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Occurrence of sulfonamide antimicrobials in private water wells in Washington County, Idaho, USA

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Abstract
Samples from six private wells formerly used as sources for drinking water by the residents of Washington County (Weiser, Idaho) were collected to assess the impact of a nearby confined animal feeding operation (CAFO) on the quality of the local groundwater. All six samples were found contaminated by two veterinary antimicrobials, sulfamethazine (at concentrations from 0.076 to 0.22 μg/l) and sulfadimethoxine (at concentrations from 0.046 to 0.068 μg/l). These groundwater samples also contained elevated concentrations of nitrate and ammonium. Three of the sampled wells have nitrate levels that exceeded the maximum contaminant level set by the US Environmental Protection Agency for drinking water, with nitrate concentration as high as 39.1 mg/l. All but one well showed nitrate, which instead contained ammonium at 1.22 mg/l. Analysis of the nitrate and ammonium in these samples by isotopic ratio mass spectrometry indicated $\delta^{15}N$ characteristic of an animal or human waste source. Results from this study underscore the role of CAFO as an important source of antibiotic contamination of groundwater.

Keywords: antibiotics, nitrate, nitrogen isotope analysis, LC/MS/MS, CAFO, groundwater

1. Introduction

It is estimated that more than 50 million pounds of antibiotics are produced each year in the United States (US), with approximately 40% used in animal production to treat diseases (therapeutic use) and increase animal weight gain (growth promoting use). In a survey conducted by the US National Animal Health Monitoring System, approximately 25% of small feedlot cattle operations and 70% of large feedlot operations used antibiotics in the feed (USDA/APHIS/Veterinary Services, 1999). A significant portion of the administered antibiotics in animals is excreted in an un-metabolized form and could result in the contamination of soil and water through cropland application of animal wastes. Recent reports of antibiotic contamination of soil, surface water, and groundwater (Halling-Sorensen et al., 1998; Kolpin et al., 2002; Christian et al., 2003) have raised public concern because low levels of antibiotics can favor the proliferation of antibiotic resistant bacteria. The use of antibiotics in animal agriculture has been linked to the increased emergence of resistant strains of pathogenic bacteria that have the potential to impact human health (Levy, 1997; NASCDUFA, 1999; Boxall et al., 2003). Resistance genes and/or antibiotic resistant bacteria can be transferred from animals to humans. In addition, bacteria can develop cross-resistance between antibiotics used in veterinary medicine with those of similar structures used exclusively in human medicine (Lathers, 2002).

Antibiotic and other pharmaceutical concentrations have been determined in various aquatic environments, including surface water and wastewater. A nationwide survey by the USGS sampled over 100 streams for the presence of antibiotics, with the surface water concen-

trations ranging from 0.02 to 0.71 μg/l with frequencies of detection as high as 27% (Kolpin et al., 2002). Another survey conducted in Germany (Hirsch et al., 1999) analyzed effluents from several Wastewater Treatment Plants, in which five antibiotics were repeatedly detected, with concentrations ranging from 0.32 to 6.0 μg/l and frequency of detection as high as 100%. Several of the same antibiotics were also detected in surface waters, with concentrations ranging from 0.03 to 1.7 μg/l and a detection frequency as high as 60%. Although antibiotics have been widely detected in wastewater and surface water, their findings in groundwater has been limited. The same study by Hirsch et al. (1999) also investigated groundwater samples, and out of 59 samples analyzed, only sulfamethazine (up to 0.16 μg/l) and sulfamethoxazole (up to 0.47 μg/l) were detected in two samples. Lindsey et al. (2001) also examined surface and groundwater samples for the presence of tetracycline and sulfonamide antibiotics, and of the six groundwater samples tested, only one revealed sulfamethoxazole (0.22 μg/l). The most prevalent detection of antibiotics in groundwater was found surrounding a landfill used as a disposal for waste from the pharmaceutical industry, with concentrations of sulfonamides as high as 10 440 μg/l close to the landfill (Holm et al., 1995).

