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Veterinary pharmaceutical residues in water resources and tap water in an intensive husbandry area in France

Lise Charuaud[a], Emilie Jardé[b], Anne Jaffrézic[c], Marine Liotaud[b], Quentin Goyat[a], Fabien Mercier[a], Barbara Le Bot[a*]

[a]Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) -UMR_S 1085, F-35000 Rennes, France

[b]Univ Rennes, CNRS, Géosciences Rennes, UMR6118, 35000 Rennes, France

[c]UMR SAS, Agrocampus Oust, INRA 35000 Rennes, France

[*]Corresponding author:

E-mail address: barbara.lebot@ehesp.fr

Postal address: EHESP/LERES, 15, avenue du Pr. Léon-Bernard, CS 74 312, 35043 Rennes cedex, France

Tel.: +33 (0)2 99 02 29 24

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Abbreviation: VPRs: Veterinary pharmaceutical residues

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KEY WORDS

Antibiotics, antiparasitic drugs, natural waters, drinking water, stanols.

ABSTRACT

In intensive livestock areas, veterinary pharmaceutical residues (VPRs) can occur in water resources, but also in tap water because treatment processes are not designed to remove these contaminants. The main objective of this study is to assess the occurrence of VPRs in water resources and tap waters in Brittany. As several identical compounds are used in both veterinary and human medicine, a toolbox (stanols and pharmaceuticals) is used to help determine the origin of contamination in the case of mixed-use molecules. Water resources samples were collected from 25 sites (23 surface waters and two groundwaters) used for tap water production and located in watersheds considered as sensitive due to intensive husbandry activities. Samples were also taken at 23 corresponding tap water sites. A list of 38 VPRs of interest was analyzed. In water resources, at least one VPR was quantified in 32% of the samples. 17 different VPRs were quantified, including antibiotics, antiparasitic drugs and anti-inflammatory drugs. Concentration levels ranged between 5 ng/L and 2946 ng/L. Mixed-use pharmaceuticals were quantified in twelve samples of water resources and among these samples nine had a mixed overall fecal contamination. In the context of this large-scale study, it appeared difficult to determine precisely the factors impacting the occurrence of VPRs. VPRs were quantified in 20% of the tap water samples. Twelve VPRs were quantified, including ten compounds exclusively used in veterinary medicine and two mixed-use compounds. Concentration levels are inferior to 40 ng/L for all compounds, with the exception of the antibiotic florfenicol which was quantified at 159 ng/L and 211 ng/L. The population of Brittany may therefore be exposed to these contaminants through tap water. These observations should be put into perspective with the detection frequencies per compound which are all below 10% in both water resources and tap water.

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1. INTRODUCTION

Intensive livestock farming leads to greater vulnerability of the animals to develop and spread diseases as they are often confined together (Hu and Cheng, 2016). To limit this issue, veterinary pharmaceuticals are often used not only for a curative goal, but also for prophylactic (Hu and Cheng, 2016). However, only veterinary drugs containing a critical antibiotic (third and fourth generation cephalosporins, fluoroquinolones) are banned for prophylactic use since 2016 (Code de la Santé Publique, 2016). When administered to the animal, veterinary pharmaceuticals may be partially metabolized in the organism. They are then excreted in urine or feces as the parent compounds and/or as metabolites. Excretion rates for veterinary antibiotics can vary from 40% for tetracyclines to 90% for sulfonamides (Kemper, 2008; Tasho and Cho, 2016). Thus, VPRs can be released to the environment either directly with urine and feces of animals in pastures or indirectly during the spreading of contaminated manure and slurry as fertilizers on fields (Bártíková et al., 2016; Boxall, 2010; Jeon et al., 2014; Kim et al., 2011; Kools et al., 2008). VPRs can then reach natural waters, i.e. surface water and groundwater, via runoff, erosion and leaching (Jaffrézic et al., 2017; Jeon et al., 2014; Kemper, 2008; Kim et al., 2011).

