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Contamination of Surface Water with Veterinary Antibiotics in Tiaoxi River Basin, East China

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The assessment of veterinary antibiotics pollution in drinking water sources is a critical issue for potential threat for human health. The occurrence and distribution of 14 selected antibiotics representing five categories (tetracyclines, sulfonamides, fluoroquinolones, macrolides and chloramphenicols) were investigated in surface waters and swine wastewater from Tiaoxi river basin using HPLC/MS/MS. The results indicated that contamination levels of the individual antibiotics in surface waters were significantly different. Tetracyclines (including tetracycline, oxytetracycline, chlortetracycline and doxycycline) and sulfonamides (including sulfadiazine, sulfamethoxazole and sulfamethazine) were the dominant antibiotics present in aqueous samples along Tiaoxi river but their concentrations varied greatly with timing and locations of sampling. The higher antibiotics concentrations were found in fishing ponds and soft-shell turtle pond than the samplings of Tiaoxi river and drinking water source, but swine wastewater with the highest antibiotics concentrations in all sampling sites. Agricultural activities (*e.g.*, swine or soft-shell turtle feeding) may be major sources of contaminants in the aqueous environment. So control measures must be done to limit antibiotics abuse in livestock farming and aquaculture and to avoid swine wastewater poured into rivers for cleaning drinking water sources and ecological health.

Keywords: Veterinary antibiotics, Surface water, Swine farm, Tiaoxi river basin.

INTRODUCTION

Antibiotics are a group of emerging organic contaminants increasingly receiving public concerns since these compounds assumed to pose adverse effects to aquatic organisms and human health[1]. Results of numerous studies have shown that concentrations of antibiotics vary greatly in the water environment with most of the levels detected within trace amounts[2]. However, even at such low concentrations, the persistent and undetectable effects induced by antibiotics could accumulate slowly and continually and finally result in irreversible change on wildlife and human beings[3]. Several studies indicated that antibiotics may cause resistance among natural bacterial populations by the antibiotic resistance genes (ARGs)[4] and not only this resistance can be transmitted to the general population that facilitates antibiotic-resistant infections, but also antibiotic-resistant bacteria can pass through the food chain from animals to humans[5].

Antibiotic compounds probably persist during the treatment processes in wastewater treatment plants (WWTPs) and diffuse from wastewater to surrounding surface water[6]. This situation would be enhanced in watersheds due to urbanization levels at the intensive agricultural activities[7]. Antibiotic levels at some sites that are directly influenced by wastewater discharge or aquaculture were found to be up to micrograms per liter[8]. Surface water was the source and sinks of antibiotics due to many antibiotics were waterborne and they were monitored in aquatic environment was important for environment evaluation. Compared with the well established sources of antibiotics from municipal sewage to the aquatic environment[1], there has been less research on the transfer of veterinary antibiotics problems from intensive agriculture to major rivers and sources of drinking water especially in developing countries.

Tiaoxi river is an anabranch of Taihu lake, the third largest freshwater lake in China. It carries a large volume of water (about 2.93×10^{10} m^3 yr^{-1}) and flows through a catchment of 4533 km and it is important drinking water sources. The intensively managed livestock farming and aquaculture systems in the region got rapid development in recent years and the densities of pig rearing and freshwater aquaculture are estimated to be up to 733 head km^{-2} and 1 ponds km^{-2} (total areas were about 5×10^4 m^2), respectively. Some antibiotics were often used in livestock farming and aquaculture and waste water was directly or indirectly discharged into the river. However, there has been little concern over this situation in contrast with the anthropogenic nutrient inputs to Taihu lake and associated eutrophication which have attracted substantial interest nationally and internationally[9] and the research on antibiotic pollution in this area was very limited. The objectives of the present study were to investigate the presence of the selected antibiotics in drinking water sources and Tiaoxi river, to study the temporal and spatial variation in concentrations of antibiotics in the river and to evaluate the contribution of veterinary antibiotics from agricultural activities to the surface waters in this region.

EXPERIMENTAL

Chemicals and standards: Fourteen selected antibiotic compounds were purchased from Sigma-Aldrich Corp., St. Louis, MO. $^{13}C_3$-caffeine solution (100 µg mL^{-1} in methanol) was obtained from Cambridge Isotope Laboratories Inc., Andover, MA. HPLC grade methanol, analytical grade formic acid (99 %), citric acid-monohydrate, sodium phosphate-dibasic anhydrous and disodium ethylene diaminetetraacetic acid (Na_2EDTA) were purchased from Sinopharm Chemical Reagent Co. Ltd., Shanghai, China. All the antibiotics were dissolved in methanol and stored in a freezer and working solutions were prepared immediately before the experiment by dilution of the stock solution. Erythromycin-H_2O, the major degradation product of erythromycin, was obtained by acidification of erythromycin[10]. Ultra-pure water was prepared with a Milli-Q water purification system (Millipore, Bedford, MA). Oasis HLB cartridges, 6mL/500mg, used for solid-phase extraction (SPE) were purchased from Water Oasis Co. (Milford, MA) and SAX cartridges (3 mL/500 mg) combined with HLB cartridges in wastewater preparation were sourced from Supelco Co., Bellefonte, PA.