Groundwater is a major source of drinking water in many parts of the United States. In the state of Idaho, a survey in 2000 by the US Geological Survey (USGS) estimated that groundwater encompasses 92% of its public and domestic water supply (Hutson et al., 2004). The development of modern agricultural practices has resulted in an increase in the number of CAFOs in the US, which without proper waste management practices may act as nonpoint sources of groundwater contamination. The study area, located in Sunnyside Area, Washington County, Weiser, Idaho, encompasses irrigated agricultural fields, a CAFO, and commercial and residential housing (Figure 1). A recent groundwater monitoring study conducted by the Idaho State Department of Agriculture showed high levels of nitrate-nitrogen (NO_3-N)

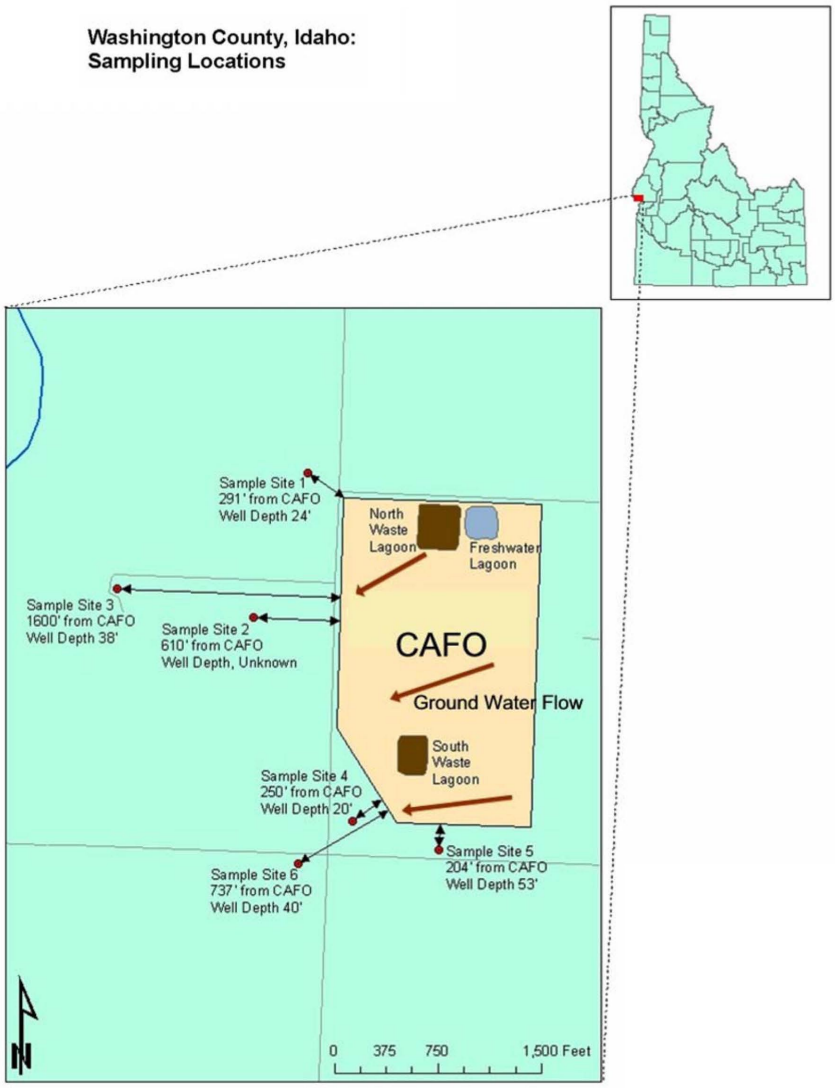
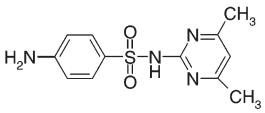
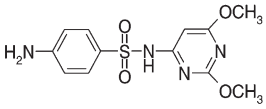
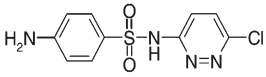
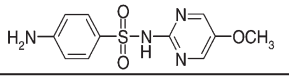


Figure 1. A map of the Sunnyside study area in Washington County, Idaho, indicating the well sampling locations, their approximate distances from the CAFO, and the approximate depths of the wells.

Table 1. Target sulfonamide drugs and a summary of their selected chemical properties

Sulfonamide	Molecular weight	CAS number	Log K_{OW}	pK_a	Structure
Sulfamethazine	278.3	57-68-1	0.25[a]	2.07[c] 7.49[c]	
Sulfadimethoxine	310.3	122-11-2	1.4[a]	2.13[c] 6.08[c]	
Sulfachloropyridazine	287.4	80-32-0	−0.52[b]	1.76[b] 5.71[b]	
Sulfamethoxine	280.3	651-06-9	nf	1.54[d] 6.69[d]	

nf = not found.
[a] Determined using shake-flask method at pH 7 (Liu et al., 2003).
[b] Blackwell et al. (2004).
[c] Qiang and Adams (2004).
[d] Taken from SciFinder Scholar Substance Identifier as calculated using Advanced Chemistry Development (ACD/Labs) software version V8.14 for Solaris (©1994–2005 ACD/Labs).

that exceeded the maximum contaminant limit of 10 mg/l set by the Environmental Protection Agency (EPA) (Tesch and Carlson, 2003b). A majority of the wells had enriched ^{15}N-isotope characteristic of an animal or human waste source of NO_3-N.

