Antibiotics in groundwater under locations with high livestock density in Germany

F. Balzer, S. Zühlke and S. Hannappel

ABSTRACT

Antibiotics are deployed in large quantities both in human and in veterinary medicine. Studies show that antibiotic residues occur in the environment (e.g. soil and surface waters). In some cases they were also detected in ground and drinking water. However, the degree of groundwater pollution by
Q1 antibiotic residues from livestock farming is unknown. Therefore Federal Environment Agency (UBA) supported a project that aimed to investigate near-surface groundwater samples in regions of high livestock density and high risk of groundwater exposure to antibiotics. By applying worst case criteria on existing sampling sites of our groundwater monitoring grid (high amounts of manure on site, high precipitation, low adsorption capacity of soils, high nitrate concentrations in ground water etc.) adequate sampling sites were identified as well as relevant antibiotics (amount of application, water solubility, biological stability etc.) by a literature review and contacts to local veterinary authorities. In total groundwater at 48 sampling sites was selected for analyses of 23 antibiotic substances. Out of the 23 antibiotics only 3 sulfonamides could be detected and quantified. With regard to the 48 sampling sites, at 39 locations no veterinary antibiotics were detected. At seven locations sulfadimidine and/or sulfadiazine was detected at low concentrations (<0.012 μg/L). Only sulfamethoxazole was repeatedly detected above 0.1 μg/L at two sites. Results show that translocation of veterinary antibiotics into near-surface groundwater in most parts of Germany does not occur above detection limits. Under unfavorable conditions leaching does occur but well below the limit values for pesticides (0.1 μg/L/0.5 μg/L). However, under some extreme conditions (to be identified by further research work) one antibiotic was present in groundwater above the pesticides limit values.

Key words | antibiotics, groundwater, livestock farming, veterinary pharmaceuticals

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INTRODUCTION

Background

The high consumption of antibiotics in human and veterinary medicine and the resulting effects for man and environment are an issue of increasing global concern. During the last years antibiotics, their metabolites and transformation products as well as antibiotic-resistant bacteria (MRSA) were detected in the environment worldwide.

In intensive livestock farming the application of pharmaceuticals, particularly antibiotics, is widespread. Today's intensive animal husbandry based on division of labour and recombination of animals during livestock breeding (e.g. mingling piglets of different offspring in pig fattening farms) results in frequent changes of location and increases stress and the risk of bacterial infections.

In 2011 Federal Office of Consumer Protection and Food Safety (BVL) published for the first time the amount of antibiotics that were supplied to veterinarians in Germany (i.e. 1.716 tons). Although since then the supplied amount (which approximately should be equal to the overall

