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Occurrence of antibiotics and hormones in a major agricultural watershed

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Abstract

Antibiotics and hormones are considered emerging environmental microcontaminants because of their potential adverse effects on ecosystems and human health. Available information on the source of these emerging contaminants in surface waters is very limited. The objectives of this study were to determine the occurrence of antibiotics and hormones in an agricultural watershed and to determine the seasonal variability of these contaminants. Water samples were collected from 15 subwatershed stations and 7 stations on the major receiving river, Choptank, Maryland, USA, over four different seasons (April, June, September and December). Antibiotics (sulfathiazole, sulfamerazine, sulfamethizole, sulfamethazine, sulfachloropyridazine, sulfamethoxazole, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline and doxycycline) and hormones (estriol, estradiol, 17α-ethynylestradiol, estrone, testosterone and progesterone) as well as arsenic which is used as feed additive were determined in these water samples. In addition, the same antibiotics were analyzed in one set of sediment samples. This study indicated that agriculture may act as a source of antibiotic residues in the aquatic environment.

Keywords: Antibiotic; Hormone; Arsenic; Watershed; Agriculture

1. Introduction

A 2002 survey conducted by the National Agricultural Statistics Service (NASS) reported that there were 104 million cattle, 8.6 billion chickens, 60 million swine, and 275 million turkeys in the United States (US) [1]. The marked intensification of animal production, particularly in the last 25 years, has resulted in increased water quality problems associated with the production and disposal of animal waste generated by these operations. Current livestock production involves the use of large amounts of different chemicals including antibiotics, hormones and metals (feed additives). These chemicals are considered emerging environmental microcontaminants because

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of their potential adverse affects on ecosystems and human health. Manure from animals is either stockpiled or immediately applied to farmland as fertilizer. When applied to a field, chemical residues in the manure can ultimately reach surface and ground water by runoff or leaching [2]. A variety of chemicals used for animal production have been detected in manure [3,4] and in surface waters [5–8].

Two commonly used classes of antibiotics for animal feeding operations are sulfonamides (SAs) and tetracyclines (TCs). While SAs are synthetic, TCs are naturally occurring or semi-synthetic. Antibiotic residues in the environment are of considerable concern because of the potential development of antibiotic-resistant bacteria. Hormones can be considered endocrine disrupting chemicals (EDCs), and laboratory studies have shown that environmental levels of EDCs can affect fish [9] and birds [10] under laboratory conditions. Animal feed additives include trace metals and metalloids such as arsenic as well as antibiotics [11]. The arsenicals include roxarsone and arsanilic acid, which improve growth performance as well as improve bird pigmentation. Arsenic has become an important environmental concern due to its potential carcinogenic properties [12], and it is also considered the number-one toxin in the USEPA list of priority pollutants [13,14].

Although these emerging contaminants have been found in surface waters, it is not clear whether the sources are urban wastewater or animal feeding operations. Also, available information on the seasonal variation of these chemicals in surface waters is very limited. The objectives of this study were twofold: to determine the occurrence of animal feed additives in an agricultural watershed and to determine the seasonal variability of these contaminants. For this purpose antibiotics (sulfathiazole, sulfamerazine, sulfamethizole, sulfamethazine, sulfachloropyridazine, sulfamethoxazole, sulfadimethoxine, tetracycline, oxytetracycline, chlortetracycline and doxycycline) and hormones (estriol, estradiol, 17α-ethynylestradiol, estrone, testosterone and progesterone), as well as feed additive metal of arsenic were determined in 15 subwatershed stations and 7 stations on the major receiving river, Choptank, Maryland, USA over four different seasons (April, June, September and December). In addition, the same antibiotics were analyzed in one set of sediment samples (only in June).