Sample collection and site description: The sampling sites (Fig. 1) are located in the Yangtze river Delta, one of the most rapidly developing and most urbanized regions of China. Most of the anthropogenic nutrients enter Taihu lake, resulting in algal blooms and restrictions on drinking water supplied for millions of people. The numbers of livestock, poultry and fish produced has increased in recent years and the use of veterinary antibiotics by agribusinesses has also increased to prevent animal diseases and promote growth. For example, a preliminary investigation found that tetracyclines (including tetracycline, oxytetracycline and chlortetracycline) and sulfadiazine are frequently used to control gastrointestinal disorders and respiratory problems. In contrast with industrialized counties, few wastewater treatment plants in China have operated treatment processes to control inputs of antibiotics to the aquatic environment.

Tiaoxi river, one of the main anabranches of Taihu lake, originates from one man-made reservoir, Qingshanhu which was constructed in 1964 and which contains more than 900 million cubic meters of fresh water and supplies the drinking water for Hangzhou city with a population of more than 6 million people, the capital city of Zhejiang province. The Tiaoxi river flows into Taihu lake. Owing to the local intensive agricultural systems, large amounts of agriculture-derived contaminants (including anthropogenic nutrient, pesticides, phthalate esters and antibiotics), are discharged to the river system and ultimately into Taihu lake.

Sampling was conducted on two occasions at typical sections along the Tiaoxi river from its source to its end at September 2010 and March 2011. The sampling sites were located at the junctions between Tiaoxi river and its tributaries to examine the inputs of antibiotics to the main stream from the tributaries (Fig. 1). To check the possible effects of agricultural activities, antibiotics in raw wastewaters from two large swine feeding operations and pond water from aquaculture (three fish ponds and one soft-shelled turtle pond) near the river were also studied, including the main stream water near the outfall of a swine farm. Water samples (about 4 L) were collected from sampling sites, stored in brown glass bottles and immediately transported to the laboratory and stored at 4 °C before analysis.

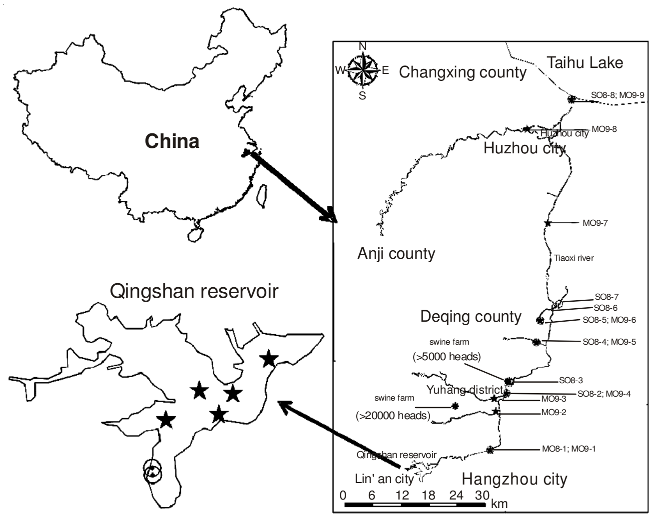

Fig. 1. Location and the sampling sites

Sample preparation: The water samples were filtered through 0.45 µm cellulose acetate membranes. One liter of water sample was acidified to pH < 3, followed by addition of 0.5 g disodium ethylene diaminetetraacetic acid (Na_2EDTA). The Oasis HLB cartridge used for the solid-phase extraction was preconditioned sequentially with 10 mL of methanol and 10 mL of ultra-pure water at a flow rate of approximately 5 mL min^{-1}. Thereafter, the samples were passed through the solid-phase extraction columns at a flow rate of approximately 3 mL min^{-1}. The HLB columns were then rinsed with 10 mL of ultra-pure water and dried under nitrogen gas for 0.5 h at a flow rate of 2-4 mL min^{-1}. After drying, each cartridge was eluted with 2 mL methanol (containing 0.1 % v/v formic acid) with retention time 2-3 min and then with 8 mL methanol with formic acid at a rate of < 3 mL min^{-1}. The analytes were collected in a 15 mL brown glass vial and the volume was reduced by purging nitrogen to below 1 mL. A 10 µL internal standard ($^{13}C_3$-caffeine, 1 mg L^{-1}) was added and mixed with the volume. The final volume in each vial was adjusted to 1 mL with methanol with formic acid and then filtered through a 0.22 µm PTFE Syringe filter (purchased from Jinteng Experiment Equipment Co., Ltd, Tianjin, China) before HPLC analysis[11,12].

SAX-HLB solid-phase extraction cartridges, pre-conditioned sequentially with methanol and ultrapure water, were set up in the tandem for sample preparation of wastewaters and pond waters. The process was similar to that used for main stream water samples with the sole exception that water samples of only 500 mL were used. After the water had passed through the combined cartridges, the SAX columns were removed and HLB cartridges were used as described above.