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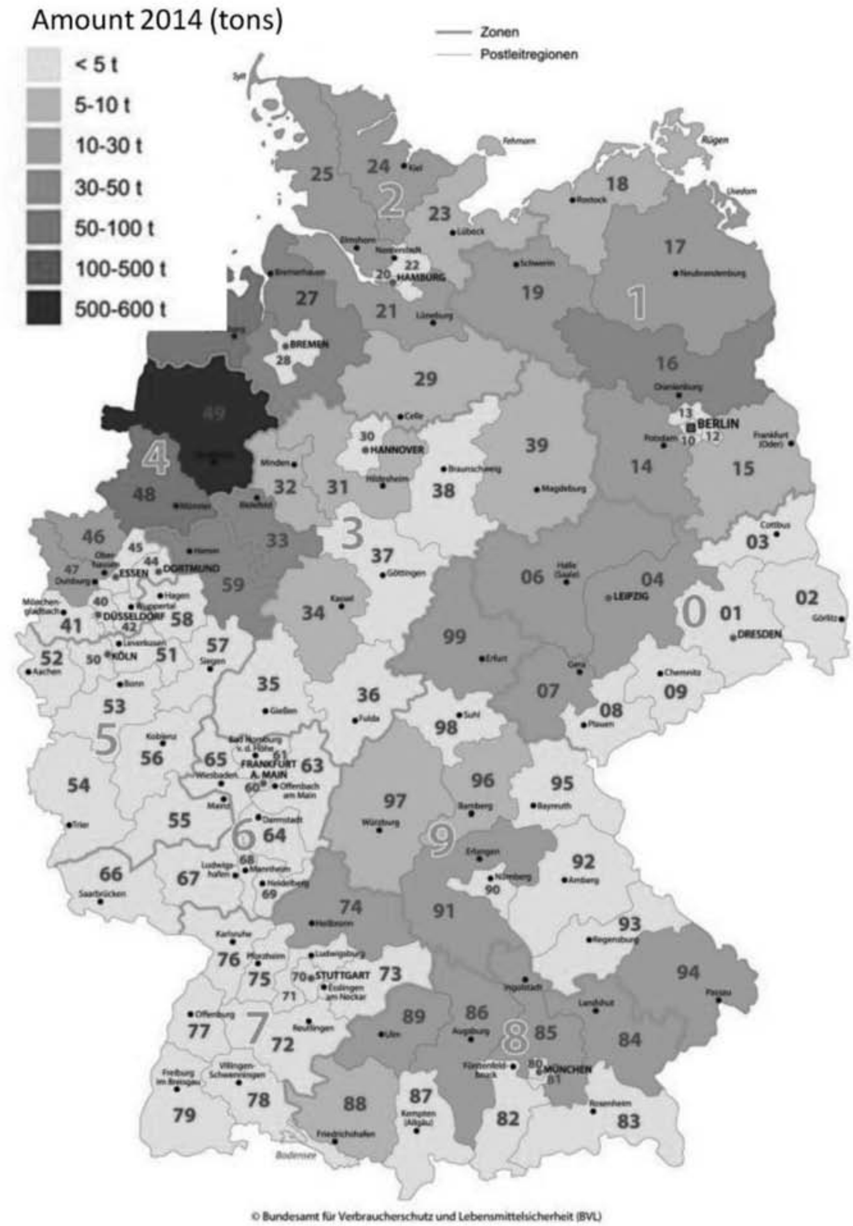
application rate) has been reduced by 478 tons in 2012, but in 2014 still 1.238 tons of antibiotics were supplied to veterinarians. This is approximately twice the amount as applied in human medicine (650 tons; Ebert *et al.* 2015).

Figure 1 shows the distribution of the supplied antibiotics in 2014. The concentration in the northwestern part of the country corresponds with the animal density in this region (Figure 2). There, stocking rate (livestock density) in livestock units/ha is up to 3–4 times higher compared to the German average.

As a reaction of the high consumption the Federal Cabinet adopted an extensive amendment to the German Medicinal Products Act in order to significantly reduce the use of antibiotics in animal husbandry. Therefore a new nationwide database shall create maximum transparency. Animal keepers have to document the use of antibiotics on their farms in greater



Q7 **Figure 1** | Supply of veterinary antibiotics per postal code area (BVL 2015).