2. Materials and methods

2.1. Study area

The Choptank River Watershed is located on the Delmarva Peninsula of the Chesapeake Bay, USA. It contains 62% agricultural land, 33% forest land, and 2% wetlands while 5% of land is developed. The Delmarva Peninsula is an agricultural center for the state of Maryland and it is dominated by the poultry industry. Approximately 35% of Maryland's cash farm income was from broilers in 2002. Maryland ranked 9th among the US states in pounds of broilers produced in 2002 with 1.4 billion pounds, and Maryland ranked 7th among the states in the number of broilers produced (585 Million in 2002). Chicken litter from the poultry houses is routinely recycled as a fertilizer on corn and soybean production fields in this region.

Parts of the Choptank River Watershed are identified as "impaired waters" under the Federal Clean Water Act. The Upper Choptank River may require preparation of Total Maximum Daily Loads (TMDLs) for nutrients and sediment, and the Choptank marine beach is impaired with respect to fecal coliform bacteria. Maryland Department of Natural Resource's results of monitoring of water quality and living resource habitat within the watershed since 1985 reveal increasing nitrate, chlorophyll *a*, total suspended solids, and decreasing Secchi depth values over time. Seasonally low oxygen levels are observed in the deeper estuarine portions of the Little Choptank River and Lower Choptank River.

This is partially an extension of the seasonal hypoxia in Chesapeake Bay as well as accelerated eutrophication in these rivers due to nutrients from unspecified sources. Agriculture is the primary land use and the leading source of nitrogen, phosphorus and sediment. The Choptank River receives significant pulses of corn and soybean herbicides, nutrients, and sediments during the spring and summer months which are associated with storm runoff events.

2.2. Sample preparation

Surface water samples were collected from 15 subwatershed stations and 7 stations on the major receiving river, Choptank, Maryland, USA over four different seasons (April, June, September and December) in 2005 (Fig. 1). All samples were filtered through 0.7 μm glass–fiber filters into bottles (for antibiotics 0.25-L amber glass, for hormones 1-L amber glass and for arsenic 1-L plastic) and stored at 4°C until they were analyzed, typically within two weeks. Water samples for arsenic analyses were acidified upon collection with trace element grade nitric acid (HNO_3) to obtain a pH < 2.0. Duplicate samples were collected for backup purposes and for laboratory replicates. Field blanks, were made from laboratory-grade organic-free water, and were also submitted to the sites and analyzed for all chemicals.

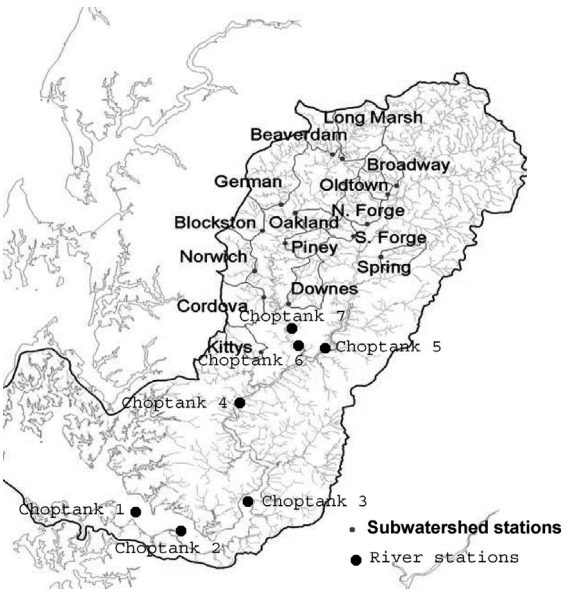

Fig. 1. Sampling stations on the Choptank watershed.

