



Presence and fate of veterinary antibiotics in age-dated groundwater in areas with intensive livestock farming[☆]

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ABSTRACT

The combination of emerging antibiotic resistance and lack of discovery of new antibiotic classes poses a threat to future human welfare. Antibiotics are administered to livestock at a large scale and these may enter the environment by the spreading of manure on agricultural fields. They may leach to groundwater, especially in the Netherlands which has some of the most intensive livestock farming and corresponding excessive manure spreading in the world. This study investigates the presence of antibiotics in groundwater in two regions with the most intensive livestock farming in the Netherlands. If so, the hydrochemical conditions were further elaborated. Ten multi-level wells with in total 46 filters were sampled, focusing on relatively young, previously age-dated groundwater below agricultural fields. Twenty-two antibiotics were analyzed belonging to the following antibiotic groups: tetracyclines, sulfonamides, trimethoprim, β -lactams, macrolides, lincosamides, quinolones, nitrofurans and chloramphenicol. The samples were analyzed for these antibiotics by LC-MS/MS ESI-POS/NEG (MRM) preceded by solid phase extraction which resulted in importantly low detection limits. Six antibiotics were found above detection limits in 31 filters in seven wells: sulfamethazine, sulfamethoxazole, lincomycin, chloramphenicol, ciprofloxacin, and sulfadiazine. The concentrations range from 0.3 to 18 ng L⁻¹. Sulfonamides were detected at all measured depths down to 23 meters below surface level with apparent groundwater ages up to 40 years old. No antibiotics were detected below the nitrate/iron redox cline, which suggests that the antibiotics might undergo degradation or attenuation under nitrate-reducing redox conditions. This study provides proof that antibiotics are present in groundwater below agricultural areas in the Netherlands due to the spreading of animal manure.

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1. Introduction

Antibiotics are used in livestock farming to prevent animal diseases and cure sick animals. They may end up in the environment as up to 90% of the parent compounds are directly excreted (Kumar et al., 2005; Massé et al., 2014; Sarmah et al., 2006). This is a growing matter of concern as antibiotic resistance is considered to be a serious threat to human welfare in the future. Infectious diseases are the third leading cause of death in Europe and a future without effective antibiotics would fundamentally change the way

modern medicine is practiced (EASAC, 2007). Once veterinary antibiotics are released in the environment they may end up in surface water via runoff and drain flow and in the groundwater due to leaching (Blackwell et al., 2009). The amount of antibiotic resistant genes in Dutch soils has increased since the industrial production of antibiotics, even though the rules on the use of antibiotics have become more strict and wastewater management has improved (Knapp et al., 2010; Schmitt et al., 2017).

The threat of antibiotic resistance is a well-known problem which goes back to the first produced antibiotics. The presence and fate of antibiotics in soils and surface water is therefore well researched and monitored. Examples of literature describing antibiotics in soils range from transport processes (e.g. Blackwell et al., 2007; Boxall et al., 2002; Wehrhan et al., 2007) to sorption processes (e.g. Boxall et al., 2002; Figueroa et al., 2004; Tolls, 2001),

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and leaching processes (Blackwell et al., 2009; Kay et al., 2005; Spielmeier et al., 2017). The presence and fate of antibiotics in surface waters were systematically studied as well (e.g. Boxall et al., 2006; Christian et al., 2003; Hirsch et al., 1999; Kemper, 2008; KWR, 2010; Ouyang et al., 2015; RIVM, 2010, 2007; Sarmah et al., 2006; Wei et al., 2011). Recently, there has been an increasing attention for the occurrence of antibiotics in groundwater (for reviews on this subject see Lapworth et al., 2012; Sui et al., 2015). However, the amount of available literature is limited when concerning the presence of antibiotics in groundwater under agricultural fields. Watanabe et al. (2010) studied the presence of antibiotics in groundwater at dairy farms in California, where they found maximum concentrations of $3.6 \mu\text{g L}^{-1}$ of sulfamethazine. These concentrations were measured underneath a manure lagoon, which contained manure of over 3000 cows. The maximum concentrations below manure-treated fields were lower with a maximum reported concentration of 110 ng L^{-1} of sulfamethazine. Sacher et al. (2001) carried out a groundwater monitoring program in Baden-Württemberg (Germany) in which samples from 105 groundwater wells were analyzed for 60 pharmaceutical compounds including 22 antibiotics. Based on a detection limit of 10 ng L^{-1} , pharmaceutical compounds were found in 39 of the 105 wells. Sulfamethazine was the only antibiotic detected in 11 samples out of 105 with a maximum concentration of 410 ng L^{-1} . Hirsch et al. (1999) researched the presence of antibiotics in the aquatic environment. One of the eight analyzed groundwater samples was close to an irrigated area and contained a concentration of 470 ng L^{-1} of sulfamethoxazole. Another sample which was more distant from the irrigated area contained a concentration of 40 ng L^{-1} . Additional samples were taken a couple of weeks later and these remarkably contained no antibiotics. From this, they concluded that the pollutant load of veterinary antibiotics to the environment is of minor importance. Hamscher et al. (2005) studied the behavior of tetracyclines and sulfonamides in soils and groundwater after repeated fertilization in an area with intensive livestock farming in northern Germany. Sulfonamides were repeatedly found in groundwater with a maximum concentration of 240 ng L^{-1} . They concluded that veterinary antibiotics were continuously leaching to groundwater under field conditions, contrary to Hirsch et al. (1999). Burke et al. (2016) recently studied the presence of antibiotics in surface water and groundwater in the catchment of a drinking water production site in Lower Saxony, Germany. In total 8 out of 26 antibiotics were detected in surface water. Trimethoprim was the only detected antibiotic in groundwater in 11 of 15 wells with concentrations ranging between 5 and 12 ng L^{-1} . Hannappel et al. (2014) and Balzer et al. (2016) researched shallow groundwater in the same region (Lower Saxony and North Rhine-Westphalia) which is characterized by high livestock density. In 39 out of the 48 sampled wells no veterinary drugs were detected. Low concentrations of sulfamethazine and sulfadiazine ($<12 \text{ ng L}^{-1}$) were found in seven wells, while sulfamethoxazole was detected with high concentrations (up to 950 ng L^{-1}) in two wells. The authors concluded that the input of veterinary drugs in this region is very rare, although under highly unfavourable conditions sulfonamide antibiotics were repeatedly detected with high concentrations.