Sulfonamides are broad-spectrum synthetic antimicrobials widely used in the livestock industry. Due the low octanol-water partition coefficients (K_{OW}), these compounds have high soil mobility and potential to contaminate groundwater. Table 1 lists the four sulfonamides that were monitored in this study and their corresponding log K_{OW} values. These antimicrobials were chosen because they are approved for use as feed additives in beef cattle in the United States (USDA/APHIS/Veterinary Services, 1999). This work was conducted in response to a request from concerned residents of the affected area who stopped using the private wells as a source of water because of the fear that their groundwater is unfit for drinking and domestic usage. The purpose of this study was to determine the concentrations of antibiotics in the private wells of the residents of Sunnyside Area in an effort to determine the source of the groundwater contamination.

2. Materials and methods

2.1. Study area

The study area is located in Sunnyside Area, Washington County, Weiser, Idaho. The land use includes residential housing, commercial businesses, irrigated agricultural fields, a CAFO, and an onion disposal site, with manure and nitrogen based fertilizers being applied to the land. The CAFO houses beef cattle, and includes at least two wastewater lagoons. Groundwater conditions are shallow, with the direction of the groundwater flow indicated in Figure 1. The soil consists first of a 15 ft layer of clay, followed by layer of sand and gravel of varying depths, under which is a second layer of clay (Tesch and Carlson, 2003a).

2.2. Sampling and sample storage

Groundwater samples were collected from six private wells located in Weiser, Idaho near the CAFO, with the sampling locations specified in Figure 1. Samples were collected in triplicate from each location. Two of the three 1-l samples for antibiotics analysis were collected in clean, baked 1-l amber glass bottles and were shipped overnight cooled in ice. Upon arrival, 50 µl (corresponding to 0.50 µg/l) of the internal standard $^{13}C_6$-sulfamethazine (Cambridge Isotope Laboratories, Andover, MA, USA) were immediately added to each sample. All samples were stored overnight at 4 °C until extraction. The third sample from each location was collected in a 1-l plastic bottle for nitrogen isotope analysis; these were immediately frozen and shipped overnight on ice to the Water Sciences Laboratory, University of Nebraska, Lincoln, Nebraska. Samples were stored frozen until analysis.

2.3. Sample preparation and analysis for sulfonamides

A solid phase extraction-LC/MS/MS method, based on a modification of a previously reported method (Batt and Aga, 2005), was optimized to separate and detect sulfonamide antimicrobials that are used in cattle, namely

sulfadimethoxine (SDM) (Fluka, St. Louis, MO, USA), sulfamethazine (SMZ), sulfamethoxazole (SMX), sulfachloropyridazine (SCP), and sulfamethoxine (SMN) (Sigma–Aldrich, St. Louis, MO, USA). Samples were pre-concentrated using solid phase extraction (SPE). Sulfonamide recoveries were determined prior to sample analysis using 60 mg Oasis HLB cartridges purchased from Waters (Milford, MA, USA) connected to a SPE manifold and vacuum pump. Five hundred milliliter solutions were prepared from NANOpure water containing the four sulfonamides and the $^{13}C_6$-sulfamethazine at a concentration of 1 µg/l. The cartridges were conditioned with 3 ml of ACN, followed by 3 ml of water, and the samples were passed through the cartridges at a rate of about 3 ml/min. The analytes were eluted twice with 3 ml of ACN into a test tube containing 50 µl of the internal standard (10 µg/ml solution of sulfamethoxazole). The eluant volume was then reduced under a stream of nitrogen at 40 °C to a volume of 0.2 ml. NANOpure water was added to reach a final volume of 1 ml, and the solution was then immediately analyzed by LC/MS/MS. The recoveries and relative standard deviations resulting from this method are listed in Table 2.