2.3. Solid phase extraction

2.3.1. Water samples

2.3.1.1. Antibiotics

Antibiotics were extracted using the method described by Lindsey et al. [5]. Samples were prepared for extraction by adding 150 μL of 40% H_2SO_4, and 1 g of disodium ethylenediaminetetraacetate (Na_2EDTA) to the bottle containing samples. To achieve dissolution of the Na_2EDTA, the bottles were agitated on an orbital shaker for 60 min at 100 rpm. Antibiotics were extracted using 60-mg HLB (hydrophilic–lipophilic balance) Oasis cartridges from Waters (Millford, MA). Cartridges were preconditioned with 3 mL of MeOH, 3 mL of 0.5 N HCl, and 3 mL of distilled water. Samples were then passed through the cartridges at 10 mL/min. After isolation, the cartridges were rinsed with 1 mL of distilled water to remove excess Na_2EDTA. The analytes were eluted into a test tube using 5 mL of MeOH. The eluents were concentrated under a flow of N_2 to a volume of 0.5 mL by evaporation. Then, 0.5 mL water was added to the tube, and the tube was vortexed for 30 s. The resulting mixture was transferred to 2 mL amber autosampler vials. Finally, 20 μL of the internal standard, 2.5 mg/L simatone, was added to each vial.

2.3.1.2. Hormones

Hormones were extracted using the method described by Laganà et al. [8]. Hormones were isolated onto 200-mg HLB (hydrophilic–lipophilic balance) Oasis cartridges from Waters Inc. (Millford, MA). The cartridges were

prewashed with 10 mL of dichloromethane:methanol (50:50, v/v), 5 mL of methanol and 10 mL of distilled water. Samples were passed through the cartridges at 10 mL/min. Then the cartridges were washed with 10 mL of water. The retained compounds were eluted with 7 mL of dichloromethane:methanol (50:50, v/v). The extracts were then evaporated to dryness under a gentle nitrogen stream in a thermostatic bath. The residues were redissolved in 200 µL of a 50:50 (MeOH:H_2O) solutions containing 0.1 mg/L of ^{13}C-estradiol as internal standard.

2.3.2. Sediment samples

Sediment samples were extracted for antibiotic analysis using the method described by Capone et al. [15]. Briefly, 1 g subsamples were extracted three times with 3 mL of 0.1 M Na_2EDTA–McIlvaine buffer by vortexing for 30 s followed by sonication for 3 min in a 100 W sonication bath (Bronson Ultrasonics, Danbury, CT). After each extraction, the extracts were subjected to centrifugation (500*g*, 5 min, 5°C), the supernatants pooled, again subjected to centrifugation (1650*g*, 20 min, 5°C), filtered and passed through 60-mg HLB Oasis cartridges from Waters cartridges after the cartridges had been previously flushed with 5 mL methanol and 10 mL 0.1 M Na_2EDTA–McIlvaine buffer. After extracts were loaded, cartridges were flushed with 20 mL distilled water, followed by sample elution using 8 mL of 0.01 M methanolic oxalic acid. Eluents were concentrated to approximately 0.5 mL by evaporation Then, 0.5 mL water was added to the tube, and the tube was vortexed for 30 s. The resulting mixture was transferred to 2 mL amber autosampler vials. Finally, 20 µL of the internal standard, 2.5 mg/L simatone, was added to each vial.

Table 1
Parent and daughter ions used for quantitation of hormones and antibiotics and MS parameters used to produce them

Compound	Parent ion (Da)	Daughter ion (Da)	Retention time (min)	Cone (V)	Collision (eV)
Sulfathiazole	256	156	3.9	20	17
Sulfamerazine	265	108	4.9	35	22
Sulfamethizole	271	156	6.9	21	19
Sulfamethazine	279	186	7.3	30	24
Sulfachloropyridazine	285	156	8.8	22	16
Sulfamethoxazole	254	156	9.1	20	16
Sulfadimethoxine	311	156	13.0	33	22
Tetracycline	445	410	6.1	20	19
Oxytetracycline	461	426	6.6	18	18
Chlortetracycline	479	444	10.1	27	22
Doxycycline	445	428	12.3	21	18
Estriol	271	133	21.9	22	18
Estradiol	255	159	22.1	14	21
17α-ethynylestradiol	279	133	22.3	24	16
Estrone	271	253	21.9	20	15
Testosterone	289	109	22.6	25	21
Progesterone	315	109	25.6	14	23
Simatone	198	124	8.9	26	20
^{13}C-Estradiol	258	159	22.1	22	18