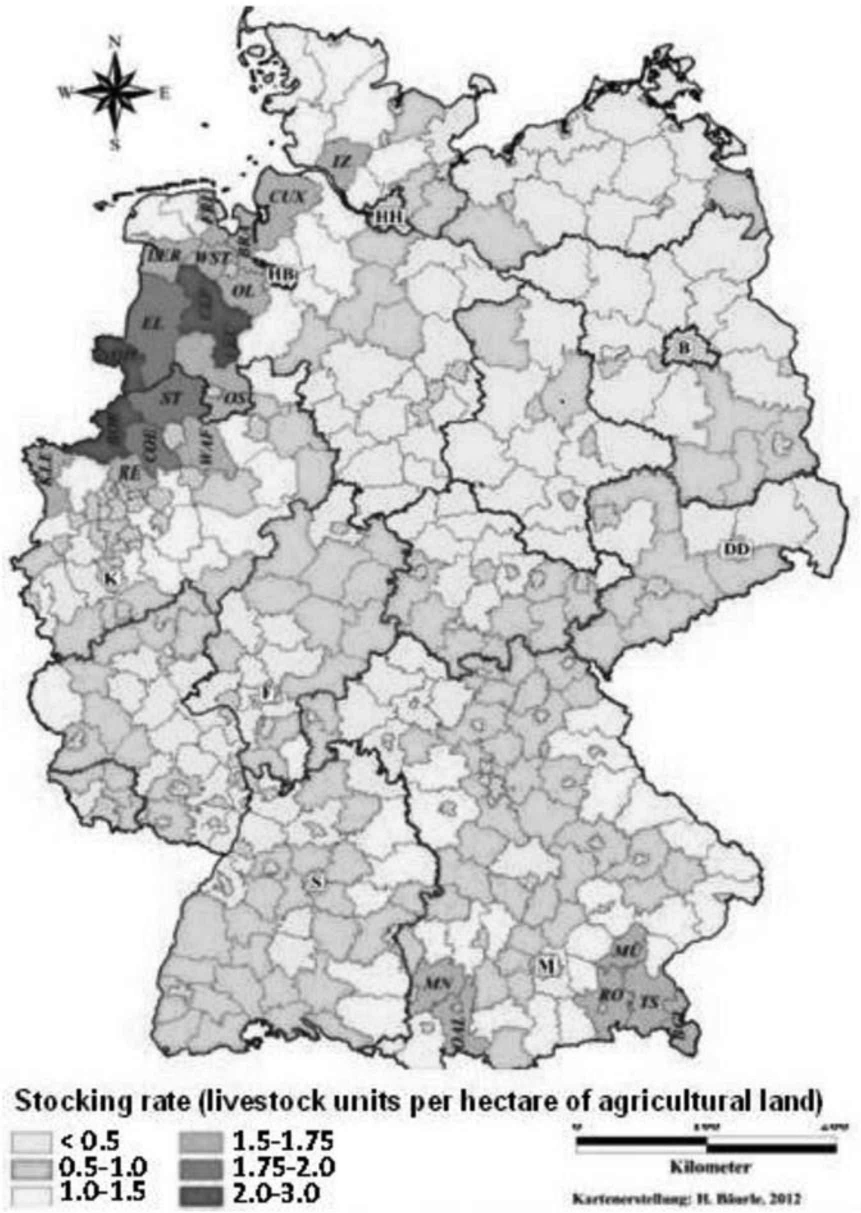


Figure 2 | Stocking rate (livestock units per hectare of agricultural land) in German counties (Bäurle & Tamasy 2012). **Q6**, **Q8**

detail and it was made more difficult to use certain active ingredients. The revised legislation entered into force in April 2014. Prospective analysis will show if the application of veterinary pharmaceuticals reduces or further steps are necessary.

Pathways into the environment

Pharmaceuticals are designed in order to reach their specific site of action in human's or animal's organism. Normally they have to overcome gastrointestinal passage. Therefore they are optimized in terms of stability and mobility and most of them are not easily bio-degradable. Thus, up to 80% of the active ingredients are excreted and may enter into the environment either unchanged or as metabolites. There, they will be transported and distributed to air, water, soil or sediment.

Figure 3 shows the different pathways for pharmaceuticals entering the environment. After being excreted human