Sample extracts were analyzed using an LCQ Advantage ion trap mass spectrometer connected to a Surveyor LC system (Thermo Finnigan, San Jose, CA, USA). The HPLC column used was a Thermo Hypersil-Keystone (Bellefonte, PA, USA) BetaBasic-18 C18 column (100 × 2.1 mm internal diameter with 3 µm particle size) equipped with a UNIPHASE™ guard cartridge (10 mm × 2.1 internal diameter with 3 µm particle size), also purchased from Thermo Hypersil-Keystone. The flow rate was 200 µl/min, the column temperature was 30 °C, and the full loop injection volume was 20 µl. The IT-MS was equipped with electrospray ionization and was operated in positive ion mode. The capillary temperature was 235 °C and the spray voltage was 5.25 kV for all applications. Nitrogen was used as a sheath gas at a flow rate of 20 and helium gas was used to induce dissociation for the collection of MS/MS data. Individual tune files were created for each standard in continuous flow mode, and the optimum capillary voltages, lens settings, collision energies, and the product ions of the highest intensity were determined (see Table 3). Separation was performed using a gradient mobile phase consisting of HPLC-grade ACN (mobile phase A) purchased from Burdick and Jackson (Muskegon, MI, USA) and water containing 0.3% formic acid (mobile phase B). Water was prepared using a NANOpure DIamond™ Ultrapure water purification system with a 0.2 µm filter, purchased from Barnstead International (Dubuque, Iowa USA). The mobile phase composition was isocratic from 0 to 2 min at 2% A, then a linear gradient from 2% to 40% A at 15 min. This composition was held for 3 min, and at 18 min, A was increased to 90% in 2 min, which was held for an additional 2 min. The conditions were returned to the initial mobile phase composition in 2 min, and a 16-min equilibration time was allowed, resulting in a total analysis time of 40 min. A MS/MS scan event was collected for each sulfonamide, and selected ion base peak chromatograms were constructed for the product ion of highest intensity.

The method limits of detection (LOD), limits of quantification (LOQ), and dynamic ranges (DR) were determined using calibration curves prepared from spiked NANOpure water samples. Five hundred milliliter volumes of NANOpure water were spiked to contain 0.1, 0.25, 1.0 and 5.0 µg of the four sulfonamides, with duplicate samples extracted at the higher levels (0.25, 1.0, and 5.0 µg) and four replicates extracted for the lowest calibration point (0.1 µg). The calibration curves were plotted, and the LOD was defined as the concentration corresponding to

Table 2. Method LOD, LOQ, DR, and the SPE recoveries from distilled water and groundwater

Sulfonamide	Method LOD (µg/l)	Method LOQ (µg/l)	Dynamic range (µg/l)	SPE recoveries in distilled water		SPE recoveries in groundwater	
				0.25 µg/l	2.5 µg/l	0.25 µg/l	2.5 µg/l
Sulfamethazine	0.02	0.12	0.02–5.0	84 ± 5%	97 ± 16%	74 ± 6%	107 ± 15%
Sulfadimethoxine	0.03	0.07	0.03–5.0	103 ± 8%	90 ± 8%	89 ± 15%	113 ± 18%
Sulfachloropyridazine	0.04	0.14	0.04–5.0	78 ± 5%	63 ± 13%	51 ± 19%	87 ± 15%
Sulfamethoxine	0.07	0.24	0.07–5.0	111 ± 6%	94 ± 31%	100 ± 8%	97 ± 26%
^{13}C-sulfamethazine	nd	nd	nd	96 ± 11%	96 ± 15%	nd nd	nd nd

Table 3. A summary of the retention time and MS/MS parameters for the target sulfonamides

Sulfonamide	Retention time (min)	Precursor ion (M-H$^+$)	Fragment ion 1	Fragment ion 2	Fragment ion 3	Collision energy (%)
Sulfamethazine	11.3	279.0	203.9	156.0	124.1	34
Sulfadimethoxine	15.8	311.2	156.0	245.1	218.0	36
Sulfachloropyridazine	13.2	285.0	156.0	108.1	92.1	30
Sulfamethoxine	11.9	281.1	215.2	156.0	188.0	36
^{13}C-sulfamethazine	11.3	285.0	203.9	nd	nd	34

nd = not detected

signal at the *y*-intercept plus three times its standard deviation. The LOQ was defined as the concentration corresponding to the signal at the *y*-intercept plus ten times its standard deviation. The DR was identified to include the LOD and the highest concentration tested (5.0 µg). Compounds demonstrated good linearity between the LODs and the highest concentration tested (Table 2).