2.4. Antibiotics and hormones analyses

Antibiotics and hormones were analyzed by LC/MS–MS. The LC instrument was a 2695 XE separations module (Waters Corp., Milford, MA) equipped with an Xterra MS C18 column (150 mm × 2.1 mm i.d., 5 μm) (Waters Corp., Milford, MA) and operated at a temperature of 45°C; the injection volume was 10 μL. For antibiotics and hormones the same mobile-phase gradient was used to separate the compounds: The respective compositions of solvents A, B and C were as follows: A, 1% formic acid–methanol (70:30, v/v); B, water; and C, methanol. The solvents were mixed as follows: 0–1 min 50% A, 50% B, 0% C; 1–12 min a linear gradient from the previous settings to 70% A, 0% B, 30% C; 12–30 min from the previous settings to 7% A, 0% B, 93% C; and finally the instrument was returned to starting conditions from 30–32 min and then allowed to stabilize for 10 min with 50% A, 50% B. The total run time was 42 min. The flow of the column was set at the rate of 0.25 mL/min. The analytes were detected using atmospheric pressure ionization-tandem mass spectrometry. The instrument was a benchtop triple quadrupole mass spectrometer (Quattro LC from Micromass Ltd., Manchester, UK) operated in electrospray ionization mode. The source parameters were as follows: capillary voltage was set at 3.0 kV and extractor voltage was set at 3 V, respectively; rf lens at 0.1 V; source and desolvation temperatures were 150 and 450°C. Liquid nitrogen was used to supply the nebulizer and desolvations gas (flow rates were approximately 80 and 600 L/h, respectively). Argon was used as collision gas to fragment the parent ions; the typical pressure of the collision cell was 2.6×10^{-3} mbar. Both high and low mass resolutions were set at 12.0 for both quadrupoles. Acquisition was done in the multiple-reaction monitoring mode (MRM) in electrospray positive (ES+) mode. The parent and daughter ions used for compound identification and quantitation are listed in Table 1 along with the optimum cone voltages and collision energies used. The detector was a photomultiplier set at 650 V. Quantitation of the SAs group of antibiotics was performed using internal standard method utilizing simatone; while calculations of TC concentrations were based on the method of standard addition described by Lindsey et al. because of matrix effects [5]. Concentrations for the hormones were calculated by internal standard method using ^{13}C-estradiol.

2.5. Arsenic analyses

Aliquots of 100 mL of the acidified samples were placed into 200 mL pre acid-washed beaker and slowly evaporated to approximately 1 mL on hot plate. The remaining sample in each beaker

Table 2
Recoveries of antibiotics and hormones from distilled water[a]

Compound	Recovery (%)	SD
Antibiotics		
Sulfathiazole	90	5
Sulfamerazine	69	19
Sulfamethizole	84	9
Sulfamethazine	75	7
Sulfachloropyridazine	74	6
Sulfamethoxazole	79	10
Sulfadimethoxine	64	7
Tetracycline	62	16
Oxytetracycline	114	21
Chlortetracycline	77	9
Doxycycline	74	10
Hormones		
Estriol	102	10
Estradiol	104	5
17α-ethynylestradiol	96	7
Estrone	99	10
Testosterone	105	14
Progesterone	106	14

[a]Recovery values are the means from duplicate samples using 0.20 and 2.0 μg/L spikes.

was quantitatively transferred to 10 mL volumetric flasks and diluted to volume with 1 N HNO_3 for analysis of arsenic. Prior to analysis, a 4 mL aliquot of digested sample was transferred to a 15 mL plastic tube where additional reagents were added as follows: 1.5 mL concentrated hydrochloric acid (trace element grade), 2.5 mL of 1.73 M sulfuric acid, 2 mL of a solution containing 5% potassium iodide and 5% ascorbic acid according to a method by Anderson and Isaacs [16]. Reagents were mixed with sample and allowed to stand for 20 min for the reduction of As(V) to As(III) to occur. Arsenic concentrations in the samples were determined using a hydride generation method with an inductively coupled plasma atomic emission spectrometer with continuous flow of a solution containing 0.5% sodium borohydride ($NaBH_4$) and 0.05% sodium hydroxide for hydride generation.