HPLC and MS-MS system: High-performance liquid chromatography-electrospray ionization tandem mass spectrometry with TQ Detector (ACQuity, Waters Corp, Milford, MA), run by Masslynx 4 software (Thermo Electron Corp., Waltham, MA), was conducted to separate residues of the antibiotics under study. Samples were separated on a Waters Acquity UPLC BEH C_{18} column (1.7 µm, 2 × 100 mm) which was maintained at 30 °C. The mobile phase A consisted of 99.9 % water and 0.1 % formic acid and mobile phase B was 99.9 % methanol mixed with 0.1 % formic acid. The gradient elution was set as follows: started at 90 % A, 0-2 min a linear gradient to 80 % A, 2-7 min 20 % A, 7-10 min linear gradient to 10 % A, then, the eluent was brought to 100 % B and maintained for 10 min. The injection volume was 5 µL and flow rate was 0.3 mL min^{-1}. Ionization was performed in the positive mode for tetracyclines, sulfonamides, macrolides and fluoroquinolones and the negative mode for chloramphenicols. MS/MS was operated at unit resolution in multiple reactions monitoring (MRM) mode. Source conditions were optimized as follows: spray voltage was +4,000 V; transfer capillary temperature, 290 °C; argon was used as collision gas at a pressure of 1 mTorr. Determination was performed in selected reactions monitoring mode using the two most intense and specific fragment ions with a scan width of 0.06 s. Optimized MS/MS parameters for the target antibiotics are shown in Table-1.

Quality control for the analysis of selected antibiotics in water samples: Concentrations in the samples were calculated by the internal standard ($^{13}C_3$-caffeine) method based on comparison between the peak area of the sum of the two monitored product ions of the target compounds and $^{13}C_3$-caffeine, except the chloramphenicols (chloramphenicol, thiamphenical and florfenicol), which were calculated by an external standard method based on the peak area of the sum of the two monitored product ions. Calibration lines of seven concentration points (1, 2, 5, 10, 20, 50 and 80 µg L^{-1} in methanol containing 0.1 % formic acid, v/v) of individual antibiotics were used for quantification. The linearity of the calibration curve in this range was confirmed ($r^2 > 0.99$).

Recovery studies for both lake water and tap water were conducted with each antibiotic and three repeats of each water body were performed. The recovery ratio was calculated by comparing the detection concentrations, which were extracted from samples spiked with a concentration of 50 ng L^{-1} in 1 L water prior to solid-phase extraction, to original concentration (50 ng L^{-1}). Method detection limits were calculated by a statistical method using student's t-variate. This statistical method multiplied the calculated standard deviation by the Student's *t*-variate for a one-sided *t*-test at the 99 % confidence interval to estimate the LOQ ($LOQ = S \times t_{(n-1,0.99)}$; where n-1 is degrees of freedom and 0.99 is the 99 % confidence). The standard deviation is calculated from the seven sequence repeats of water samples spiked with the concentration which is near the detection limit (approximately 0.1 µg L^{-1} for each target) determined from the signal/noise ratio. Recovery and method detection limits study of water are shown in Table-2.

TABLE- 1
OPTIMIZED Ms/MS PARAMETERS
FOR THE TARGET ANTIBIOTICS

Compounds	Molecular Weight	Parent (*m/z*)	Daughter (*m/z*)	Cone (V)	Collision (V)
Tetracycline	445	445	154	30	35
			410	20	35
			427	10	30
Oxytetracycline	460	461	381	30	40
			426	20	40
			443	10	40
Chlortetracycline	479	479	154	30	35
			444	20	35
			462	15	35
Doxycycline	445	445	154	30	35
			410	20	35
			428	15	35
Sulfadiazine	250	251	92	30	30
			156	15	30
Sulfamethoxazole	253	254	92	30	30
			108	20	30
Sulfamethazine	278	279	92	30	35
			156	20	35
			186	20	35
Norfloxacin	319.	320	276	20	40
			302	20	40
Ofloxacin	361	362	302	20	40
			261	20	40
Enythromycin-H_2O	734	735	158	35	35
			576	20	35
Roxithromycin	837	838	158	39	35
			679	20	35
Chloramphenicol	323	3218	152	20	30
			257	10	30
Thiamphenical	356	3548	185	20	35
			290	10	35
Florfenicol	358	3568	185	20	30
			336	10	30

TABLE-2
RECOVERY AND METHOD DETECTION
LIMITS (MDL) STUDY OF WATER

Compounds	Lake water recovery (%) n = 3		Drink water recovery (%) n = 3		MDL (ng/L)
	Average (%)	Standard Deviation (%)	Average (%)	Standard Deviation (%)	
Tetracycline	84.4	7.7	87.4	8.7	0.1
Oxytetracycline	75.5	6.3	73.3	7.4	1.0
Chlortetracycline	77.2	10.4	77.2	12.2	1.0
Doxycycline	69.7	8.9	62.5	7.8	0.1
Sulfadiazine	88.9	23.2	74.2	7.4	0.1
Sulfamethoxazole	84.1	17.7	74.2	10.1	0.1
Sulfamethazine	80.7	0.4	77.5	3.7	0.1
Norfloxacin	79.9	6.7	82.9	2.2	0.1
Ofloxacin	90.7	43.1	77.0	12.0	0.1
Enythromycin-H_2O	74.9	6.0	84.0	1.9	0.1
Roxithromycin	71.4	9.8	68.0	10.8	0.1
Chloramphenicol	92.5	31.0	111.0	35.9	1.0
Thiamphenical	77.9	7.3	73.1	9.6	1.0
Florfenicol	92.5	24.9	88.2	12.4	1.0

RESULTS AND DISCUSSION

Occurrence of selected antibiotics in surface waters: The concentrations of 14 antibiotics along the Tiaoxi river on the two sampling occasions in 2010 and 2011 are presented at Table-3. Most of the compounds were below 80 ng L^{-1} in river water and drinking water sources except for the special sampling site (outfall of swine farm), where chlortetracycline and sulfadiazine were up to 1.1×10^3 and 3.5×10^3 ng L^{-1}, respectively. Tetracyclines (including tetracycline, oxytetracycline, chlortetracycline and doxycycline) and sulfonamides (including dulfadiazine, sulfamethoxazole and sulfamethazine) were the dominated types of antibiotics detected in these surface water samples, in agreement with the trend found in wastewaters from swine farms (Table-3), but significant difference were showed among sewage and other sampling sites for them ($P < 0.01$). Soft-shell turtle pond water also contained high levels of dulfadiazine (359.8 ng L^{-1}) in contrast with the fish pond water which had a similar range of antibiotics concentrations with the main stream water. Wastewater discharge from swine farms may have a large influence in Tiaoxi river because the higher values were observed at the main stream water near the swine farm and it was the major reason for higher concentrations of these antibiotics in downstream.