To determine the influence of the sample matrix on the SPE methods, groundwater from a local private drinking well (Orangeville, New York) was tested using the optimized SPE conditions. Two 1 l aliquots were spiked in duplicate to obtain final concentrations of 0.25 and 2.5 µg/l. Fifty microliters (corresponding to 0.50 µg) of the $^{13}C_6$-sulfamethazine was added to each sample, and the samples were extracted as described above. The compounds exhibited similar recoveries in the spiked groundwater as they did in NANOpure water (Table 2). Quantification of analytes was based on internal calibration curves constructed from the SPE extraction of 500 ml spiked NANOpure water samples. The area ratio of the base peak product ion of each sulfonamide (m/z = 203.9, 156.0, 156.0, and 215.2 for SMZ, SDM, SCP, and SMN, respectively) to that of the internal standard (203.9 for the $^{13}C_6$-sulfamethazine) versus concentration were plotted to construct the calibration curves. The addition of the $^{13}C_6$-sulfamethazine prior to extraction would account for any losses during the extraction procedure, any differences in matrix effects between standards and samples, and any differences in ionization or injection volumes during LC/MS/MS analysis.

2.4. Nitrate-nitrogen isotope analysis

Samples for nitrogen isotope analysis were prepared and analyzed using the steam distillation method (Bremner and Keeney, 1965) with modification of Gormly and Spalding (1979). Nitrate and ammonium concentrations were determined during sample preparation by titration with standardized acid. Nitrogen isotopes were measured on purified nitrogen gas prepared by reacting the concentrated distillate from the samples with lithium hypobromite on a high vacuum preparation system. All isotope measurement were conducted using an Optima dual inlet isotope ratio mass spectrometer (GV Instruments, Manchester, UK) with results expressed relative to atmospheric nitrogen by the formula:

$$\delta^{15}N(‰) = \frac{{}^{15}N/{}^{14}N_{sample} - {}^{15}N/{}^{14}N_{atm}}{{}^{15}N/{}^{14}N_{atm}} \times 1000$$

The working nitrogen standard was calibrated daily against an atmospheric nitrogen standard, with a measured precision of ±0.1‰. Overall analytical precision is estimated at ±0.2‰ determined from preparation and analysis of an ammonium sulfate standard. Accuracy of nitrogen isotope determination was verified through analysis of USGS and IAEA reference standards obtained from NIST.

3. Results and discussion

3.1. Detection of sulfonamides

Duplicate samples from six private wells were analyzed according to the validated SPE-LC/IT-MS/MS method described above. Although no sulfachloropyridazine or sulfamethoxine were detected, sulfamethazine and sulfadimethoxine were present in all wells sampled. Concentrations of sulfamethazine ranged from 0.046 to 0.067 µg/l, while concentrations of sulfamethazine ranged from 0.076 to 0.22 µg/l. Analysis of an extracted blank revealed no trace of the target sulfonamides, indicating no carry over from the extraction procedure or the LC column. The MS/MS method collected all the fragmentation information for the precursor ions identified in Table 2, allowing high confidence in the identification of target analytes at trace levels based on the multiple fragment ions produced. The characteristic fragment ions for the two sulfonamides were observed in all samples (Figure 2), and the summary of results for the groundwater samples is listed in Table 4. These wells are located down-gradient or in close proximity (less than 1600 ft) to the CAFO (Figure 1). Pharmaceutical testing was previously conducted by the Idaho State Department of Agriculture (ISDA) on samples taken from the CAFO waste lagoon and a CAFO water well in 2003 (Tesch and Carlson, 2003b). The CAFO waste lagoon contained 43.353 µg/l sulfamethazine and 2.033 µg/l sulfadimethoxine, while the CAFO well water contained 0.310 µg/l sulfamethazine and 0.107 µg/l sulfadimethoxine. Sulfonamides are a synthetic antimicrobial, so their detection in groundwater is not expected from natural sources, unlike some of the antibiotic classes used in animal growth promotion. These four sulfonamide drugs are also not approved for human usage (USFDA, 2004) and therefore their detection is an indication of contamination from animal sources.

The sorption and transport of sulfachloropyridazine has been determined in different soil systems (Boxall et al., 2002). Since the structures and physical properties of sulfonamides are very similar (Table 1), sulfamethazine, sulfadimethoxine, and other sulfonamides can be expected to exhibit similar behaviors. Based on previously reported soil sorption coefficients (log K_D) for sulfachloropyridazine, which is 1.8 kg^{-1} in clay loam and 0.9 kg^{-1} in sandy loam, sulfachloropyridazine is considered to be highly mobile in soil. Similarly, sulfamethazine (pK_a 7.49) and sulfadimethoxine (pK_a 6.08) can be expected to be highly mobile in the sand/clay soil present in the study area. The values of K_D for sulfachloropyridazine in both types of soil decreased with an increase in soil pH, as can be expected due to the predominance of the anionic form of sulfachloropyridazine at pH values greater than 5.7. The addition of manure results in an increase in soil pH due to the high ammonia content. The pH of manure-fertilized soils in temperate climates are typically between 6 and 7.5 (Boxall et al., 2002), and within this pH range, most sulfonamides are ionized and hence increased soil mobility can be expected.