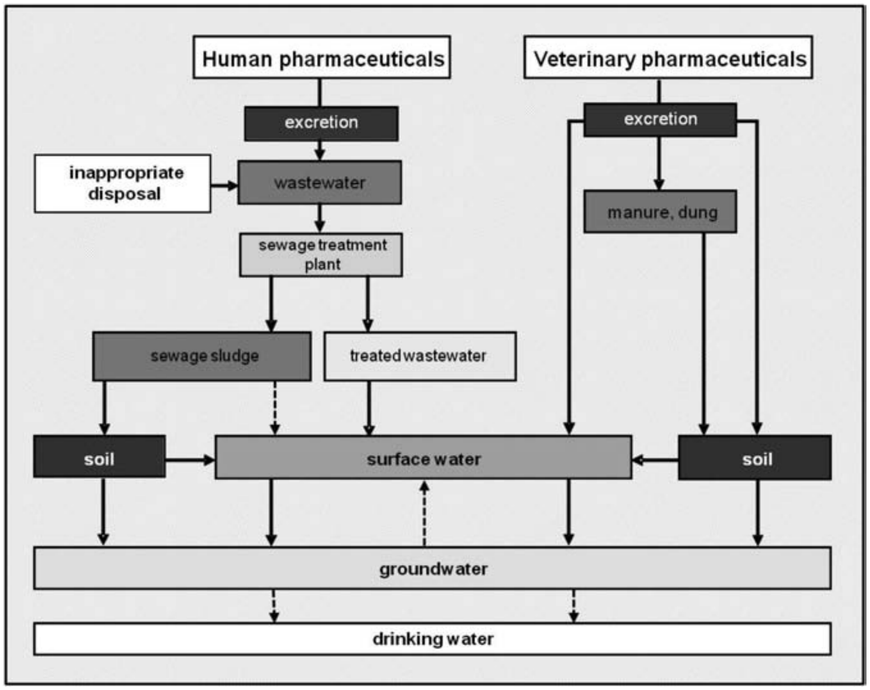


Figure 3 | Different pathways for human and veterinary pharmaceuticals entering the aquatic environment (Ebert *et al.* 2015).

pharmaceuticals and metabolites enter the municipal sewer system and end up in sewage treatment plants. Yet during sewage treatment some of these substances may not be completely eliminated, depending on their polarity, water solubility and persistence. They enter surface waters with the treated wastewater and pose a risk entering the groundwater. Others may enter into the environment via the disposal of sewage sludge on agricultural soils; they may thus become prone to runoff to surface waters as well as leaching to groundwater after rainfall (Monteiro & Boxall Q2 2010).

Veterinary pharmaceuticals are mostly released into the environment by spreading of manure and the application of organic fertilizers (Boxall *et al.* 2004). After percolation through the soil and unsaturated zone, they may reach shallow groundwater. Additionally, they may reach adjacent surface water bodies via runoff from fertilized farmlands.

Numerous studies show that antibiotic residues occur in manure and slurry (Kreuzig *et al.* 2007; Ratsack *et al.* 2013), in soils (Hamscher *et al.* 2004; Foerster *et al.* 2009), in leachates and in surface waters (Zhang *et al.* 2014). In some cases they were also detected in both groundwater and drinking water (Hirsch *et al.* 1999; Bartelt-Hunt *et al.* 2011; Cheng *et al.* 2015; Ma *et al.* 2015).

Goal of the project

The extent of groundwater pollution by antibiotic residues from livestock farming in Germany is unknown. Monitoring programs or a threshold value for groundwater do not exist. Particularly as groundwater is a vital resource for drinking water contamination by pollutants such as antibiotic residues should be avoided.

Therefore Federal Environment Agency supported a project that aimed to investigate near-surface groundwater samples in regions of high livestock density (stocking rates) and a high risk of groundwater exposure to antibiotics (Hannappel *et al.* 2014a, 2014b). To identify suitable sampling sites within the existing groundwater monitoring grid in Germany worst-case criteria were developed to foster a high probability of leaching of veterinary antibiotics to the groundwater. Thus, the results should not reflect a situation representative for Germany but focus on worst case scenario.

MATERIALS AND METHODS

Selection of veterinary pharmaceuticals

In the beginning a preliminary literature study was carried out to identify the veterinary pharmaceuticals of highest probability for emission into the groundwater. To select suitable pharmaceuticals for our purpose the following information was compiled and criteria applied:

- Highest amount of veterinary pharmaceuticals applied in Germany, especially within the areas of high livestock density in North Rhine-Westphalia and Lower Saxony
- Specific characteristics of the substances influencing emission and fate within the environment (e.g. high water solubility, long half-life time, only moderate sorption to soil)
- Findings of antibiotics in groundwater in literature

As a result 23 relevant antibiotics serve most of the purposes and were selected. They belong to the groups of tetracyclines, sulfonamides, macrolides, fluorchinolones and lincosamides (see Table 1).

Additionally the antiepileptic carbamazepine was chosen as a tracer for inputs of human medication. It is currently detected widespread all over Germany in near-surface groundwater because it is poorly removed during wastewater treatment, thus enters surface water and from there groundwater bodies, and is highly mobile in subsoils. The application of a tracer was necessary because some antibiotics, e.g. sulfamethoxazole (SMX), are used both in human and veterinary medicine (on both pathways shown in Figure 3). In case of detecting an antibiotic active ingredient without finding carbamazepine at the same time, most probably the antibiotics resulted not from human but from veterinary application.