Compared with data reported in the literature (Fig. 2), the range of concentrations for tetracyclines (including tetracycline, oxytetracycline, chlortetracycline and doxycycline in this study) observed in Tiaoxi river were similar to the reported values but showed a higher frequency of detection. Sulfonamides (including dulfadiazine, sulfamethoxazole and sulfamethazine) were lower than most reported data, in particular at the locations that were directly influenced by sewage effluent or agricultural activities[11,15]. As the extreme dulfadiazine values were detected in wastewater and river water around the outfall of a swine farm (153.5×10^3 and 3.5×10^3 ng L^{-1}, separately), the relatively low concentrations of the compound in river water were possibly due to rapid dilution in the main river by the large volume of water carried by the river (about 2.93×10^{10} m^3 yr^{-1}). Fluoroquinolones (norfloxacin and ofloxacin) and macrolides (ETM-H_2O and roxithromycin) occurring below the method detection limits or at low levels in Tiaoxi river is probably due to the limited availability of these antibiotics in this region resulting in low detection in swine wastewater and aquaculture water. Chloramphenicol (CPC), was officially prohibited antibiotic and was also detected in the river water, likely attributable to the illegal use of these drugs in animal husbandry or aquaculture. More research is required to highlight the unique features of the distributions of these antibiotics in this region.

Presence of selected antibiotics in the lake as a drinking water source: Concentrations of antibiotics in Qingshan lake were unexpectedly high because the lake is the supply of drinking water for Hangzhou city with a population of over 6 million. The range of 14 studied antibiotics in different sites of the reservoir was < 0.1 to 73.5 ng L^{-1} at the two sampling occasions (Table-3) and the predominant contaminants were tetracyclines (oxytetracycline, chlortetracycline and doxycycline). As the drinking water source or the water of a large lake (contains more than 900 million cubic meters fresh water), the antibiotic concentrations were much higher than in the pristine area of Cache la Poudre river in northern Colorado in the United States, where no antibiotic residuals were found and Western lake Erie basin (United States)[13], where median values of ETM-H_2O, sulfamethazine and sulfamethoxazole detected in the whole field were below 1.2, 0.2 and 0.7 ng L^{-1}, respectively[16]. Levels of tetracycline and oxytetracycline pollution in the water of Qinshan lake were higher than in the lake water located at the similar latitude in central China (< 12 ng L^{-1} for tetracycline and < 10 ng L^{-1} for oxytetracycline)[17]. Other analytes (sulfonamides, macrolides and fluoroquinolones) detected in this lake ranged from < 0.1 to 25.4 ng L^{-1} and were consistent with the source of the River Ely (South Wales, UK), where sulfamethoxazole was below 1 ng L^{-1} and ETM-H_2O ranged < 0.5 to 37 (ng L^{-1})[18]. The use of chloramphenicol is restricted in aquaculture because of its possible side effects on consumers was mostly found (3.6--5.9 ng L^{-1}) at this drinking water source sampled in March 2011[19]. The widespread detection of this compound at concentrations above those (below 2 ng L^{-1}) of the rivers Taff and Ely in South Wales in the UK and lake water in Hubei Province in central China (around 2 ng L^{-1})[17,18], indicates the possibility of the illegal use of these drug in animal husbandry or aquatic products around