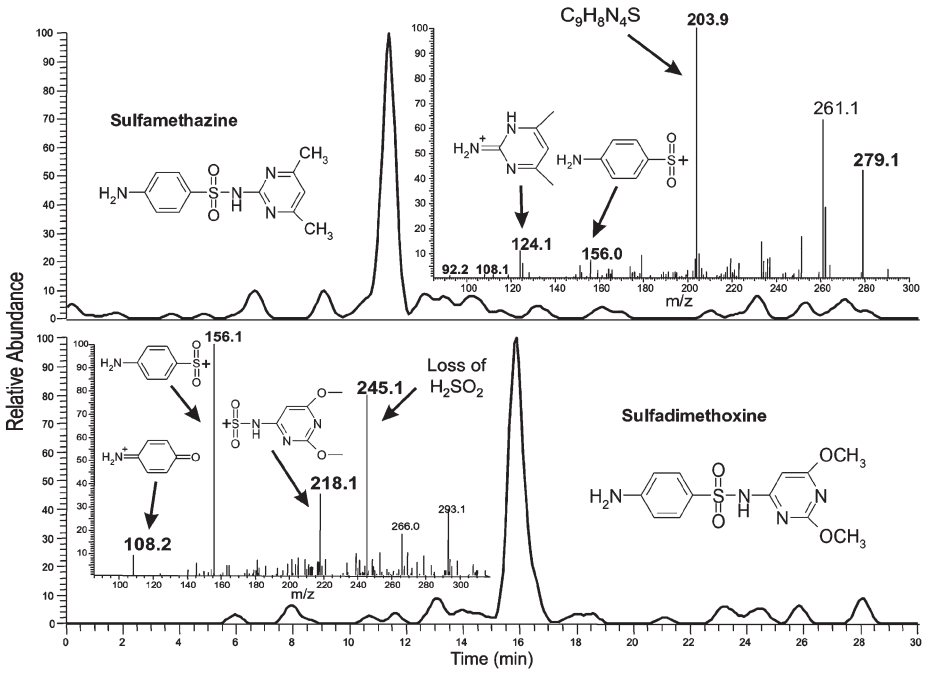


Figure 2. The chromatogram and corresponding MS/MS spectrum of the sample from Location 4 showing the chromatographic peak and characteristic fragment ions for sulfamethazine and sulfadimethoxine, which were detected at 0.078 and 0.050 μg/l, respectively.

Table 4. A summary of the sulfonamides, nitrate, ammonium, and nitrogen isotope results in groundwater samples from Washington County

Location	Well depth (ft)	Sulfamethazine (μg/l)	Sulfadimethoxine (μg/l)	NO_3-N (mg/ml)	^{15}N-NO_3 (‰)	NH_4-N (mg/ml)	^{15}N-NH_4 (‰)
Well 1	24	0.076 ± 0.9%	0.047 ± 0.0%	6.83	5.45	< 0.10	nm
Well 2	na	0.215 ± 0.3%	0.068 ± 1.0%	18.7	11.5	< 0.10	nm
Well 3	38	0.077 ± 0.9%	0.051 ± 0.0%	39.1	12.9	< 0.10	nm
Well 4	15–20	0.078 ± 0.0%	0.050 ± 1.4%	21.6	12.4	< 0.10	nm
Well 5	53	0.085 ± 0.8%	0.046 ± 0.0%	< 0.10	nm	1.22	12.22
Well 6	40	0.076 ± 0.0%	0.050 ± 0.0%	1.28	11.5	< 0.10	nm

nm = not measured, na = not available

The concentrations of sulfonamides found in the groundwater samples from the sampled private wells are within the range of the reported EC_{10} for sulfonamides that causes considerable reduction in the number of soil bacteria. The EC_{10}, which is the effective solution concentration resulting in a 10% inhibition of microbial activity, for the sulfonamide sulfapyridine was found to be 160 and 0.14 ng/g for two different sandy soil samples (Thiele-Bruhn and Beck, 2005). It has been shown in the said study that at these environmentally relevant concentrations of antibiotics a temporary selective pressure on soil microorganisms could take place. Furthermore, it was also shown that antibiotics with a low sorption coefficient (K_D) also displayed a greater effect on the soil microorganisms due to an increase in their bioavailability, indicating that highly mobile antibiotics, such as the sulfonamides, have a large potential to cause significant effects on soil microorganisms (Thiele-Bruhn and Beck, 2005).