The Netherlands has some of the highest livestock densities and corresponding animal manure production and application in the European Union and among OECD countries (Oenema et al., 2005; Bouraoui et al., 2011). The sales of veterinary antibiotics in the Netherlands peaked in 2007 (MARAN, 2017), which was at that time the highest in terms of sold antibiotics per kg of biomass of the major animal groups (pigs, poultry, and cattle) among the ten European countries which regularly publish this data (Grave et al., 2010). However, the presence and fate of antibiotics in

groundwater in the Netherlands is not well known. Table 1 lists studies which found antibiotics in groundwater in the Netherlands, where the detection limits varied considerably. For most of these studies the main goal was not to research the presence of antibiotics in agricultural areas; Vissers and van Gelderen (2016) focused on human antibiotics in urban areas and de Weert and Smedes (2015) and Chitescu et al. (2012) researched innovative methods for the detection of antibiotics. van Schijndel et al. (2009) researched 4 wells in agricultural areas in the province of Drenthe where livestock farming is not as intensive as in other areas in the Netherlands and antibiotics were detected in only 1 well. Based on the recent international literature, the intensive nature of Dutch livestock farming, and the historical veterinary antibiotic usage we anticipated that antibiotics are present more frequently and in higher concentrations than the results of the mentioned studies in areas with intensive livestock farming.

The objective is to test whether antibiotics are present in groundwater in regions with one of the most intensive livestock farming areas worldwide and, if so, under which hydrochemical conditions. We sampled groundwater from multi-level observation wells that were previously age dated using tritium-helium (Visser et al., 2007a, 2007b; Zhang et al., 2009; Broers et al., 2009), with the aim to better understand the leaching of antibiotics to groundwater and the processes that may attenuate the concentrations. To our knowledge, this is the first study that relates the concentrations of veterinary antibiotics to groundwater age, allowing to interpret the leaching patterns of antibiotics in relation to the application history.

2. Methods and materials

2.1. Study area and well selection

Two regions were studied in the Netherlands: the Gelderse Valley in the provinces of Utrecht and Gelderland, and the eastern part of Noord-Brabant and northern part of Limburg. Both areas are characterized by unconsolidated, mainly sandy deposits in the upper 20 m of the subsurface and shallow water tables, ranging between 1 and 3.5 m below surface.

The Gelderse Valley was formed in the Saalian period (150 ka BC) as a valley between two ice pushed ridges at the southern fringe of the Saalien land ice mass and the shallow subsurface is dominated by Late Pleistocene predominantly sandy deposits from periglacial and coastal origin (Willemen et al., 2008). Shallow groundwater (down to a depth of 30 m) is characterized by neutral pH and moderate hardness. The redox-status is generally Fe-anoxic, but oxic waters are frequently present within the first few tens of meters of the subsoil (Griffioen et al., 2013). The area is approximately 750 km^2 of which agriculture covers about 71% of the land use (533 km^2). The area has a high livestock density covering about 20% of the Dutch poultry production (Willemen et al., 2008).

Noord-Brabant and northern Limburg are together one of Europe's centers of pigs breeding and are notorious for a large surplus of manure production by intensive livestock farming. The surplus manure is partly exported to other regions and neighboring countries in order to meet the compliance regime for the EU Nitrates Directive (European Commission, 1991). The area is approximately 3000 km^2 of which agriculture covers 62% (1860 km^2). The shallow subsurface in the eastern part of Noord-Brabant and northern Limburg consists of Pleistocene, fluvial sand and gravel deposits from the Meuse River covered by a 2–30 m thick layer of fine sands and loam deposited in the Middle and Upper Pleistocene epoch. This region is vulnerable to groundwater pollution from agriculture since contaminants easily reach the deeper aquifers and the subsurface has a low acid buffering capacity (Broers, 2004; Fest

Table 1
List of studies in which antibiotics were detected in groundwater in the Netherlands.

Study	Number of sites	Number of contaminated samples	Detected antibiotic	Maximum concentration (ng L ⁻¹)	Detection limit (ng L ⁻¹)
van Schijndel et al., 2009	8	1	Sulfonamides	1.3	1
Chitescu et al., 2012	3	Not mentioned	Sulfamethoxazole	50–75	10
de Weert and Smedes, 2015	2	2	Sulfadiazine	30	10
			Sulfamethazine	21	10
Visser & van Gelderen, 2016	60	3*	Oxytetracycline	>100	100

*reconnaissance survey with lower precision measurements and high detection limit.

et al., 2007; Visser et al., 2009a, 2007b). The shallow groundwater in this region (down to 30 m) is generally acid (pH 4.0 to 5.5). The upper 10–15 meter often contains nitrate but the redox status turns into Fe-anoxic below that depth when the recharging groundwater reaches layers with high organic carbon or iron sulfides (Griffioen et al., 2013).

Ten multi-level observation wells were selected to be sampled. These wells are part of provincial and national monitoring networks (e.g. Broers, 2002; Broers & Van Der Grift, 2004), except for two wells which were installed to monitor groundwater quality near a drinking water site in Oostrum, Limburg (Zhang et al., 2009). The screens of the observation wells are 2" wide, which enables the use of a submersible pump for taking high quality samples for age dating (Visser et al., 2007b). Five of the ten wells additionally include mini-filter screens across the entire depth range, making it possible to establish high resolution concentration-depth profiles. The well completion of mini-filter wells 40, 41 and 1863 is different from wells 271 and 377, as the former have clay seals between the individual mini-screens that prevent preferential, vertical flow along the well, whereas the risk for this flow must be recognized for the latter wells. Water levels in the observation wells during sampling varied between 1 and 3.5 m below surface, indicating the limited thickness of the unsaturated zone in our study area.

We primarily used wells that we previously age dated with tritium-helium (Visser et al., 2007a, 2007b; Zhang et al., 2009; Broers et al., 2009) and that have time series of groundwater composition. From this it is known that the sampled water is young and derived from rainwater infiltration under agricultural fields. The selected wells were always at locations that could not be influenced by point or diffuse sources other than agricultural land use. We excluded wells that were close to ditches draining the shallow or deeper groundwater, as to prevent the converging of old groundwater into the samples screens. The locations of the ten selected wells are shown in Fig. 1. Detailed information on the filter depths, locations and groundwater ages is given in Supporting Information 1.