TABLE-3
CONCENTRATIONS (ng L^{-1}) OF ANTIBIOTICS IN SURFACE WATER AND SWINE WASTEWATER IN TIAOXI BASIN

Antibiotics (abbreviation)	Samples for September 2010				Samples for March 2011				
	Tiaoxi river (n=8)	Drinking water source (n=2)	Fish pond (n=3)	Swine wastewater (n=3)	Tiaoxi river (n=9)	Drinking water source (n=5)	Soft-shell turtle pond (n=1)	Special site [a](n=1)	Swine wastewater (n=5)
Tetracycline	Nd-6.9	Nd-0.6	0.2-1.4	59.6-967.6	Nd-10.0	0.2-2.8	7.8	620.7	6.8-41.6×10^3
Oxytetracycline	0.5-60.8	15.1-16.7	Nd	780-12.1×10^3	Nd-15.2	Nd-4.4	Nd	Nd	Nd-1.3×10^3
Chlortetracycline	Nd-27.8	Nd-29.6	Nd-61.4	1.2-13.7×10^3	Nd-71.6	30.2-73.5	68.7	1.1×10^3	164.5-7.9×10^3
Doxycycline	Nd-6.6	7.2-44.8	Nd-58.6	377.0-3.6×10^3	Nd-5.3	0.6-5.0	14.4	69.3	562.5-2.6×10^3
Sulfadiazine	Nd-4.8	Nd-0.2	Nd-42.8	10.4-28.8	Nd-17.5	Nd-25.4	359.8	3.5×10^3	6.9-153.5×10^3
Sulfamethoxazole	Nd-5.4	Nd-11.5	Nd-9.0	1.6-36.0	1.7-9.6	3.2-5.9	Nd	61.3	Nd-3.6
Sulfamethazine	Nd-11.9	0.4-2.2	7.0-13.6	223.6-675.4	0.5-18.2	2.2-3.9	0.7	11.1	16.2-61.3
Norfloxacin	Nd-1.8	Nd	Nd	Nd	1.5-8.2	Nd-5.4	2.7	28.5	Nd-28.9
Ofloxacin	Nd-2.2	Nd-0.7	Nd	Nd-94.2	1.0-10.8	1.0-5.6	2.4	25.6	17.2-29.7
Enythromycin-H_2O	Nd	0.1	Nd	Nd-0.2	Nd-0.3	Nd-0.5	0.6	7.9	Nd-3.1
Roxithromycin	Nd-5.1	Nd-4.7	Nd	Nd-26.6	Nd-6.3	1.9-3.9	10.3	6.9	2.5-20.2
Chloramphenicol	–	–	–	–	Nd-11.6	3.6-5.9	2.5	7.2	Nd-1.3
Thiamphenical	–	–	–	–	Nd-8.7	Nd-10.0	1.8	6.5	Nd-38.1
Florfenicol	–	–	–	–	Nd-7.7	Nd-7.8	Nd	Nd	Nd-20.2

[a]Represent a sample site located at Tiaoxi river nearby the outfall of a swine farm; Nd means below the method detection limits; "—" means no data available in this sample time

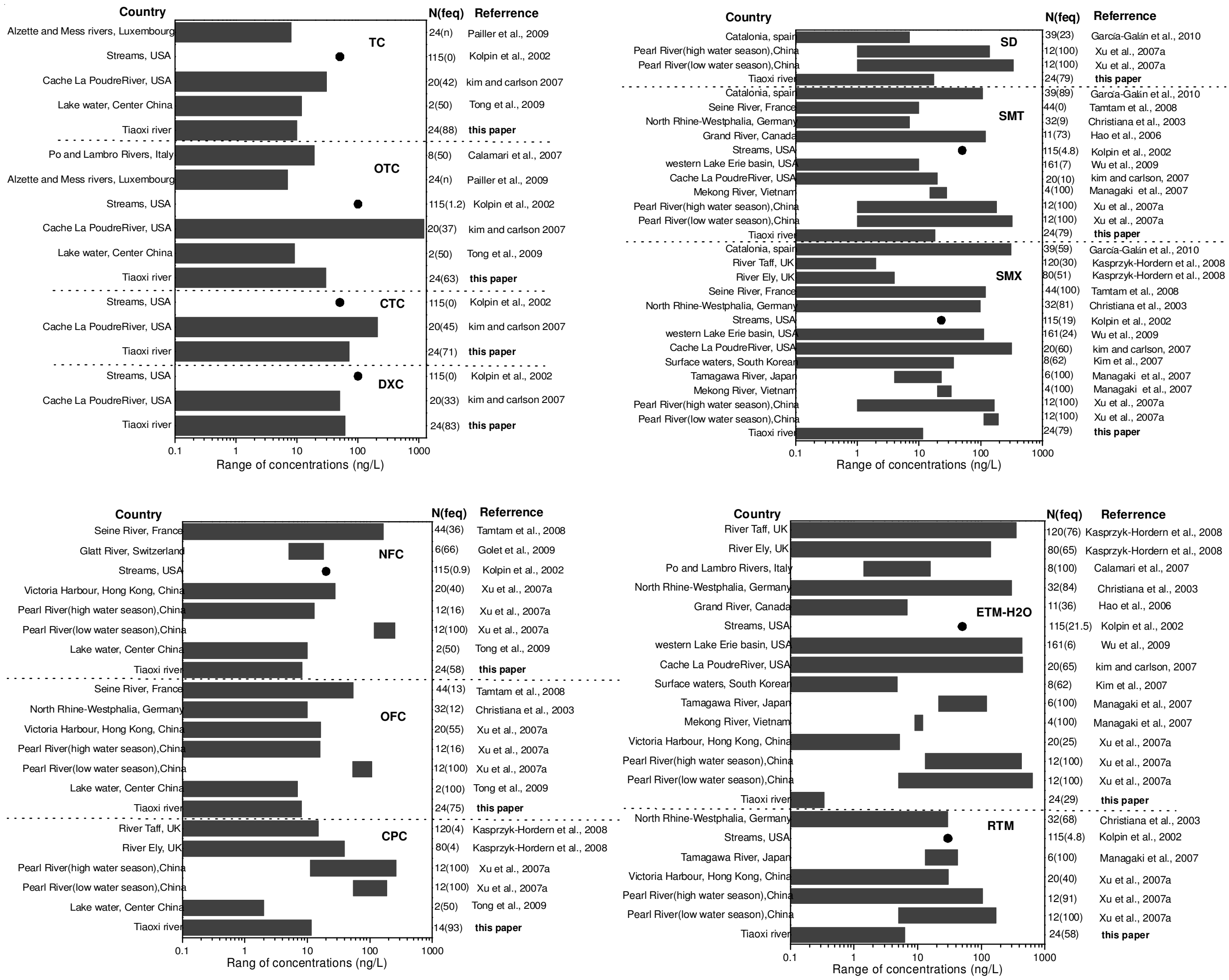

Fig. 2. Range of antibiotics concentrations reported by publications and observations in the paper. N (freq) means the sample number (frequency of detection); the value of streams of USA was showed on reported levels[20]

the reservoir. The presence of antibiotics in drinking water source should be of great public concern due to the unknown health effects of chronic low-level exposure.