Microbial resistance induced by antibiotics in groundwater at low concentrations from manure applications has been observed (Huysan et al., 1993). A recent study identified tetracycline antibiotic resistant genes from eight different classes of genes in wastewater lagoons from a swine production facility (Chee-Sanford et al., 2001). Furthermore, these resistance genes were also found to leach into the groundwater, and were detected as far as 250 m from the swine operation lagoons. Not only can pathogenic resistant bacteria be transferred from animals to humans, but resistance genes can be transferred from the bacteria of animals to human pathogens in human intestinal flora (Lathers, 2002). The use of antibiotics in agriculture can also be the cause of cross-resistance between those antibiotics used strictly in veterinary medicine and human medicine (Lathers, 2002). For example, bacteria that develop resistance to the veterinary sulfonamides, such as sulfamethazine and sulfadimethoxine, may potentially be resistant to sulfamethoxazole and other human sulfonamide counterparts because of their similarity in chemical structures. Therefore, the detection of sulfonamides in groundwater and its long-term effects on the microbial ecology in the water

supply that is used for domestic and agricultural purposes deserve attention and further study.

3.2. Nitrate and nitrate-nitrogen analysis

Five of the six wells sampled contained detectable levels of nitrate. Three samples exceeded the maximum contaminant level of 10 mg/l for NO_3-N for drinking water set by the US EPA, with levels ranging between 18.7 and 39.1 mg/l (Table 4). Ammonium-N was detected in the sixth well at 1.2 mg/l NH_4-N. Nitrogen isotope composition of nitrate ranged between +11.5‰ and +12.8‰ δ^{15}N-NO_3 in four samples, while the fifth well measured +5.45. The δ^{15}N-NH_4 in the remaining well was also enriched at 12.2‰ relative to atmospheric nitrogen.

Exposure to high levels of nitrates has been associated with methemoglobinemia, or "blue baby syndrome" in infants. Nitrate exposure has also been found to correlate with increased levels of birth defects and some cancers that result from the formation of nitrosamines (Pye et al., 1983; Collins, 1996). Elevated nitrate levels in groundwater can result from many sources including plant and soil nitrogen, human wastes, and the application of fertilizers. In general each source can be characterized by its nitrogen isotope composition (δ^{15}N). Animal waste contains urea, which is readily converted to ammonia. Volatilization of this ammonia results in fractionation and enrichment of the ^{15}N isotope in the remaining nitrogen. Nitrification of this ammonia before and after land application often results in contamination of groundwater with nitrate that retains this isotope composition (Kendall and McDonnell, 1998). Nitrate from animal or human waste sources generally has a δ^{15}N above +10‰, soil and plant nitrogen has a δ^{15}N between +5‰ and +10‰, while synthetic fertilizer derived from atmospheric nitrogen has a δ^{15}N between −5‰ and +5‰. A δ^{15}N between +5‰ and +10‰ can also indicate a mixed source, such as the combination of animal wastes and fertilizer (Tesch and Carlson, 2003b). In addition, bacterial denitrification in groundwater results in significant enrichment of nitrate from both fertilizer and animal waste (Gormly and Spalding, 1979; Kendall and McDonnell, 1998).

Wells 2, 3, 4, and 6 are all down gradient from the CAFO, with three of those four locations having nitrate levels greater than 18 mg/l and, assuming the effect of denitrification is minimal, all exhibited δ^{15}N characteristic of animal or human waste sources. Well 1 is located northwest of the CAFO and while it contained an elevated concentration of nitrate, it did not exceed the 10 mg/l limit. This well also revealed a nitrogen isotope level that indicates an organic soil nitrogen or mixed source. Since sulfamethazine and sulfadimethoxine were also detected at this location, it is probable that the groundwater in this location has been impacted by animal waste, and contamination could be the result of a mixed source, such as animal waste and fertilizer. Well 5 did not contain detectable nitrate concentration, but ammonium with a δ^{15}N suggesting animal or human waste was observed. Ammonium is generally not mobile in soils and does not leach to groundwater unless the soils are excessively fertilized. Ammonium is typically held in soils until nitrified and/or used by crops unless soils are well drained and the exchange capacity of the soil is exceeded. For example, both nitrate and ammonium have been detected near intensive swine feeding operations in North Carolina and Oklahoma (Becker et al., 2002; Karr et al., 2002). Thus, the presence of ammonium in well 5 is a further indication of groundwater contamination from animal waste.