2.2. Sample collection and analysis

The groundwater samples were collected in April and May of 2016 and February of 2017. The 2-inch screens were sampled using a Grundfos MP1 submersible pump. The 1-inch filters and mini-filters were sampled using an Eijkelkamp peristaltic pump where, if possible, three filters above each other were pumped at the same time to ensure laminar horizontal groundwater flow towards the filters while sampling the middle filter in order to avoid cross-contamination between adjacent filters.

Field parameters (pH, temperature, electrical conductivity (EC), dissolved oxygen and total dissolved gas (TDG)) were measured continuously during pumping in a flow-through cell. The groundwater was sampled when these parameters reached stable values or after a pumping time of 1.5 h. Alkalinity (HCO₃) was determined in the field by titration using a HACH AL-DT field set. The antibiotic

samples were collected in 250 ml glass bottles, which were flushed with groundwater before sampling. Groundwater was also collected for analysis of major anions (Cl, NO₃, SO₄, PO₄), cations (Na, K, Mg, Ca, Fe, Mn, NH₄), and trace elements (Al, As, Ba, Cd, Cr, Cu, Ni, Pb, Sr, Zn) in 100 ml PE bottles. Samples for the cations including trace elements were filtered through a 0.45 µm filter and acidified. The samples were stored for a maximum of two days in a cool and dark environment before being analyzed.

The samples were analyzed at the joint TNO/University Utrecht Central Environmental Laboratory (Utrecht Castel). Analysis of trace metal and major cations was conducted with inductively coupled plasma mass spectrometry (ICP-MS) following the NEN-EN-ISO 17294-1 and NEN-EN-ISO 17294-2 standard. Ammonium and phosphate were analyzed by continuous flow analysis (CFA) following NEN-EN-ISO 11732 and NEN-EN-ISO 15861-2. The other anions were detected by ion chromatography (IC) following NEN-EN-ISO 10304-1.

The samples for antibiotics were prepared by adding 2 g CH₃CO₂NH₄ (Sigma Ammonium acetate for molecular biology, A1542-250G) to reach a buffered pH of 7. The solid phase extraction was done using Waters Oasis HBL 6 cc columns. The cartridges were conditioned by eluting 5 mL CH₃OH (FLUKA Methanol, LC-MS chromasolv[®], 34966-2.5L) and 5 mL of Milli-Q water (buffered to a pH of 7 with CH₃CO₂NH₄). The samples (200 mL) were subsequently loaded on the cartridges. The analytes were desorbed by eluting with 10 mL CH₃OH. The samples were then concentrated under a stream of nitrogen to a volume of approximately 0.2 mL. Next, 0.4 mL milli-Q water was added and CH₃OH was added until the total volume of the samples equaled 1 mL. The samples were stored at –18 °C before further analysis.

The 22 analytes and internal standards were detected by an Agilent 1260 series high-performance liquid chromatographer using a 100 × 2.1 mm, 2.6 µm Kinetex column (Phenomenex, Utrecht, the Netherlands) coupled with an Agilent 6460 triple quadrupole LC/MS with Jetstream Electron Spray Ionisation (ESI) and multiple reaction monitoring (MRM). A sample volume of 5 µL was injected with a column temperature of 60 °C and a flow rate of 200 µL min⁻¹. The sample was eluted with a gradient of methanol (eluent A) and 1 mM ammonium fluoride with 0.01% acetic acid in Milli-Q water (eluent B) with flow rates of 0.5 mL min⁻¹. Eluent A was increased from 5% to 90% in 10 min and maintained for 3 min. After this it is decreased to 5% in 0.1 min and maintained for 1.9 min to complete the cycle of 15 min. Mass spectrometry was performed with a gas temperature of 350 °C and a flow rate of 7 L min⁻¹. Sheath gas temperature was set at 350 °C with a flow rate of 12 L min⁻¹. The capillary voltage was set at 3500 V.

The target compounds were determined with one precursor ion and two product ions. For information about mass-to-charge ratios, retention times and ratios see Supporting Information 2. Calibration was done before measuring the samples with known amounts of the analytes in 9 steps with concentrations ranging between 0.1 and 50 ng mL⁻¹. The limit of detection (LOD) and limit of quantification (LOQ) of the analytes were determined with signal-to-noise



Fig. 1. Locations of the selected wells. Two wells with close proximity (wells 40 and 41) are shown as one dot.

ratios of 1:3 and 1:10 respectively. Average recoveries and concentrations for the LOD and LOQ are given in [Supporting Information 2](#). The method resulted in values for LOQ ranging between 0.3 and 18 ng L⁻¹.

2.3. Groundwater ages

The groundwater ages for the wells in Noord-Brabant and Limburg were determined by [Visser et al. \(2007a, 2007b\)](#) and [Zhang et al. \(2009\)](#). The age of the groundwater was determined by the ratio of tritium and helium (³H/³He) in the water. Tritium decays to tritiogenic helium and the ratio of ³H to ³He is used to derive the apparent groundwater age. This is defined as:

$$t_{\frac{3H}{3He}} = \lambda^{-1} \ln \left(\frac{3He^*}{3H} + 1 \right) \quad (1)$$

where $t_{3H/3He}$ is the apparent groundwater age in years, ³H and ³He* are the concentrations of tritium and tritiogenic helium in Tritium Units (TU), and λ is the decay constant for tritium (0.05626 y⁻¹) ([Solomon et al., 1993](#)). [Visser et al. \(2009b, 2007a\)](#) corrected the apparent ages of the current well selection for the process of degassing of N₂ and He that originated from the process of denitrification in those wells. Apparent ages are a good proxy for the

true groundwater ages in the aged-dated observation wells, because mixing is restricted in the screens of limited length and the groundwater flow is generally downward in the recharge areas investigated here ([Broers and van der Grift, 2004](#)).