Presence and distribution of antibiotics along Tiaoxi river: All the selected antibiotics were detected in this river (Table-3), depending on sampling location and season. The maximum concentrations of antibiotics on the sampling occasions were up to 71.6 ng L^{-1}. Tetracyclines (sum of tetracycline, oxytetracycline, chlortetracycline and doxycycline) were the main contaminants along Tiaoxi river but their concentrations varied among sampled sites (Fig. 3). This variation was probably the result of inter alia, contaminants inputs from agricultural activities and river dilution capacity at sampling sites[13]. As the strong sorption of tetracyclines to particulate materials in water results in active deposition and sedimentation[14], the velocity distribution of the river might be another factor contributing to variation. Widespread inputs of veterinary antibiotics from swine farms to the Tiaoxi river may occur as a result of the high density of swine habitation (up to733 head km^{-2}) and the large number of pigs (approximately 2.5 million head yr^{-1}) in this region, judging from the more frequent observation of this type of antibiotic in swine wastewater.

Sulfonamides (sum of dulfadiazine, sulfamethoxazole and sulfamethazine) were another notable class of compounds detected along the river and they also varied among sampling sites (Fig. 3). The concentrations of this category were one order of magnitude lower than in the Pearl river (a very large big river in south China), where the median concentrations of dulfadiazine, sulfamethoxazole and sulfamethazine were 38, 37 and 67 ng L^{-1}, respectively[11]. Considering the very large amounts of domestic wastewater input to the Pearl river, the lower values of these analytes in Tiaoxi river were dependent on agricultural activities as evidenced by the extremely high concentration of dulfadiazine observed in swine wastewater and pond water of soft-shelled turtles, in contrast with significantly higher concentrations at municipal sewage wastewaters in south China[12]. Low detection of dulfadiazine in Tiaoxi river, apart from the heavier contamination with dulfadiazine at the main stream near outfall of a swine farm, was likely due to high dilution by this river and because of its supposed weak sorption and high mobility[21]. In general, higher concentrations and detection frequencies of this class of compounds were found in March 2011, probably due to the lower flow conditions and lower water temperatures which might enhance the persistence of these compounds in aqueous environment[13].

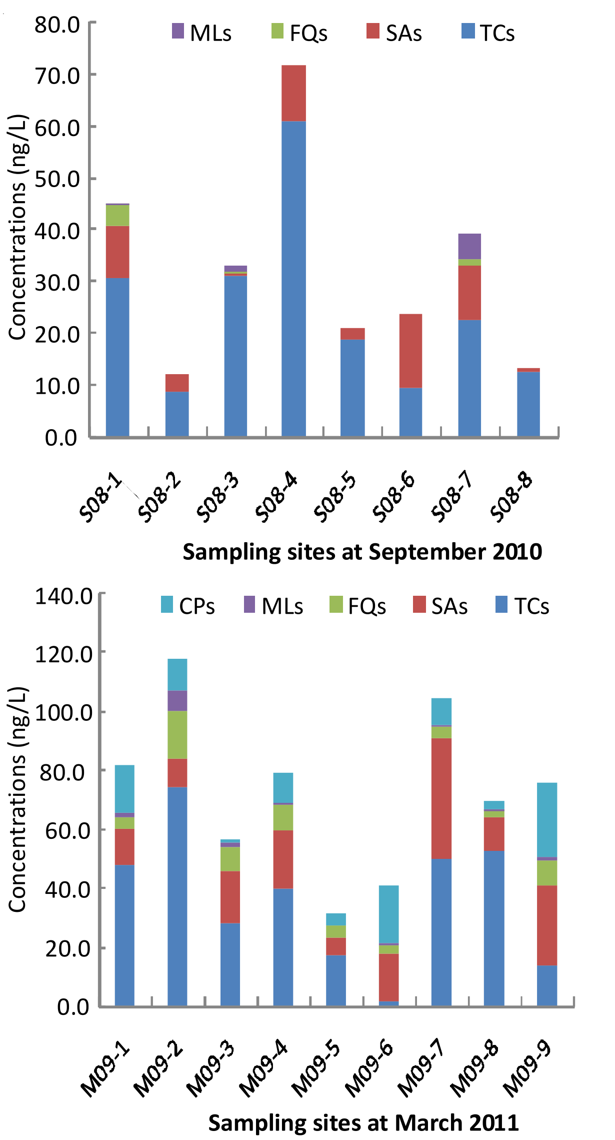

Fig. 3. Distributions and occurrence for categories of selected antibiotics along the Tiaoxi river. tetracyclines = tetracyclines (included tetracycline, oxytetracycline, chlortetracycline and doxycycline), Sas = sulfonamides (included sulfadiazine, sulfamethoxazole and sulfamethazine), FQs = fluoroquinolones (contained norfloxacin and ofloxacin), MLs = macrolides(included erythromycin-H_2O and roxithromycin), CPs = chloramphenicols (included chloramphenicol, thiamphenicol and florfenicol)

Macrolides and fluoroquinolones (<10 ng L^{-1} for each selected compound) in this river were one or two orders of magnitude lower than those in the Pearl river in south China[11], Taff and Ely rivers in UK[18], Tamagawa river in Japan[17], Cache la Poudre river in the US and streams water in the US[13,20] (Fig. 2). Macrolides and fluoroquinolones used for both human and animals may be contributed by either municipal wastewater treatment or animal farms. However, the low concentration of selected antibiotics was measured at swine wastewater and main stream water near the outfall of a swine farm and it is still uncertain whether the residues of these compounds in Tiaoxi river are sourced from domestic sewage or intensive farms.