The nitrate and nitrogen isotope results found in the present study are consistent with those found by the ISDA in both November 2002 and April 2003 (as summarized in Table 5), suggesting that the groundwater quality of the Sunnyside area is still being affected by the CAFO waste management practices. An assessment of the water quality of the Sunnyside Area of Washington County, Idaho by the ISDA in November 2002 found 73% of the 22 domestic wells sampled had NO_3-N levels that exceeded the MCL (10 mg/l) for drinking water. It was also found that of the 19 wells selected for nitrogen isotope analysis, 58% of the wells exhibited δ^{15}N values in the range indicating contamination from a human or animal waste source (Tesch and Carlson, 2003a). Because enriched δ^{15}N of nitrate can also result from bacterial denitrification, oxygen isotope analysis was also performed by the ISDA. Bacterial nitrification also enriches the amount of ^{18}O in addition to ^{15}N in a 2:1 ratio, and it was found that the ratio of ^{18}O to ^{15}N was much less than 2:1, indicating that the δ^{15}N values over 10‰ were most likely due to animal or human waste sources and not bacterial denitrification.

Follow-up testing in the Sunnyside area of Washington County was also conducted by the ISDA in April of 2003, where it was found that 69% of the 22 wells tested exceeded the 10 mg/l limit. The maximum nitrate concentration observed was 43 mg/l (Tesch and Carlson, 2003b).

Table 5. A summary of the nitrate concentration and nitrate-nitrogen isotope results reported by the ISDA as adapted from Tesch and Carlson (2003b)

Nitrate concentration range (mg/l)	November 2002 # wells sampled (% detection)	April 2003 # wells sampled (% detection)
0.0 to 10.0	6 (27.3%)	8 (30.8%)
10.0 to 20.0	8 (36.3%)	9 (34.6%)
20.0 to 30.0	6 (27.3%)	6 (23.1%)
>30.0	2 (9.1%)	3 (11.5%)
Total	22 (100%)	26 (100%)
Mean value	15.9 mg/l	15.2 mg/l
Median value	14 mg/l	13.5 mg/l
Maximum value	37 mg/l	43 mg/l
δ^{15}N Values (‰) (potential source)		
−5 to 5 (commercial fertilizer)	1 (5.3%)	0 (0%)
+5 to +10 (organic soil nitrogen or mixed source)	7 (36.8%)	4 (20%)
>+10 (animal or human waste)	11 (57.9%)	16 (80%)
Total	19 (100%)	20 (100%)

Twenty of the wells with elevated levels of nitrate were also selected for nitrogen isotope analysis, and this time it was found that 80% of the wells had $\delta^{15}N$ values characteristic of an animal or human waste source. The ratio of ^{18}O to ^{15}N was also retested and once again found to be much less than 2:1, indicating that the $\delta^{15}N$ values over +10‰ were not due to bacterial denitrification. Ammonia levels were also measured, since ammonia and other nitrogenous compounds are typically oxidized to nitrates. Although elevated ammonia levels are not commonly found in groundwater, ammonia concentrations ranging from 0.1 to 8.50 mg/l were detected in 12 of the tested wells surrounding the CAFO in April 2003. This assessment of water quality also discussed sodium and sulfate testing results from samples taken in November 2002, and it was found that six wells tested located down gradient of the CAFO had high concentrations of sodium (410–520 mg/l), high concentrations of sulfate (460–600 mg/l) and high concentrations of nitrate (9.1–37 mg/l), suggesting that the south lagoon is a potential source of nitrate contamination.

4. Conclusion

The detection of two sulfonamide antimicrobials approved strictly for use in veterinary medicine in water wells provides evidence of groundwater contamination from an animal waste source, as substantiated by the elevated nitrate levels, the detection of ammonium, and the nitrate-nitrogen and ammonium-nitrogen isotope ratios observed. Our present study underscores the importance of CAFOs as a source of antibiotics and nitrate contamination of groundwater, making this water supply unsuitable for drinking. The fate of antibiotics in groundwater is not known as most reports on the occurrence of antibiotics in the environment have been primarily in surface waters. The development and transfer of antibiotic resistance among microorganisms poses potential health risks to humans, since resistance genes can be transferred to pathogenic bacteria and cross-resistance to similar sulfonamides used only in human medicine can be developed.

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