3. Results and discussion

Our recovery values ranged from 62% to 114% for antibiotics and from 96% to 106% for hormones (Table 2). The average recoveries for antibiotics and hormones were 78% and 102%, respectively. These recoveries are comparable to values reported by others [5,8].

A summary of the antibiotics from a total of 26 water samples from 7 river stations and 56 water samples from 15 subwatershed stations collected from April 2005 through December

Table 3
Concentrations of sulfonamides (SAs) class antibiotics in surface water samples (μg/L)

Stations	Sulfathiazole				Sulfamerazine				Sulfamethizole			
	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4
River stations												
Choptank 1	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 2	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 3	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 4	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 5	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 6	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
Choptank 7	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
Subwatershed stations												
Kittys	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
Cordova	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
Norwick	0	0	0	0	0	0	0	0	0	0	0	0
Blockston	0	0	0	0	0	0	0	0	0	0	0	0
Piney	0	0	0	0	0	0	0	0	0	0	0	0
Oakland	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
German	0	0	0	0	0	0	0	0	0	0	0	0
Beaverdam	0	0	0	0	0	0	0	0	0	0	0	0
Cong marsh	0	0	0	0	0	0	0	0	0	0	0	0
Broadway	0	0	0	0	0	0	0	0	0	0	0	0
Oldtown	0	**0.004**	0	0	**0.694**	**0.005**	0	0	0	**0.005**	0	0
Spring	0	0	0	0	0	0	0	0	0	0	0	0
North forge	0	0	0	0	0	0	0	0	0	0	0	0
South forge	0	0	0	0	0	0	0	0	0	0	0	0
Downes	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0

n.m.: not measured.

2005, is shown in Tables 3 and 4. Only sulfamethoxazole (19% detection; 5 out of 26 samples) and sulfadimethoxine (12% detection; 3 out of 26 samples) in the SAs group were detected in the river stations at concentrations ranging from 0.001 to 0.002 μg/L (avg. 0.001 μg/L) and 0.001 to 0.003 μg/L (avg. 0.002 μg/L), respectively (Table 3). Similarly, the most frequently detected SAs were sulfamethoxazole (5% detections) and sulfadimethoxine (14% detections) in the subwatershed stations at concentrations ranging from 0.005 to 0.007 μg/L (avg. 0.006 μg/L) and 0.001 to 0.009 μg/L (avg. 0.003 μg/L), respectively. The highest concentration for SAs was detected for sulfamerazine, 0.694 μg/L, at the April collection.

Chlortetracycline (19% detection) and oxytetracycline (15% detection) were the most frequently detected of the TCs group of antibiotics in the river stations (Table 4). The concentrations ranged from 0.011 to 0.034 μg/L (avg. 0.016 μg/L) and 0.006 to 0.047 μg/L (avg. 0.016 μg/L), respectively. Tetracycline and doxycycline were detected only once (4% detection) in the river stations at concentrations of 0.005 and 0.020 μg/L, respectively. There was no trend towards lower concentration moving from upstream to downstream as one might expect. Chlortetracycline (21% detection) and oxytetracycline (18% detection) were the most frequently detected TCs class antibiotics at the subwatershed stations. The concentrations ranged from 0.001 to 0.180 μg/L (avg. 0.020 μg/L) and 0.005 to 0.084 μg/L (avg. 0.053 μg/L), respectively. Tetracycline and doxycycline were detected (5%) in the subwatershed