We estimated the ages of the groundwater at the depth of the mini screens from the measured tritium-helium apparent ages of the 2"-diameter screens in the same multi-level wells, by assuming that the travel time distribution with depth conforms to an exponential shape, corresponding to the following relation which is valid for homogeneous and isotropic aquifers:

$$t(z) = \frac{\varepsilon D}{N} \ln \left(\frac{D}{D-z} \right) \quad (2)$$

where $t(z)$ is the groundwater age in years at depth z , ε is the porosity, D is the aquifer depth in meters, N is the recharge rate in m yr⁻¹ and z is the depth below the water table in meters ([Raats, 1981, 1977](#); [Zhang et al., 2009](#)). Following [Visser et al. \(2009b, 2007a\)](#) and [Zhang et al. \(2009\)](#), we assume that the groundwater age at a certain depth does not change with time as long as the groundwater flow regime does not change with time. In our study area this is a good assumption given the boundary conditions of the groundwater flow systems which are driven by precipitation excess infiltrating in phreatic aquifers.

2.4. Data interpretation

Groundwater quality data was used to check if the groundwater in the selected wells was influenced by agriculture. This was based on the groundwater oxidation capacity (OXC), which is defined as:

$$\text{OXC} = 5[\text{NO}_3^-] + 3.5[\text{SO}_4^{2-}] \quad (3)$$

where the nitrate and sulfate concentrations and OXC are expressed in meq L⁻¹ (Postma et al., 1991). The OXC indicates the capacity of the groundwater to oxidize reduced compounds in the subsurface. It is based on the redox reaction between nitrate and pyrite, which is known to determine nitrate and sulfate concentrations in much of shallow groundwater in the Netherlands (e.g. Zhang et al., 2009):



The advantage using the OXC defined this way, is that the OXC is a conservative variable when NO₃ bearing groundwater is subject to denitrification in association with pyrite oxidation where NO₃ disappears and SO₄ gets mobilized. There is no official groundwater quality standard for the OXC, but a threshold value of 7.0 meq L⁻¹ is used to distinguish between water that is significantly influenced by agricultural pollution from water that is not (Broers, 2004; Zhang et al., 2009). An OXC of 7.0 meq L⁻¹ refers to water with 87 mg L⁻¹ nitrate and no sulfate, or water with 96 mg L⁻¹ sulfate and no nitrate, or a combination of nitrate and sulfate with concentrations between those thresholds.

The antibiotic concentrations were compared to the groundwater concentrations of redox-sensitive O₂, NO₃ and Fe presence under different redox conditions. These parameters can be used to identify oxic, suboxic and anoxic groundwater and therefore the transport and fate of heavy metals and organic micropollutants.

3. Results and discussion

3.1. Presence of antibiotics in groundwater

In total 31 out of the 46 filters and seven out of the ten selected wells contained antibiotics above the LOD (see Supporting Information 3). Six out of the 22 investigated antibiotics were detected in the wells. The antibiotics were detected in four wells in Noord-Brabant, two in Limburg and one in the Gelderse Valley. Fig. 2 shows the concentrations of the detected antibiotics and the frequency at which the antibiotics were found above the LOD and LOQ. Sulfamethazine was most frequently found: above LOD in 26 filters and above LOQ in 22 filters with concentrations ranging between 0.3 and 12.5 ng L⁻¹. Sulfamethoxazole has the highest detected concentration (18 ng L⁻¹) but the concentrations were less evenly distributed compared with sulfamethazine; the second highest concentration of sulfamethoxazole is considerably lower at 3.3 ng L⁻¹. Lincomycin and sulfadiazine were both detected once above LOQ with concentrations of 2.5 ng L⁻¹ and 1.5 ng L⁻¹, respectively. Chloramphenicol and lincomycin were also detected above LOD, but not above LOQ.

Fig. 3 shows the relation between screen depth and apparent ³H/³He groundwater age for the wells which apparent groundwater ages were known. Groundwater age typically increases with depth as was elaborated by Visser et al. (2007b) and Zhang et al. (2009) for some of these wells specifically. The figure also shows that antibiotics were detected at all measured depths down to 23 m and in groundwater aged up to 40 years.

Fig. 4 details the concentrations for the six detected antibiotics versus the ³H/³He recharge year, which is obtained by subtracting

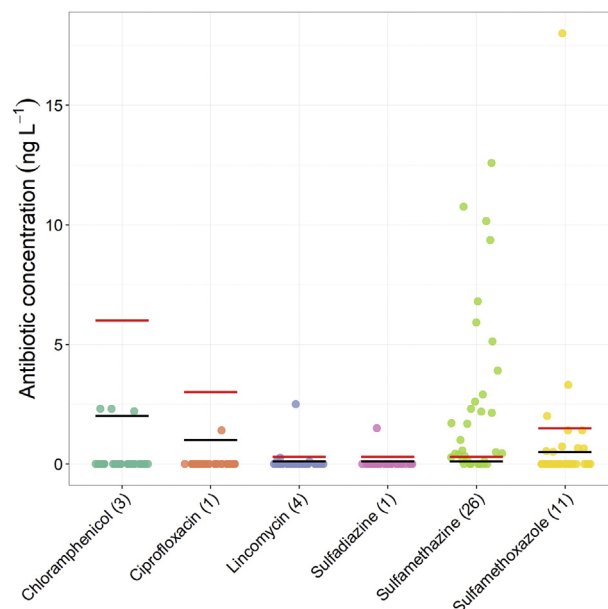


Fig. 2. Jitterplot of the concentrations of the detected antibiotics. The black lines indicate the limit of detection (LOD), red lines indicate the limit of quantification (LOQ). The numbers behind the antibiotics indicate the number of times that the antibiotic was found above detection limit. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the ³H/³He apparent age from the sample date. Sulfamethazine is detected, although with a low concentration (0.43 ng L⁻¹), in water of 40 years old. The higher antibiotic concentrations are found for groundwater which has infiltrated from 1995 onward. A LOWESS smooth (locally weighted scatter plot smoothing; Cleveland, 1979) was tailored to the sulfamethazine concentrations as this is the most detected antibiotic. The LOWESS smooth has a span parameter of 0.75. The upper and lower lines are the upper and lower quartile of the distribution where 50% of the data lies between the lines, these indicate the spread and symmetry of the data around the middle LOWESS. The middle LOWESS smooth increases gradually from 1990 onward and decreases for more recent years. The upper LOWESS shows a more defined peak around 2005–2007 and declines for more recent years, which generally corresponds with the known sales history of sulfonamide antibiotics in the Netherlands (see Fig. 5). The corresponding pattern is a remarkable signal, given the limited dataset available, but is by no means final proof that the concentrations in all groundwater under farmlands has been decreasing since 2007, as the LOWESS approach represents a statistical summary for a limited dataset.