A previous study performed on VPRs in waters from nested watersheds from agricultural headwater to water framework management outlet in Brittany (Jaffrézic et al., 2017) demonstrated that VPR contamination is indeed an issue, as animal-specific pharmaceuticals were detected at all sampling dates upstream and downstream from a wastewater treatment plant and at concentrations higher than those for human-specific pharmaceuticals (i.e. maximum concentrations of 181 $ng.L^{-1}$ and 1450 $ng.L^{-1}$ for sulfamethazine and flunixin respectively). VPRs also have been widely quantified worldwide in natural waters (either surface water or groundwater) from $ng.L^{-1}$ to $\mu g.L^{-1}$ during the last decade (García-Galán et

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al., 2010; Hu et al., 2014; Iglesias et al., 2014; Kim et al., 2016; Luo et al., 2011; Ok et al., 2011; Tong et al., 2014, 2009; Wei et al., 2011; Yao et al., 2017, 2015; Zhou et al., 2016). As data on VPR behavior in drinking water treatment plants (DWTPs) is scarce (Charuaud et al., 2019), there is a public health concern as the human population may be exposed to these compounds via tap water, especially when tap water is produced with surface water resources from intensive livestock areas. In Brittany, surface water is the major resource (75%) for water production; while the national ratio is 64% groundwater and 36% surface water (ARS Bretagne, 2017). However, no data is available concerning the occurrence of VPRs in tap water even though it is a high-risk region. This lack of information is also true at international level, where very few studies have been conducted on VPRs in tap waters (Leung et al., 2013; Ye et al., 2007). Due to the highly varied physico-chemical properties of VPRs, their low concentration levels in the environment and the complexity of environmental matrices, it is difficult to develop an optimal multi-residue analysis method for a large number of compounds, especially when they belong to different therapeutic classes. There are many analytical methods dedicated to veterinary antibiotics (Gao et al., 2016; Hu et al., 2014; Tong et al., 2009; Wei et al., 2011; Xue et al., 2015; Yao et al., 2017; Zhang et al., 2014) and some dedicated to antiparasitic drugs (Krogh et al., 2008; Thompson et al., 2009; Zrnčić et al., 2014), but few include different therapeutic classes (Iglesias et al., 2012). There is therefore a need to develop specific analytical methods and the selection of the VPRs needs to be performed according to the veterinary uses on the study site (Soulier et al., 2015).

In addition, the contamination originating from veterinary medicine cannot always be distinguished from that arising from human medicine, since several identical compounds are used in both veterinary and human medicine. It is therefore necessary to develop tools to help determine the origin of contamination in the case of mixed-use molecules. The monitoring of fecal contamination in natural water samples may be of interest.

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Several tools have been developed and efficiently applied to distinguish human and animal fecal contamination in environmental matrices, such as microbiological markers (Ahmed et al., 2016; Derrien et al., 2012; Gourmelon et al., 2010; Heaney et al., 2015; Ohad et al., 2015; Raith et al., 2013; Seurinck et al., 2005; Solecki et al., 2011), viral markers (Cole et al., 2003; Gourmelon et al., 2010; Jofre et al., 2014; Lee et al., 2009; Muniesa et al., 2009; Tyagi et al., 2009) or chemical markers such as fecal stanols (Biache and Philp, 2013; Derrien et al., 2012; Gourmelon et al., 2010; Harrault et al., 2014; Jardé et al., 2018; Jeanneau et al., 2011; Leeming et al., 1996; Shah et al., 2007; Tran et al., 2015; Tyagi et al., 2009), pharmaceuticals or other compounds related to human consumption (caffeine, nicotine, artificial sweeteners) (Madoux-Humery et al., 2013; Tran et al., 2015; Wade et al., 2015). Markers of fecal contamination are often used in association in what is called a “Fecal Source Tracking (FST) Toolbox” (Gourmelon et al., 2010; Tran et al., 2015; Devane et al., 2018) in order to gain more certainty. Stanols seem to fulfill various criteria to be useful tracing tools. Stanols are sensitive and specific markers of fecal contamination. Their distribution in feces will vary according to the specie's diet, the ability of animals to biosynthesize endogenous sterols and the composition of the intestinal flora which will converts sterols into stanols by biohydrogenation (Leeming et al., 1996). This species distribution, called "stanol fingerprint", has been successfully used, via stanol ratio analysis or by multivariate analysis, to distinguish human and animal contamination in water (Biache and Philp, 2013; Derrien et al., 2012; Gourmelon et al., 2010; Harrault et al., 2014; Jardé et al., 2018; Jeanneau et al., 2011; Leeming et al., 1996; Shah et al., 2007; Tran et al., 2015; Tyagi et al., 2009). Besides, stanols demonstrate a significant persistence in the environment (Harrault et al., 2014) and sufficient concentrations to be detected. Regarding the limitations of the use of stanols, some studies report a difficulty in identifying the source of fecal contamination via the "stanol fingerprint" in the case of watersheds affected by several sources of pollution (Shah et al. 2007).

