chen, J., Wang, J., Ma, Z., Han, P., Luan, Y., Lu, A., 2015. Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment. Sci. Total Environ. 522, 101–107.
Liu, P., Piao, X.S., Thacker, P.A., Zeng, Z.K., Li, P.F., Wang, D., Kim, S.W., 2010. Chito-oligosaccharide reduces diarrhea incidence and attenuates the immune response of weaned pigs challenged with Escherichia coli K88. J. Anim. Sci. 88, 3871–3879.
Lützhøft, H., Halling-Sorensen, B., Jorgensen, S.E., 1999. Algal toxicity of antibacterial agents applied in Danish fish farming. Arch. Environ. Contam. Toxicol. 36, 1–6.
Ma, X.K., Shang, Q.H., Wang, Q.Q., Hu, J.X., Piao, X.S., 2019. Comparative effects of enzymolytic soybean meal and antibiotics in diets on growth performance, antioxidant capacity, immunity, and intestinal barrier function in weaned pigs. Anim. Feed Sci. Technol. 248, 47–58.
Mahmoud, M.A.M., Abdel-Mohsein, H.S., 2019. Hysterical tetracycline in intensive poultry farms accountable for substantial gene resistance, health and ecological risk in Egypt-manure and fish. Environ. Pollut. 255, 113039.
Oliver, J.P., Gooch, C.A., Lansing, S., Schueler, J., Hurst, J.J., Sassoubre, L., Crossette, E.M., Aga, D.S., 2020. Invited review: fate of antibiotic residues, antibiotic-resistant bacteria, and antibiotic resistance genes in US dairy manure management systems. J. Dairy Sci. 103, 1051–1071.
Pan, L., Zhao, P.F., Ma, X.K., Shang, Q.H., Xu, Y.T., Long, S.F., Wu, Y., Yuan, F.M., Piao, X.S., 2017. Probiotic supplementation protects weaned pigs against enterotoxigenic Escherichia coli K88 challenge and improves performance similar to antibiotics. J. Anim. Sci. 95, 2627–2639.
Park, S., Choi, K., 2008. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. Ecotoxicology 17, 526–538.
Schlüsener, M.P., Bester, K., 2006. Persistence of antibiotics such as macrolides, tiamulin and salinomycin in soil. Environ. Pollut. 143, 565–571.
Sun, J., Zeng, Q., Tsang, D.C.W., Zhu, L.Z., Li, X.D., 2017. Antibiotics in the agricultural soils from the Yangtze river delta, China. Chemosphere 189, 301–308.
Suresh, G., Das, R.K., Brar, S.K., Rouissi, T., Ramirez, A.A., Chorfi, Y., Godbout, S., 2018. Alternatives to antibiotics in poultry feed: molecular perspectives. Crit. Rev. Microbiol. 44, 318–335.
Veeck, G., Shaohua, W., 2000. Challenges to family farming in China. Geogr. Rev. 90, 57–82.
Vermillion Maier, M.L., Tjeerdema, R.S., 2018. Azithromycin sorption and biodegradation in a simulated California river system. Chemosphere 190, 471–480.
Wang, J., Ben, W., Yang, M., Zhang, Y., Qiang, Z., 2016. Dissemination of veterinary antibiotics and corresponding resistance genes from a concentrated swine feedlot along the waste treatment paths. Environ. Int. 92–93, 317–323.
Wei, R.C., Ge, F., Huang, S.Y., Chen, M., Wang, R., 2011. Occurrence of veterinary antibiotics in animal wastewater and surface water around farms in Jiangsu Province, China. Chemosphere 82, 1408–1414.
Wei, R., He, T., Zhang, S., Zhu, L., Shang, B., Li, Z., Wang, R., 2019. Occurrence of seventeen veterinary antibiotics and resistant bacterias in manure-fertilized vegetable farm soil in four provinces of China. Chemosphere 215, 234–240.
Xie, H., Wang, X., Chen, J., Li, X., Jia, G., Zou, Y., Zhang, Y., Cui, Y., 2019. Occurrence, distribution and ecological risks of antibiotics and pesticides in coastal waters around Liaodong Peninsula, China. Sci. Total Environ. 656, 946–951.
Xu, B., Mao, D., Luo, Y., Xu, L., 2011. Sulfamethoxazole biodegradation and biotransformation in the water–sediment system of a natural river. Bioresour. Technol. 102, 7069–7076.
Xu, W., Yan, W., Li, X., Zou, Y., Chen, X., Huang, W., Miao, L., Zhang, R., Zhang, G., Zou, S., 2013. Antibiotics in riverine runoff of the Pearl River Delta and Pearl River Estuary, China: concentrations, mass loading and ecological risks. Environ. Pollut. 182, 402–407.
Yang, Y., Owino, A.A., Gao, Y., Yan, X., Xu, C., Wang, J., 2016. Occurrence, composition and risk assessment of antibiotics in soils from Kenya, Africa. Ecotoxicology 25, 1194–1201.
Zhang, H.Y., 2018. Family farm is the basic direction of economic development of farmers in China. Rural Work Newslett. (4), 12–15, 2018.
Zhang, Q.-Q., Ying, G.-G., Pan, C.-G., Liu, Y.-S., Zhao, J.-L., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ. Sci. Technol. 49, 6772–6782.
Zhang, M., Liu, Y.-S., Zhao, J.-L., Liu, W.-R., He, L.-Y., Zhan, J.-N., Chen, J., He, L.-K., Zhang, Q.-Q., Ying, G.-G., 2018. Occurrence, fate and mass loadings of antibiotics in two swine wastewater treatment systems. Sci. Total Environ. 639, 1421–1431.
Zhang, J., Lu, T., Chai, Y., Sui, Q., Shen, P., Wei, Y., 2019. Which animal type contributes the most to the emission of antibiotic resistance genes in large-scale swine farms in China? Sci. Total Environ. 658, 152–159.
Zhao, L., Dong, Y.H., Wang, H., 2010. Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. Sci. Total Environ. 408, 1069–1075.
Zhi, S., Zhou, J., Yang, F., Tian, L., Zhang, K., 2018. Systematic analysis of occurrence

and variation tendency about 58 typical veterinary antibiotics during animal wastewater disposal processes in Tianjin, China. Ecotox. Environ. Saf. 165, 376–385.
Zhou, L.-J., Ying, G.-G., Liu, S., Zhang, R.-Q., Lai, H.-J., Chen, Z.-F., Pan, C.-G., 2013a. Excretion masses and environmental occurrence of antibiotics in typical swine and dairy cattle farms in China. Sci. Total Environ. 444, 183–195.
Zhou, L.-J., Ying, G.-G., Zhang, R.-Q., Liu, S., Lai, H.-J., Chen, Z.-F., Yang, B., Zhao, J.-L., 2013b. Use patterns, excretion masses and contamination profiles of antibiotics in a typical swine farm, south China. Environ. Sci.: Process. Impacts 15, 802–813.
Zhou, L.-J., Ying, G.-G., Liu, S., Zhao, J.L., Yang, B., Chen, Z.F., Lai, H.J., 2013c. Occurrence and fate of eleven classes of antibiotics in two typical wastewater treatment plants in South China. Sci. Total Environ. 452–453, 365–376.