The concentrations detected in this study were relatively low (<20 ng L⁻¹) compared with the maximum concentrations of the studies mentioned before. However, our results suggest more frequent hits under areas with intensive livestock farming (65% detection frequency). The frequent detection of sulfamethazine is evident since it is one of the most used veterinary antibiotics, has a low sorption coefficient (see Table 2), and has an excretion rate of approximately 90% (Halling-Sørensen et al., 2001; Pinna et al., 2012). However, the detection of chloramphenicol in three samples is surprising considering that its use has been banned for food producing animals in the EU since 2010 (European Commission, 2010). The three contaminated samples were taken from 2 wells within close proximity (wells 40 and 41 in Oostrum, Limburg) sampled six days after each other. Two of the three detections occur in old groundwater (19 and 25 years old) which infiltrated before the ban on chloramphenicol. However, one detection occurred in a

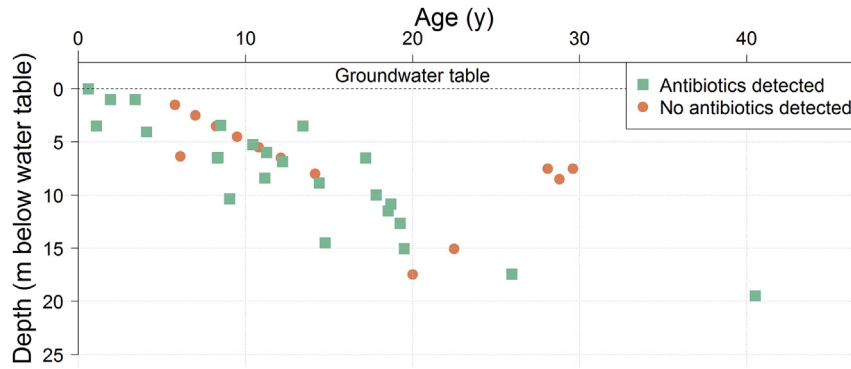


Fig. 3. Plot which shows the relation between the depth of the detected antibiotics (measured from the average groundwater table) and the corresponding apparent groundwater ages at these depths.

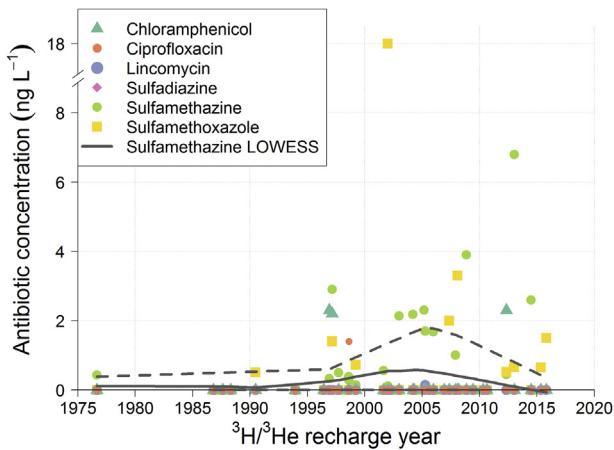


Fig. 4. Plot of the concentrations of the six detected antibiotics versus the $^3\text{H}/^3\text{He}$ recharge year. A LOWESS regression line is tailored to the sulfamethazine concentrations. The dotted lines indicate the upper and lower LOWESS smooth.

sample with an apparent groundwater age of 2 years which, at the time of sampling, had infiltrated in 2014. The detection of chloramphenicol in this young groundwater could be due to several possibilities: illegal use of chloramphenicol as a veterinary antibiotic, the land could be fertilized with manure from non-food producing animals, or this detection is an analytical error (the detections are above LOD but not LOQ). However, the reported results are in compliance with 2002/657/EC (performance of analytical methods and the interpretation of results) and the wells were sampled and analysed on separate days which makes analytical errors unlikely.

Most of the detected antibiotics are not exclusively used for veterinary purposes and could therefore also be of human origin. However, the wells were intentionally selected within infiltration areas which are known to have a downward flow direction from the agricultural fields towards the deeper groundwater, without nearby superficial drainage networks (Broers and van der Grift, 2004; Visser et al., 2007a). The absence of ditches and water courses prevents water to infiltrate from surface water and prevents

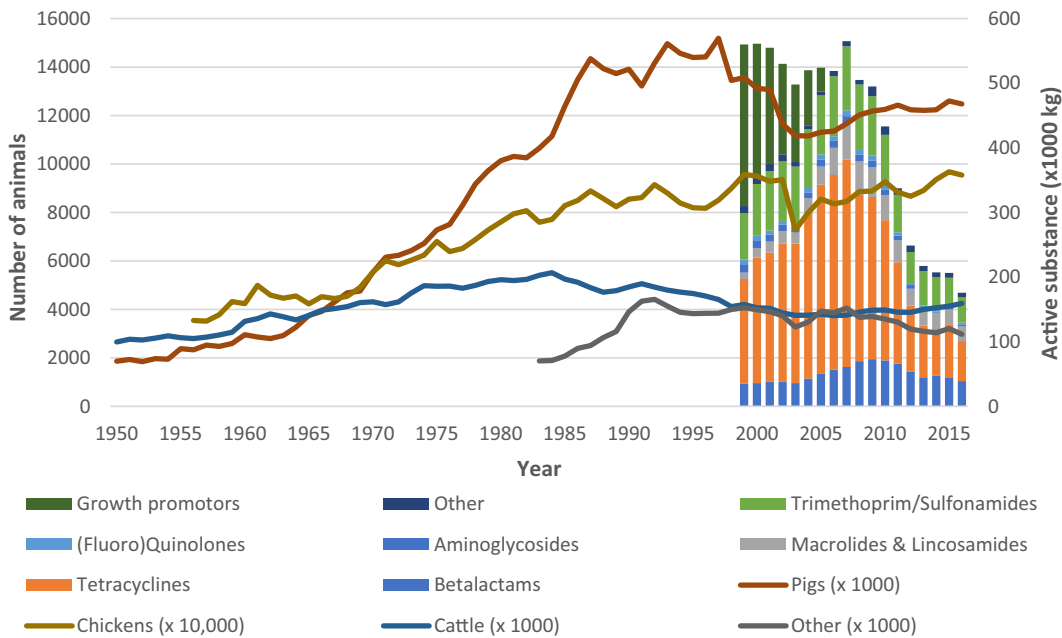


Fig. 5. Combined plot of the number of animals in Dutch livestock farming (lines, left axis) and the sold amount of antibiotics shown as kg of active substance (bars, right axis). “Other” animals include horses, ducks, sheep, and turkeys. Cattle includes dairy cows and veal calves. Note the scale for the amount of chickens (x 10,000 instead of x 1000 for the other animals). Source number of animals: (CBS, 2017), source antibiotic sales: (MARAN, 2017; Speksnijder et al., 2015).