Table 3 (*continued*)

Sulfamethazine				Sulfachloropyridazine				Sulfamethoxazole				Sulfadimethoxine			
April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4
0	0	0	0	0	0	0	0	0	0	0	**0.001**	0	0	**0.003**	0
0	0	0	0	0	0	0	0	0	0	0	**0.002**	0	0	**0.001**	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	**0.001**	0	0	**0.001**	0
n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	**0.001**	n.m.	0	0	0
n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	**0.001**	n.m.	0	0	0
0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	**0.001**	n.m.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	**0.007**	0	0	0	0	0	0	0
0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	**0.001**	n.m.
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0.001**	0
0	0	0	0	0	0	0	0	0	0	0	**0.007**	0	**0.002**	0	0
0	**0.006**	0	0	0	**0.005**	0	0	0	**0.005**	0	0	0	**0.009**	**0.002**	0
0	0	0	0	0	0	0	0	0	0	0	0	0	**0.002**	**0.001**	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0

Table 4
Concentrations of tetracyclines (TCs) class antibiotics in surface water samples (μg/L)

Stations	Tetracycline				Oxytetracycline				Chlortetracycline				Doxycycline			
	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4
River stations																
Choptank 1	0	0	0	0	0	0	0	0	0	0	0	**0.034**	0	0	0	0
Choptank 2	0	0	0	0	0	0	**0.007**	0	0	0	0	**0.011**	0	0	0	0
Choptank 3	0	0	0	0	0	0	**0.007**	0	0	0	0	**0.013**	0	0	0	0
Choptank 4	0	**0.005**	0	0	0	**0.047**	0	0	0	**0.016**	0	0	0	**0.020**	0	0
Choptank 5	0	0	0	0	0	**0.006**	0	0	0	0	0	**0.007**	0	0	0	0
Choptank 6	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
Choptank 7	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
Subwatershed stations																
Kittys	0	0	0	n.m.	**0.388**	**0.009**	0	n.m.	0	**0.020**	0	n.m.	0	0	0	n.m.
Cordova	0	0	0	n.m.	**0.045**	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
Norwick	0	0	0	0	**0.059**	0	0	0	0	0	0	0	0	0	0	0
Blockston	0	0	0	0	0	0	0	0	**0.010**	0	0	**0.011**	**0.039**	0	0	0
Piney	0	0	0	0	0	0	0	0	0	0	**0.007**	**0.005**	0	0	0	0
Oakland	0	0	0	n.m.	0	**0.005**	0	n.m.	0	0	0	n.m.	0	0	0	0
German	0	0	0	0	0	0	0	0	0	0	0	**0.001**	0	0	0	0
Beaverdam	0	0	0	0	**0.084**	0	0	**0.0010**	0	**0.001**	0	**0.006**	0	0	0	0
Cong marsh	0	0	0	0	0	0	0	0	0	0	0	**0.008**	0	0	0	0
Broadway	0	0	0	0	0	**0.007**	0	**0.0024**	0	**0.004**	0	0	0	0	0	0
Oldtown	0	**0.003**	0	0	0	**0.017**	0	0	0	0	**0.180**	**0.003**	0	**0.013**	0	0
Spring	0	**0.002**	0	0	0	**0.009**	0	0	0	**0.005**	**0.020**	**0.005**	0	0	0	0
North forge	0	0	0	0	**0.013**	0	0	0	0	0	**0.067**	**0.002**	0	0	0	0
South forge	**0.001**	0	0	0	**0.049**	0	**0.007**	0	**0.058**	**0.005**	0	**0.005**	**0.146**	0	0	0
Downes	n.m.	0	0	0	n.m.	0	**0.007**	0	n.m.	0	**0.013**	**0.011**	n.m.	0	0	0

n.m.: not measured.

Table 5
Concentrations of antibiotics in sediments for June 29 sampling (μg/kg dry weight (D.W.))