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Secondly, because of their hydrophobic nature, stanols are easily absorbed on soils and are not a suitable marker for monitoring fecal contamination of groundwater (Tran et al. 2015).

The analysis of indirect markers of fecal contamination as pharmaceuticals can be a complementary tool to trace the sources of contamination of a given sample. Indeed, only the study of pharmaceuticals can establish contamination from both human and animal origin, as there are drugs specific of human use and others used only in veterinary medicine. The other indirect chemical markers only highlight human contamination (i.e. caffeine, nicotine).

The relevance of pharmaceuticals as fecal markers depends largely on many contributing factors, including land use patterns, population, amount consumed of each type of pharmaceutical, characteristics of pollution sources, hydrology and geology of the study area (Tran et al., 2015). Carbamazepine (anti-epileptic and neuroleptic) and diclofenac (anti-inflammatory) are pharmaceuticals widely used in human medicine. They are not completely degraded during wastewater treatment plant processes and are often quantified in water resources (Wade et al., 2015; Zhang et al., 2008). Moreover, carbamazepine and diclofenac have been quantified in water resources in Brittany in previous studies (Jaffrezic et al. 2017; Mompelat et al., 2011). For those reasons, carbamazepine and diclofenac can be adequate markers of fecal human contamination in water resources.

The main objective of this study is to assess the occurrence of VPRs in water resources, and corresponding tap waters, in watersheds characterized by intensive livestock farming and recycling of animal waste on soils. The secondary objectives are to identify the sources of contamination through use of chemical markers (stanols and pharmaceuticals) to determine the origin of the fecal contamination associated with the presence of VPRs, and to identify the factors related to the occurrence of VPRs. For these purposes, several stages were necessary to select the VPRs of interest and drinking water treatment plants with high husbandry pressure in Brittany and then to develop a suitable method of analysis.

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2. MATERIAL AND METHODS

2.1. Sampling sites and sampling strategy

The investigated area is the region of Brittany in northwest France. Agriculture holds an important place in Brittany, both economically (agricultural or agro-alimentary production) and in terms of land use. About 60% of the territory of Brittany is dedicated to agricultural activities (1 630 536 hectares out of a total regional area of 2 750 667 hectares), and it is the leading French region for livestock production (Chambres d'Agriculture de Bretagne, 2017). In 2016, there were approximately 7 million pigs, 2.1 million head of cattle and 89 million poultry, representing respectively 56%, 11% and 33% of national production (Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt Bretagne, 2017a). Brittany is also one of the French regions where aquaculture activities are important, particularly for salmonid farming (Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt Bretagne, 2017b).

The climate is temperate oceanic, with mean annual rainfall between 617 mm and 1490 mm depending on the station concerned. The morphogeological characteristics of Brittany make it a unique region for the production of drinking water. The granite and the schists, basement rocks, contain aquifers with little capacity. These superficial resources are vulnerable, especially to anthropogenic pollution from agricultural, industrial and domestic sources, as sites are often located downstream of these activities. Groundwater also experiences these pressures because most of the drinking water treatment plants pump at shallow depths (75% of groundwaters are collected at a depth of less than 12 meters) (Groupe de Travail du Plan Régional Santé Environnement 2, 2015).

Water resource samples were collected from 25 sites (23 surface waters and two groundwaters) used for tap water production and located in watersheds considered as

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sensitive due to intensive husbandry activities. These 25 sites are geographically distributed over the territory of Brittany and are likely to cover all local specificities in terms of husbandry. Surface water samples were taken from 21 different rivers. Samples were also taken at 23 corresponding tap water sites. There are fewer tap water sites (23) than water resources (25), as some DWTPs operate on several sites. In practice, the samples were collected in 23 DWTPs, at the inlet and the outlet of the plant. Table S1 shows the surrounding livestock pressure on the selected sites. Five sampling campaigns (SC) were performed (in March 2017, May 2017, September 2017, January 2018 and May 2018). All sites could not been sampled during the five sampling campaigns. Table S1 presents which sites were sampled during which sampling campaigns. The purpose of the sampling strategy was to reflect variations in water regime (low water or high water) and to collect during manure/slurry spreading times. Table 1 shows the sampling periods and the characteristics of the five sampling campaigns.