Table 2
Hydrochemical properties of the analysed antibiotics, categorized by their respective groups. Included are the octanol-water partition coefficient ($\log K_{OW}$), solubility (S), and the sorption coefficient (K_d). 1: (Tolls, 2001), 2: (Hamscher, 2006), 3: (Sarmah et al., 2006), 4: (Banzhaf et al., 2012), 5: (García-Galán et al., 2010), 6: (Zhang et al., 2014), 7: (Wishart et al., 2018), 8 (Rabølle and Spliid, 2000).

Antibiotic Group	Antibiotic	Log K_{OW}	S (g L^{-1})	K_d (L kg^{-1})
Tetracycline	Doxycycline	-0.02 ²	0.63 ²	
	Oxytetracycline	-1.22 ¹	1 ¹	417–1026 ⁸
	Tetracycline	-1.19 ¹	1.7 ¹	400–1620 ¹
Sulfonamide	Sulfachloropyridazine	0.31 ²	7 ²	0.9–1.8 ³
	Sulfadiazine	-0.09 ²	0.077 ²	1.4–2.8 ³
	Sulfamethazine	0.89 ¹	1.5 ¹	0.6–3.1 ¹
	Sulfamethoxazole	0.89 ⁴	0.61 ⁴	0.22 ⁵
Trimethoprim	Trimethoprim	0.91 ²	0.4 ²	6.73–9.21 ⁶
	Amoxicillin	0.87 ²	4 ²	
B-lactam	Cefuroxime	-0.16 ⁷		
	Azithromycin	4.02 ⁷		
Macrolide	Clarithromycin	3.16 ⁷		
	Erythromycin	3.06 ⁷	2 ⁷	
	Roxithromycin			
	Tylosin	3.5 ¹	5 ¹	8.3–128 ⁸
Lincosamide	Lincomycin	0.56 ²	0.9 ²	
	Ciprofloxacin	0.4 ¹	30 ¹	430 ¹
Quinolone	Flumequine	1.7 ¹	0.07 ¹	310 ¹
	Levofloxacin			
	Ofloxacin	0.35 ¹		
	Furazolidone	-0.04 ⁷	0.04 ⁷	
Other (Nitrofurantoin)	Chloramphenicol	1.14 ¹	2.5 ¹	0.2–0.4 ¹

farmers to use surface water for irrigation. Therefore, sewage treatment plants are not a likely source of the antibiotics. Furthermore, the application of sludge from sewage treatment plants on agricultural fields as a fertilizer has been banned in the Netherlands by law since the 1st of January 1995 (LNV/VRM, 1998) and application of sewage sludge on maize fields indeed stopped since the introduction of that law (CBS et al., 2016). Most of the antibiotics that we detected are derived from recharge years after the ban (see Fig. 4) so sewage sludge is not a possible source for these detections. There are two detects with apparent ages that respond to recharge years from before the ban: sulfamethazine at 0.43 ng L^{-1} in 40 years old water and sulfamethoxazole (above LOD but not above LOQ) in water of 25 years old. For these two detections, a human origin through the application of sewage sludge cannot be excluded.

The application history of veterinary antibiotics in Dutch agriculture leads to no surprise that these are detected in groundwater. Antibiotics have been used for veterinary purposes in the Netherlands since the 1950s (Speksnijder et al., 2015) so the detection in groundwater up to 40 years old is certainly possible, since it would have infiltrated in the 1970s. The number of animals in Dutch agriculture increased substantially between 1965 and 1990 but has since then levelled off (Fig. 5). In 2016 there were 95,400,000 chickens, 12,479,000 pigs and 4,251,000 cows in the Netherlands. For more recent years (1999–2016), the sales of antibiotics have been monitored by FIDIN (the federation of the Netherlands veterinary pharmaceutical industry). Antimicrobial growth promoters, used as an additive in animal feed, constituted to almost half of the total antibiotic sales in 1999. Their usage has since declined as it has been banned in the EU since 2006. The decline in growth promoters was partly substituted by an increase in therapeutic antibiotic usage (MARAN, 2007). The antibiotic sales peaked in 2007 with a total sold amount of 565,000 kg. Since then, the sales of antibiotics have declined by 69% down to 176,000 kg in 2016 following Dutch agricultural management policies to discourage the use (MARAN, 2017).

The most used antibiotic group in Dutch agriculture, tetracyclines, are not detected in groundwater in this study. Tetracyclines have, as a group, the highest sorption coefficients of the antibiotics

measured in this study (see Table 2) and can be considered to be rather immobile ($K_d > 50 \text{ L kg}^{-1}$; Tolls (2001)) and are often found in soils but not deeper in groundwater (e.g. Watanabe et al. (2010)). It is not surprising that these are not detected in groundwater. Most of the antibiotics detected in this study have low sorption coefficients and high solubilities, and antibiotics are generally as a group not hydrophobic (low $\log K_{OW}$ values, see Table 2). An exception is the detection of ciprofloxacin, that has a high sorption coefficient (430 L kg^{-1} ; Tolls (2001)), but was occasionally reported in groundwater by others as well (e.g. Boy-Roura et al. (2018)).