Interestingly, chloramphenicol was mostly found (frequency of detection was 91 %) at all locations along Tiaoxi river in March 2011. Chloramphenicol has been banned from use in livestock breeding since 2002 in China because of its plastic anaemias in humans. The level of chloramphenicol in river water (<1-11.6 ng L^{-1}) was higher than Taff and Ely rivers in UK (below 2 ng L^{-1}) and lake water in Hubei province in central China (around 2 ng L^{-1})[17,18], but was far lower than detected in the Pearl river (up to 266 ng L^{-1})[11]. Because of the very low concentration of this compound in municipal wastewater[12,22], there were no more samplings of municipal wastewater in the paper and the level in the aquatic environment was probably due to illegal usage in aquaculture or animal husbandry in this region. Thiamphenical and florfenicol are substitutes for chloramphenicol and were present in some aqueous samples.

Although there is no evidences for the relationship between concentration and incidence of antibiotic resistance in this region, tetracycline resistance genes have been claimed to be widespread in environmental matrices (more than 38 tetracycline resistance genes) and had encoding ribosomal protection proteins in environmental matrices[23,24]. This suggests the possible spread of bacterial resistance in the natural environment. The occurrence of tetracyclines antibiotic resistance genes in the surface waters of northern subtropical in east China should be monitored in the future.

Evaluation of contribution of veterinary antibiotics discharged from intensive farms: Concentrations of the antibiotics in Tiaoxi river, main stream water near the outfall from a swine farm and raw wastewater from pig farms were compared in Fig. 4. Some of dominant contaminants tetracycline, oxytetracycline, chlortetracycline and sulfadiazine were two orders of magnitude lower in the main stream of the Tiaoxi river than in the wastewater. Dilution by the large water mass of Tiaoxi river may explain this, in view of the high concentrations at main stream water near the farm outfall. Exclusively associated with suspended particles in water, active deposition and sedimentation might be another explanation for the relatively low concentrations in aqueous samples as some classes of antibiotics have strong sorption to particulate materials (*e.g.*, tetracyclines and fluoroquinolones)[14,25].

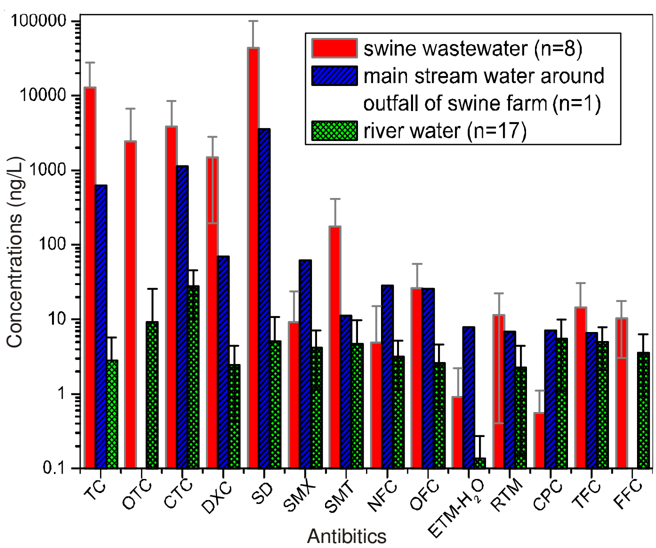

Fig. 4. Antibiotic concentrations in swine wastewater, main stream water near the outfall of a swine farm and surface waters along Tiaoxi river

We detected substantial concentrations of chlortetracycline in swine wastewater and stream water, consistent with results for swine wastewater in north China and manure investigations in eight Chinese provinces[26,27]. Chlortetracycline was also detected in animal manures in Germany, Turkey, Australia and Canada[28-31]. It suggested that chlortetracycline can be used as a semi-quantitative marker in these countries as a higher proportion of chlortetracycline indicates higher inputs of livestock wastes. This contrasts with situation in the Mekong Delta where the sulfamethazine was used as a molecular marker of livestock wastes in Vietnam[15]. Because of high levels detected of sulfamethazine (up to 675.4 ng L^{-1}) in swine wastewater in sampling surveys and high levels of this compound (about 323 ng L^{-1}) in sewage treatment plants in south China[12], it cannot be used as a marker of livestock wastes in China. This is consistent with the results from United States, where sulfamethazine has been detected in rivers influenced by urban and municipal wastewater treatment plants[32]. However, detailed information should be evaluated for the use of chlortetracycline as a marker specific to livestock wastes because of complex physico-chemical prosperities of this compound in environmental matrices, such as transformation to other isomers against the conventional analysis method (HPLC system), which determine the analyte by its retention time.