Stations	Sulfa-thiazole	Sulfa-merazine	Sulfa-methizole	Sulfa-methazine	Sulfa-chloropyridazine	Sulfa-methoxazole	Sulfadi-methoxine	Tetra-cycline	Oxytetra-cycline	Chlortetra-cycline	Doxy-cycline
Choptank 4	0	0	0	**0.816**	0	**0.102**	0	0	0	**10.00**	0
Choptank 5	0	0	0	0	0	**0.145**	0	0	0	**4.12**	0
Choptank 6	0	0	0	0	0	0	0	0	0	**2.07**	0
Choptank 7	0	0	0	0	0	**0.047**	0	0	0	**2.17**	0

Table 6
Concentrations of hormones in surface water samples (μg/L)

Stations	Estriol				Estradiol				17α-Ethynylestradiol			
	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4
River stations												
Choptank 1	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 2	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 3	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 4	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 5	0	0	0	0	0	0	0	0	0	0	0	0
Choptank 6	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
Choptank 7	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
Subwatershed stations												
Kittys	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
Cordova	0	0	**0.012**	n.m.	0	0	0	n.m.	0	0	0	n.m.
Norwick	0	0	0	0	0	0	0	0	0	0	0	0
Blockston	0	0	0	0	0	0	0	0	0	0	0	0
Piney	0	0	0	0	0	0	0	0	0	0	0	0
Oakland	0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
German	0	0	0	0	0	0	0	0	0	0	**0.002**	0
Beaverdam	0	0	0	0	0	0	0	0	0	0	0	0
Cong marsh	0	0	0	0	0	0	0	0	0	0	0	0
Broadway	0	0	0	0	0	0	0	0	0	0	0	0
Oldtown	0	0	0	0	0	0	0	0	0	0	0	0
Spring	0	0	0	0	0	0	0	0	0	0	0	0
North forge	0	0	0	0	0	0	0	0	0	0	0	0
South forge	0	0	0	0	0	0	0	0	0	0	0	0
Downes	n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0

n.m.: not measured.

stations at concentrations ranging from 0.001 to 0.003 μg/L (avg. 0.002 μg/L) and 0.013 to 0.146 μg/L (avg. 0.066 μg/L), respectively.

At river and subwatershed stations at least one antibiotic was detected at least once. River stations Choptank 6 and 7 had only one detection (3% detection) whereas Choptank 2, 4 and 5 had the most detection (9% detection) for antibiotics. Norwick and German subwatershed stations had the minimum detection (3% detection) for antibiotics while Broadway, Oldtown, Spring and South forge subwatershed stations had more than 10% (11%, 32%, 16% and 16%, respectively) detections. Kittys, Blockston, Piney, Beaverdam, Broadway, Oldtown, Spring, North forge, South forge and Downes were detected more than 10% for TCs class antibiotics (25%, 19%, 13%, 25%, 19%, 31%, 31%, 19%, 44% and 25%, respectively) and Oldtown was the most frequently detected compound among SAs (32% detection). In addition, all the SAs were detected at the Oldtown subwatershed station.

The same percentages of detections were found for all antibiotics in the river and subwatershed stations; except sulfamethoxazole. Also, the average antibiotic concentrations in the

Table 6 (*continued)*

Estrone				Testosterone				Progesterone			
April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4	April 1	June 29	Sep 26	Dec 4
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	**0.016**	0	0	0	0	0	0
n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0
0	**0.013**	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
0	0	0	0	0	0	0	0	0	0	**0.012**	0
0	0	0	0	0	0	0	0	0	0	**0.014**	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	n.m.	0	0	0	n.m.	0	0	0	n.m.
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	**0.012**	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0.020	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
n.m.	0	0	0	n.m.	0	0	0	n.m.	0	0	0

subwatershed stations were higher than the average antibiotic concentrations in the river stations where more dilution occurs. Seasonally there appears to be more samples with positive detections for antibiotics in the December collections and chlortetracycline appears to top the list with 14 out of 19 (74%) samples. September and June come next with 32% detection.