de Weert and Smedes (2015) researched one well that was also sampled in this study (well 1841) in June of 2015. They found sulfamethazine concentrations of 21 and 16 ng L^{-1} at respective depths of 3.5 and 22 m below surface. The shallowest filter at 3.5 m did not yield water in our study, but three other filters of the multi-level well were sampled in February of 2017. Our current results show sulfamethazine concentrations of 3.9 and 0.43 ng L^{-1} at 9 and 22 m depth respectively, and one detection of sulfamethazine above LOD but below LOQ at 14 m depth. The difference between the concentrations at 22 m depth ($0.43 \text{ vs } 16 \text{ ng L}^{-1}$) is remarkable considering the analyses were done in the same laboratory using the same methods, but the hit at 22 m depth with an apparent age of 40 years was confirmed. It is unknown whether temporal fluctuation in manure application and leaching from the agricultural field causes such a concentration difference. In the study of Weerts and Smedes the hit of sulfamethazine at 22m depth was accompanied by high concentrations of the pesticides 2,6-dichlorobenzamide (BAM, $918 \mu\text{g L}^{-1}$) and kresoxim-methyl ($122 \mu\text{g L}^{-1}$) and lower concentrations of bentazon ($8 \mu\text{g L}^{-1}$), which confirms that agricultural pollution has impacted the groundwater with pesticides and antibiotics up to this depth and age range.

The general relation between the antibiotic concentrations and impact of agricultural land use on the groundwater quality was also confirmed when evaluating the relations between OXC and antibiotics (see supporting information 3 & 4). Thirty-five out of the 46 filters (76%) exceed the threshold value of 7 meq L^{-1} that indicates agriculturally polluted groundwater. Most of the positive detections of antibiotics relate to water with an OXC exceeding 7 meq

L^{-1} ; antibiotics were detected in water with OXC ranging from 2 to 30 meq L^{-1} . The OXC from groundwater samples of wells 329 and 376 did not exceed 7 meq L^{-1} ; these are also two of the three wells in which no antibiotics were found. Flow directions are probably different than anticipated and groundwater is probably not infiltrated at the surrounding agricultural fields. The third well which contained no antibiotics, well 271, has some of the highest values for OXC (up to 30 meq L^{-1}). Given the strong signal for agricultural influence, the absence of antibiotics in this well is suggested to stem from either retarding or transforming processes, the use of antibiotic-free manure, or from the use of artificial fertilizers instead of animal manure. There are also several detections of sulfamethazine and sulfamethoxazole below the threshold of 7 meq L^{-1} , most from a well that we suspect to suffer from preferential flow along the borehole (well 377).

3.2. Redox conditions

Redox conditions may be expected to influence the concentration of antibiotics. Therefore, we studied the depth patterns and relations between redox indicators and the antibiotics. Well 40 is one of five researched wells which include mini-filters, and can thus be used to establish detailed depth profiles that may elucidate redox processes impacting the antibiotics. Fig. 6 shows depth plots of sulfamethazine, NO_3 , SO_4 , O_2 and Fe concentrations for well 40 in Oostrum, Limburg (concentrations for all samples are included in Supporting Information 3). Here, the concentration of sulfamethazine increases gradually down to a depth of 15 m, below which the concentration sharply declines to below the detection limit at 17.5 m depth. The sulfamethazine concentrations in the normal filters were higher at similar depths than the concentrations in the mini-filters although in the same order of magnitude. The difference can be explained by the fact that the normal filters are 2 m long and thus represent a mixed concentration between 5 and 7 m depth for the first filter and 7.5–9.5 m depth for the second filter. The nitrate concentrations are high, exceeding 250 mg L^{-1} . Fig. 6 also shows that nitrate gets reduced together with dissolved O_2 in a process coupled to pyrite oxidation, as indicated by the increased sulfate concentration and appearance of iron below the reaction front at approximately 16 m depth (see also Zhang et al. (2013, 2009) who describe the same well). The shifts in the redox-sensitive inorganic solutes correspond with a decrease in sulfamethazine, suggesting that sulfamethazine gets degraded or

attenuated at the same redox transition.

Moreover, Fig. 7 shows the concentrations of the detected antibiotics versus the concentrations of redox-sensitive groundwater solutes. Antibiotics are typically present in groundwater containing O_2 and NO_3 , representing oxic and suboxic conditions (Fig. 7a and b). Here, nitrate concentrations are often high, far exceeding the European drinking water standard and EU groundwater standard of 50 mg L^{-1} and reflect the leaching of nitrate from agricultural land in the infiltration areas. However, no linear relation was observed between O_2 or NO_3 concentrations and the antibiotic concentrations. This is in line with research done by García-Galán et al. (2010) who found no relation between sulfonamide antibiotics and nitrate in two groundwater bodies in Catalonia (Spain). Speciation calculations with PHREEQC (Parkhurst and Appelo, 2013) were executed to determine the oxidation state of the iron present in the samples. For an assumed chemical equilibrium with iron(III) oxide-hydroxide (log saturation index of goethite = 0) the concentrations of Fe(III) are negligible to Fe(II) (see supporting information 5). Assuming a more soluble form of iron(III) oxide-hydroxide (log saturation index of goethite = 3, so 1000 times more soluble) increases the Fe(III) concentration somewhat, but it remains negligible to the Fe(II) concentrations. This indicates that for all samples iron is present as Fe(II) and not as Fe(III). In our current study, antibiotics were absent in all but a few of our groundwater samples containing iron (Fig. 7c). The absence of antibiotics in iron-containing groundwater, plus the clear concentration-depth relation in Fig. 6 suggests that degradation of the studied antibiotics is redox-dependent and mainly occurring under nitrate-reducing conditions. This must hold as antibiotics appear in groundwater across the complete age range found. Our field results, therefore, confirm previous lab and field studies that studied the redox sensitivity of antibiotics (Banzhaf et al., 2012; Barbieri et al., 2012; Heberer et al., 2008). The first study showed in a column experiment that sulfamethoxazole degrades under nitrate reducing redox conditions. This was also reported by Heberer et al. (2008) who found that sulfamethoxazole was almost completely eliminated under anoxic conditions during riverbank infiltration at a drinking water production site. Our results suggest that this is not only the case for sulfamethoxazole but also for the other antibiotics detected. Contrary results have also been reported: a laboratory experiment to simulate bank filtration by Baumgarten et al. (2011) found that the removal of sulfamethoxazole was more effective under aerobic than under anoxic and anaerobic conditions. They