Table 1 | Limit of detection (LOD) and LOQ of antibiotics selected

Substance	LOD [ng/l]	LOQ [ng/l]
Sulfamethoxypyridazine	2	6
4-OH-Sulfadiazine	5	16
Sulfadiazine	2	4
Sulfathiazole	2	6
Sulfamethazine	2	6
Trimethoprim	2	6
Sulfadimidine	1	3
Sulfadoxine	2	6
Sulfamethoxypyridazine	2	6
N-Ac-Sulfadiazine	2	6
Sulfachloropyridazine	6	18
SMX	4	10
Sulfadimethoxine	2	6
Tetracycline	6	18
4-epi-Tetracycline	6	18
Oxytetracycline	12	30
4-epi-Oxytetracycline	12	30
Chlortetracycline	15	50
4-epi-Chlortetracycline	15	50
Enrofloxacin	3	12
Lincomycin	2	6
Tilmicosin	3	10
Tulathromycin	3	10
Erythromycin	2	6
Carbamazepine*	0.1	0.3

*Tracer for waste water influence.

Selection of sampling sites

Based on a realistic worst-case scenario with regard to possible inputs of antibiotics into near-surface groundwater, appropriate groundwater sampling points were selected from the existing groundwater monitoring network operated Q3 by the German Federal States (The kind and efficient assistance of colleagues from the competent Federal States' authorities of Lower Saxony, Northrhine Westfalia, Saxony and Bavaria is highly appreciated by the authors).

For the selection the following criteria were addressed:

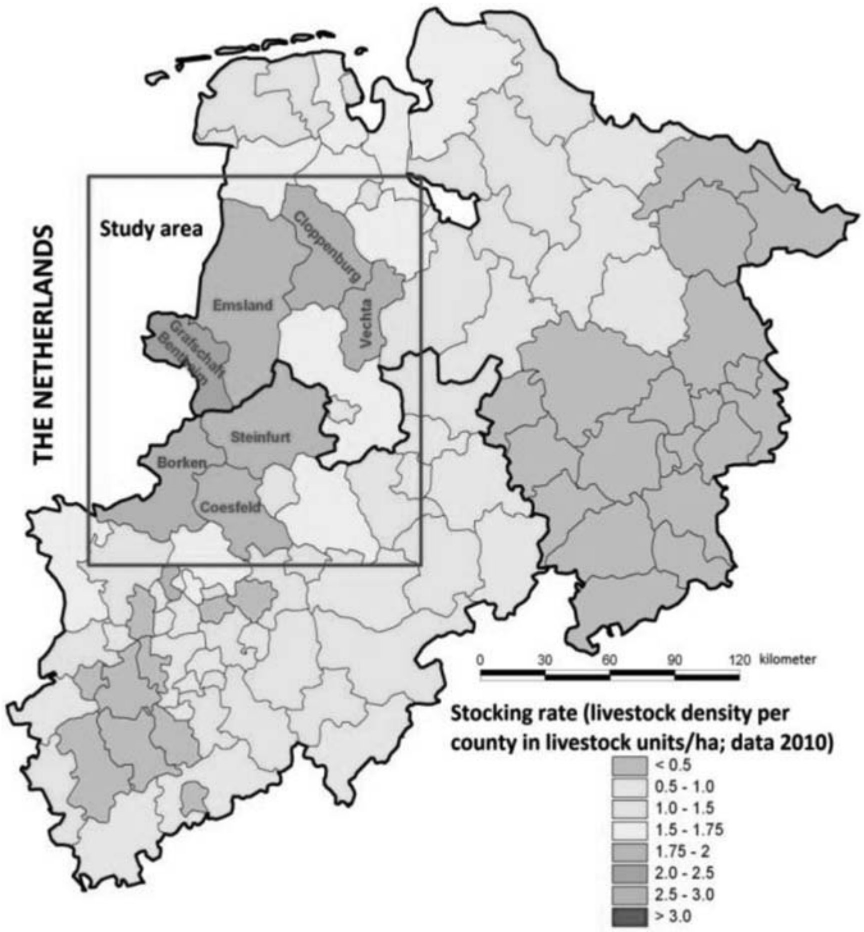
- Very high livestock density (>1.75 livestock units/hectare utilized agricultural area (GV/ha*LF)) and intensive application of manure and slurry
- Sandy soils with low gross field moisture capacity of the total unsaturated zone above groundwater. Thus resulting in low natural protective function of the near-surface groundwater layer on first groundwater floor
- Low depth of groundwater surface (<5 meters below soil surface) causing short percolation time for leachates through the unsaturated zone