Conclusion

The results obtained in this study showed that these detected antibiotics could lead to drinking water pollution risk in Tiaoxi river basin, especially for the tetracyclines (tetracycline, oxytetracycline, chlortetracycline and doxycycline) which are likely derived from the livestock wasters and agricultural activities (*e.g.*, swine or soft-shell turtle feeding) may be major sources of contaminants. So these wastewaters should be controlled and treated before discharged into common rivers. Furthermore, we only investigated and analyzed the concentrations of selected antibiotics in the study and it was not enough to reveal the dangerous for human health. More studies should be done to show the occurrence and fate of veterinary antibiotics in this region and it was important for the risk assessments of these compounds in the aquatic environment or human health.

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REFERENCES

1. I. Michael, L. Rizzo, C.S. McArdell, C.M. Manaia, C. Merlin, T. Schwartz, C. Dagot and D. Fatta-Kassinos, *Water Res*., **47**, 957 (2013).
2. S. Kaplan, *Crit. Rev. Environ. Sci. Technol*., **43**, 1074 (2013).
3. B. Liu, W. Liu, X. Nie, C. Guan, Y. Yang, Z. Wang and W. Liao, *J. Environ. Sci. (China)*, **23**, 1558 (2011).
4. Y.G. Zhu, T.A. Johnson, J.Q. Su, M. Qiao, G.X. Guo, R.D. Stedtfeld, S.A. Hashsham and J.M. Tiedje, *Proc. Natl. Acad. Sci. USA*, **110**, 9 (2013).
5. F. Canada-Canada, A. Munoz de la Pena and A. Espinosa-Mansilla, *Anal. Bioanal. Chem*., **395**, 987 (2009).
6. Y.S. Chen, H.B. Zhang, Y.M. Luo and J. Song, *Environ. Monit. Assess*., **184**, 2205 (2012).
7. S. Zheng, X. Qiu, B. Chen, X. Yu, Z. Liu, G. Zhong, H. Li, M. Chen, G. Sun, H. Huang, W. Yu and D. Freestone, *Chemosphere*, **84**, 1677 (2011).
8. J. Fick, H. Söderström, R.H. Lindberg, C. Phan, M. Tysklind and D.G.J. Larsson, *Environ. Toxicol. Chem*., **28**, 2522 (2009).
9. L. Guo, *Science*, **317**, 1166 (2007).
10. C.S. McArdell, E. Molnar, M.J. Suter and W. Giger, *Environ. Sci. Technol*., **37**, 5479 (2003).
11. W.H. Xu, G. Zhang, S.C. Zou, X.D. Li and Y.C. Liu, *Environ. Pollut*., **145**, 672 (2007).
12. Y. Zhang, J.L. Zhou and B. Ning, *Water Res*., **41**, 19 (2007).
13. S.C. Kim and K. Carlson, *Environ. Sci. Technol*., **41**, 50 (2007).
14. J. Tolls, *Environ. Sci. Technol*., **35**, 3397 (2001).
15. S. Managaki, A. Murata, H. Takada, B.C. Tuyen and N.H. Chiem, *Environ. Sci. Technol*., **41**, 8004 (2007).
16. C.X. Wu, J.D. Witter, A.L. Spongberg and K.P. Czajkowski, *Water Res*., **43**, 3407 (2009).
17. L. Tong, P. Li, Y.X. Wang and K.Z. Zhu, *Chemosphere*, **74**, 1090 (2009).
18. M. Deborde and U. von Gunten, *Water Res*., **42**, 13 (2008).
19. H.T. Lai, J.H. Hou, C.I. Su and C.L. Chen, *Ecotoxicol. Environ. Saf*., **72**, 329 (2009).
20. D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber and H.T. Buxton, *Environ. Sci. Technol*., **36**, 1202 (2002).
21. S. Thiele-Bruhn, T. Seibicke, H.-R. Schulten and P. Leinweber, *J. Environ. Qual*., **33**, 1331 (2004).
22. Q. Sui, J. Huang, S. Deng, G. Yu and Q. Fan, *Water Res*., **44**, 417 (2010).
23. X.X. Zhang, T. Zhang and H.H.P. Fang, *Appl. Microbiol. Biotechnol*., **82**, 397 (2009).
24. T. Kobayashi, F. Suehiro, B. Cach Tuyen and S. Suzuki, *FEMS Microbiol. Ecol*., **59**, 729 (2007).
25. E.M. Golet, I. Xifra, H. Siegrist, A.C. Alder and W. Giger, *Environ. Sci. Technol*., **37**, 3243 (2003).
26. W.W. Ben, Z.M. Qiang, C. Adams, H.Q. Zhang and L.P. Chen, *J. Chromatogr. A*, **1202**, 173 (2008).
27. L. Zhao, Y.H. Dong and H. Wang, *Sci. Total Environ*., **408**, 1069 (2010).
28. G. Hamscher, S. Sczesny, H. Höper and H. Nau, *Anal. Chem*., **74**, 1509 (2002).
29. A. Karci and I. Balcioglu, *Sci. Total Environ*., **407**, 4652 (2009).
30. E. Martínez-Carballo, C. González-Barreiro, S. Scharf and O. Gans, *Environ. Pollut*., **148**, 570 (2007).
31. M.O. Aust, F. Godlinski, G.R. Travis, X.Y. Hao, T.A. McAllister, P. Leinweber and S. Thiele-Bruhn, *Environ. Pollut*., **156**, 1243 (2008).
32. K.G. Karthikeyan and M.T. Meyer, *Sci. Total Environ*., **361**, 196 (2006).