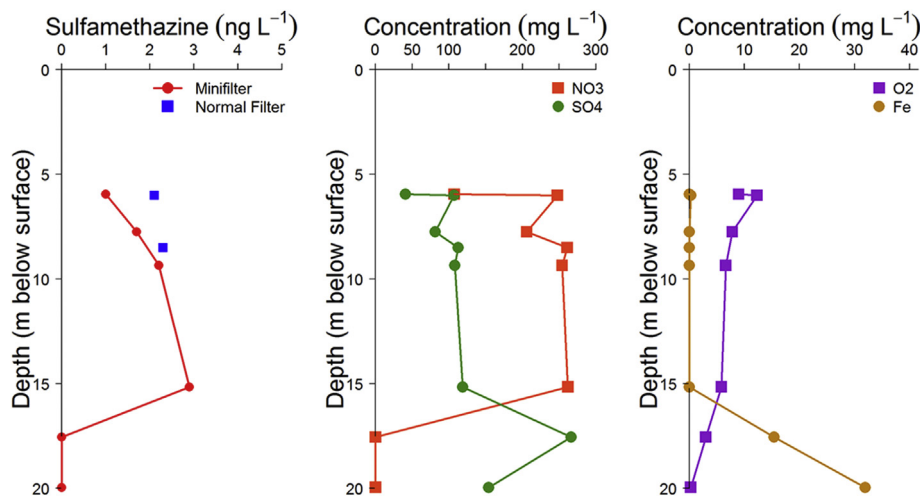


Fig. 6. Concentration - depth profiles for sulfamethazine, NO_3 , SO_4 , O_2 and Fe for well 40 in Oostrum, Limburg.

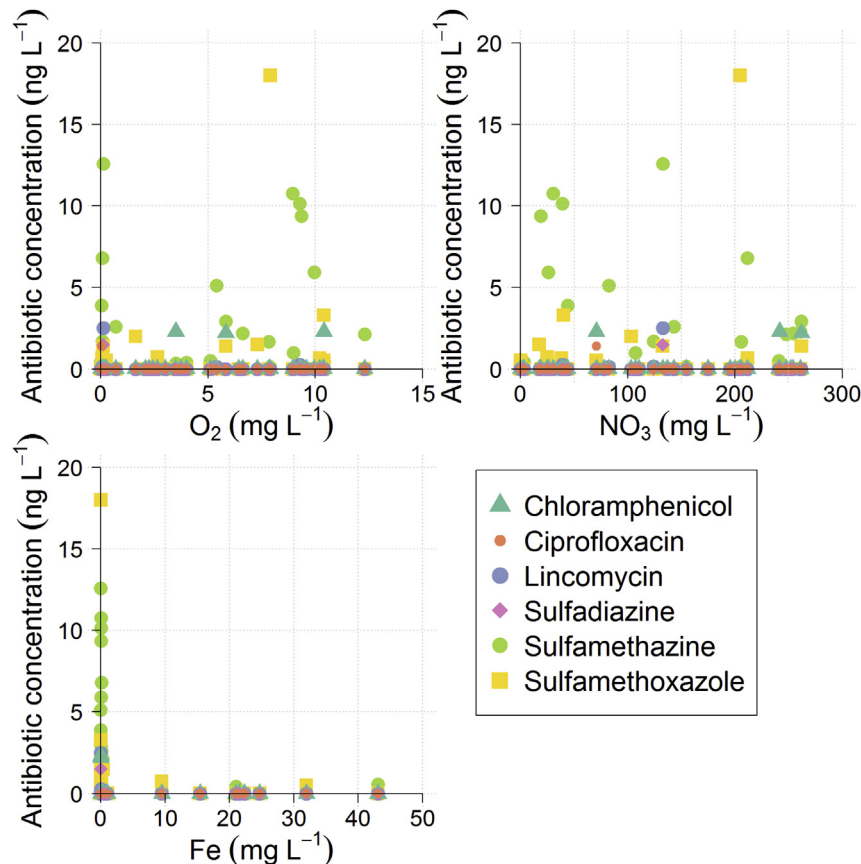


Fig. 7. Concentrations of the detected antibiotics versus the concentrations of the redox-sensitive groundwater solutes.

concluded that the presence of sulfamethoxazole in groundwater was not an indication of its persistency but of insufficient residence time or unfavorable redox conditions; contrary to their work we could infer that insufficient residence time may not be a prime reason in our study as sulfamethoxazole was found in water up to 26 years old.

4. Conclusions

The aim of our research was to characterize the presence of antibiotics under agricultural fields in relation to groundwater age and the processes that may control their fate in a groundwater environment. We therefore studied the presence and fate of antibiotics in one of the most intensive livestock areas worldwide in a set of advanced multi-level observation wells that were previously age dated using tritium-helium. Our results show that certain types of antibiotics leach to great depths and are even present in rather old groundwater. Six types of antibiotics were detected above detection limits, four of which above quantification limits with a concentration range of 0.30 up to 18 ng L⁻¹. Antibiotics were present in a majority of the sampled filters (31 of 46 samples). Sulfamethoxazole and sulfamethazine were encountered most often. The antibiotics were found at all measured depths down to 23 m and in groundwater up to 40 years old. These findings reflect the known application history of antibiotics in the Dutch veterinary system and indicate that leaching of antibiotics from agricultural fields on which manure is applied is a pathway for pollution of the aquatic environment.

Our study suggests that the attenuation of concentrations is controlled by redox process in the groundwater environment. Hits

of antibiotics correspond to groundwater that contains oxygen and medium to high concentrations of nitrate (10–270 mg L⁻¹). The relation between the antibiotics and Fe showed that the antibiotics were no longer present when the groundwater passed the nitrate/iron redoxcline and became anoxic. This result suggests that the antibiotics were degraded under nitrate-reducing conditions, an observation that seems to be supported by three studies at especially laboratory scale.

Given the systematic detections of antibiotics in groundwater below agricultural fields, we recommend the systematic monitoring of antibiotics in groundwater in Europe's regional and national monitoring networks, for example as part of the surveillance monitoring for the EU Water Framework Directive (EC 2000). Low detection limits are a prerequisite for such programs as our results indicate that the concentrations are often in the range of 0.5–20 ng L.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2018.05.085>.

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