- Very high nitrogen contents in groundwater (nitrate and/or ammonia) above EU thresholds (according to Water Framework Directive and Groundwater Directive) which indicates intensive agriculture in the surrounding of the sample sites.

Applying these criteria 48 sampling points were selected for further investigation. 39 locations represent aquifers with unconsolidated sediments whilst nine sites are composed of consolidated rock. Figure 4 shows the location of the main study area, where 40 selected sampling points are located. In addition to the sampling points in the northwestern part of the country, four sampling points in Bavaria and four in Saxony were selected, followed the same selection criteria. These study areas are not shown in Figure 4.

Sample collection, preparation and analysis

In 2012 and 2013 at least two groundwater samples (altogether more than 100 ground water samples) were taken from each of the 48 sampling points by pumping the groundwater until reaching hydraulic and hydrochemical criteria (fixed values of pH, redox, salinity and temperature) and filling the water in dark 2 Liter glass bottles. These bottles were preserved at 4 °C until the analysis in the laboratory within few days. They were analyzed with multi-methods, based on solid phase extraction as well as liquid chromatographic separation coupled to detection by mass spectrometry (LC-MS-MS). The detection by triple quadrupole MS allows both, high selectivity and high sensitivity for the unambiguous identification and quantification of the pharmaceutical residues. Multiple reaction



Q9 **Figure 4** | Location of the study area.

monitoring mode was used monitoring at least two characteristic fragments of each compound. The limits of detection were in low ng/l-range for all substances (see Table 1).

RESULTS AND DISCUSSION

The total of the groundwater samples represent young recently developed groundwater with a site-typical total mineralization. The available time for degradation and transformation is comparatively short. The leaching probability of antibiotics into the groundwater can hence be considered to be high. This correlates with the above-mentioned worst-case scenario for site and sampling point selection.

To summarize only 3 sulfonamides (sulfadiazine, sulfadimidine and SMX) of 23 investigated antibiotics were found with concentrations above the limit of quantification. Tetracycline and all other antibiotics could not be verified in groundwater samples.

With regard to the 48 investigated groundwater sampling points:

- At 39 locations no detections of veterinary antibiotics were ascertained.
- At 2 locations sulfadimidine was detected at very low concentrations; less than limit of quantification (LOQ) (less than 4 ng/l).
- At 5 locations sulfadimidine/sulfadiazine was determined at low concentrations; >LOQ but <100 ng/l.