The concentrations of antibiotics from a total of four sediment samples from four river stations collected in June, 2005 are given in Table 5. Chlortetracycline (100% detection) and sulfamethoxazole (75% detection) were the most frequently detected of the antibiotics. The concentrations ranged from 2.1 to 10.0 μg/kg dry weight (D.W.) (avg. 4.6 μg/kg) and 0.10 to 0.15 μg/kg D.W. (avg. 0.10 μg/kg D.W.), respectively. Sulfamethazine was detected 25% at a concentration of 0.82 μg/kg D.W. Chlortetracycline and sulfamethoxazole were also the most frequently detected antibiotics among TCs and SAs classes in water samples in the river stations. However, oxytetracycline, which gave the second highest concentration in water samples in the river stations, was not detected in sediment samples.

A summary of the hormones from a total of 26 water samples from 7 river stations and 56 water samples from 15 subwatershed stations collected from April 2005 through December 2005, is shown in Table 6. Estradiol was not detected at all in the river and subwatershed stations, whereas progesterone was detected the most. Only testosterone was detected in the river stations, while none of the river and subwatershed stations was detected more than once. Also the hormones were less detected in the river where more dilution occurs.

The Oldtown branch had the second highest average arsenic concentration (all trips averaged) (Table 7) among the watersheds and it is also the location where the most antibiotics (32% detection) were detected.

Table 7
Concentrations of metals in surface water samples (μg/L)

Stations	As			
	April 1	June 29	Sep 26	Dec 4
River stations				
Choptank 1	0.09	0.46	0.98	0.20
Choptank 2	0.13	0.44	1.22	0.27
Choptank 3	0.13	0.58	1.08	0.35
Choptank 4	0.31	0.61	0.62	0.58
Choptank 5	0.31	0.54	0.31	0.41
Choptank 6	n.m	0.64	0.27	0.39
Choptank 7	n.m	0.52	0.33	0.24
Subwatershed stations				
Kittys	0.55	0.48	0.45	0.46
Cordova	0.43	0.37	0.17	0.34
Norwick	0.49	0.46	0.25	0.48
Blockston	0.36	0.26	0.15	0.26
Piney	0.20	0.17	0.12	0.12
Oakland	0.23	0.26	0.14	0.20
German	0.58	1.51	2.45	0.52
Beaverdam	0.35	0.28	0.15	0.13
Cong marsh	0.31	0.48	0.29	0.19
Broadway	0.30	0.60	0.72	0.16
Oldtown	0.46	1.45	0.18	0.28
Spring	0.20	0.16	0.07	0.16
North forge	0.49	0.34	0.17	0.28
South forge	0.21	0.33	0.18	0.18
Downes	0.26	0.17	0.06	0.11

4. Conclusions

The Choptank agricultural watershed was monitored to determine the occurrence and possible aqueous loadings of antibiotics, hormones and arsenic in this system. The same percentages in detections were found for all antibiotics in the river and subwatershed stations; except sulfamethoxazole. Also, the average antibiotic concentrations in the subwatershed stations were higher than the average antibiotic concentrations in the river stations where more dilution occurs. Chlortetracycline, which is very widely used to promote growth at low doses and to control various types of bacterial infections at higher doses, was the most frequently detected antibiotics in the water and sediment samples. This suggest that there is likely a transport of this antibiotic from poultry litter amended fields to adjacent waterbodies, as there are no other potential sources (e.g., wastewater treatment plants) within these tributaries. Because of their widespread use, this compound may be a useful tracer of poultry litter contaminants. Hormone levels were mostly below the detection limit. It appears that sediment samples also provide good long-term monitoring for antibiotics Therefore, for future studies sediment samples should be analyzed as well as water samples. This study indicates that agriculture may serve as a source of antibiotic residues to the aquatic environment.

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