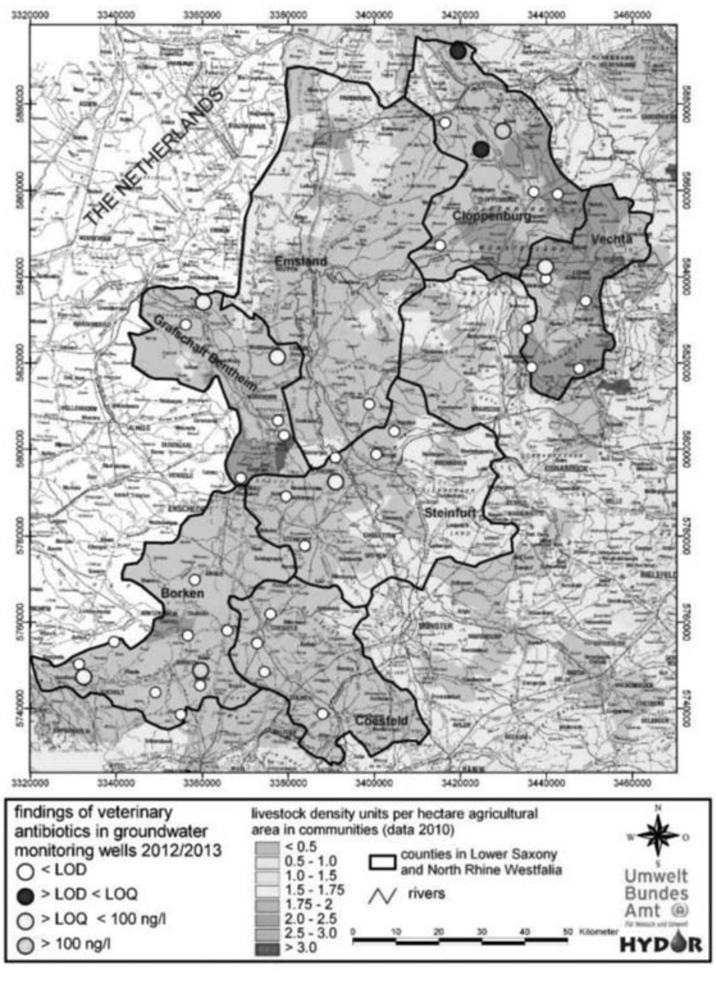


Figure 5 | Map of sampling sites and findings of antibiotics in northwestern Germany. Q10

- At 2 locations SMX was found at high concentrations; ≥100 ng/l.

Figure 5 shows the 40 sampling points and findings of antibiotics in groundwater in the northwestern part of Germany. These 8 sites in Bavaria and Saxony are not represented in the map, as no antibiotics could be detected there.

At seven sampling points the veterinary antibiotics sulfadiazine and sulfadimidine could be found at concentrations slightly above the detection limit with a maximum concentration of 11 ng/l. One of the sampling points represented an aquifer of consolidated carbonate rocks. This is the first documented detection of an antibiotic within such geological formations. In total, no distinct natural conditions for the slight leaching could be identified at these sampling points.

At two sampling points higher concentration of SMX was found repeatedly. One site is located in North Rhine-Westphalia and was already investigated in 2008 and 2009. Together with former results our investigation showed that SMX was detected in near-surface groundwater in concentrations above 200 ng/l. By now constant high concentrations of SMX can be confirmed during a period of six years ($n = 8$). The other sampling point is located in Lower Saxony. While the first sampling (10/2012) did not contain antibiotic residues the second sampling (5/2013) detected 138 ng/l SMX and a third sample (9/2013) contained 950 ng/l SMX. In contrast to above mentioned sulfadimidine and sulfadiazine, tendency to sorption is less for SMX.

Additionally, it has been already shown, that insufficient residence time or unfavorable redox conditions enables SMX in groundwater after bank filtration from contaminated surface water (Baumgarten *et al.* 2011). Both locations with high concentrations of SMX show site specific characteristics of the underground (low buffering- and degradation capacities), resulting from the lithology and hydrochemistry. If the SMX originated from application of manure to the field (veterinary medicine) or from leaky sewage drain by adjacent households (human medicine) could not be concluded so far. Further studies and data from samples of sewage drain are necessary to clear that point.

CONCLUSIONS

Based on a worst-case approach we can conclude that leaching of veterinary antibiotics into near-surface groundwater in most parts of Germany (taking into account the criteria of natural site factors, agricultural intensity and hydrogeological terms) does not regularly occur above detection limits. However, under unfavorable conditions leaching does occur but mostly well below the limit values for pesticides. Nevertheless, under some extreme conditions (to be identified by further research work) the presence of one antibiotic in groundwater above the pesticides limit values was shown.

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Author Queries

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Q2 Monteiro *et al*. (2010) has been changed to Monteiro & Boxall (2010) as per the reference list.

Q3 As per the journal style, footnote is not allowed. Hence we have moved the footnote text to the text part.

Q4 Please provide publisher name for Hamscher *et al*. (2004).

Q5 Please list all the authors name for Zhang *et al*. (2014).

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