[Table 1]

A total of 199 samples were collected for analysis, including 105 natural waters (97 surface waters and eight groundwaters) and 94 tap waters.

For VPR analysis, water resources or tap water were sampled in 1 L amber glass bottles containing 200 µL of a stabilizing agent (ascorbic acid; 20 g/L) in order to block the effect of oxidants such as free chlorine. 1 L of surface water or 2 L of groundwater or tap water were sampled in sterilized plastic bottles for stanol analysis. 500 mL of water resources were sampled in sterilized plastic bottles for dissolved organic carbon (DOC) analysis. 500 mL of water resources were sampled in sterilized plastic bottles with 60 mg of thiosulfate sodium for microbiological analyses. All samples were kept in ice boxes during transport to the laboratory.

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2.2. VPRs of interest

A list of 38 VPRs of interest was defined according to veterinary practices in Brittany, animal targets, routes of administration, pharmacokinetics, mobility from soil to water, persistence in water and analytical feasibility (Charuaud et al., 2016).

Those 38 VPRs include several therapeutic classes such as antibiotics (21: amoxicillin, ampicillin, cefquinome, chlortetracycline, doxycycline, enrofloxacin, erythromycin, florfenicol, flumequine, lincomycin, marbofloxacin, neospiramycin, oxolinic acid, oxytetracycline, spiramycin, sulfadiazine, sulfadimethoxine, sulfamethazine, tilmicosin, trimethoprim, tylosin), antiparasitic drugs (10: clorsulon, diazinon, dicyclanil, eprinomectin, flubendazole, ivermectin, levamisole, triclabendazole, triclabendazole sulfone, triclabendazole sulfoxide), anticoccidians (3: toltrazuril, toltrazuril sulfone, toltrazuril sulfoxide) and anti-inflammatory drugs (4: dexamethasone, flunixin, ketoprofen, meloxicam). Among these 38 residues, five are metabolites (neospiramycin, toltrazuril sulfone, toltrazuril sulfoxide, triclabendazole sulfone, triclabendazole sulfoxide). Moreover, carbamazepine and diclofenac were integrated into the method as tracers for inputs of human medicine. Chemical structures of VPRs and their physico-chemical properties are described in Table S2.

2.4. VPR analysis

Chemicals, reagents and stock solutions are described in Supplementary information S3.

At the laboratory, 200 mL of water samples were filtered through glass fiber filters of 0.7 µm diameter (Millipore) and stored at 4 °C if the extraction could be performed in the next 48 hours. However, the samples from the first two sampling campaigns were stored at -20 °C for one to five months before analysis. Samples from the other three sampling campaigns were

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extracted within 48 hours after sampling, according to XP T90-223 guidelines (Afnor, 2013a), and analyzed within 14 days.

Immediately prior to the extraction, sample pH was adjusted to pH2 with nitric acid (67%, VWR), and 100 µL of an internal standards mix solution (0.5 mg/L) was added to each sample.

Samples were extracted by solid phase extraction with a robot GX-274 ASPEC^{TM} (Gilson) on Strata-X cartridge (200 mg, 6 mL, Phenomenex). The cartridges were conditioned with 6 mL of acetonitrile (ACN) / methanol (MeOH) (90:10 v/v) followed by 6 mL of acidified ultrapure water (pH2). Each 200 mL sample was loaded onto a cartridge at a flow rate of 10 mL/min. Cartridges were then rinsed with 10 mL of ultrapure water to eliminate impurities, and vacuum-dried for 15 minutes. Analytes were eluted with 10 mL of ACN/MeOH (90:10 v/v) fractioned in four portions (4 x 2.5 mL). Eluates were then transferred into glass tubes to be evaporated until the drop under N_2 at a temperature of 30 °C. The residues were reconstituted with 0.5 mL of H_2O/ACN (87:13 v/v) and 25 µL of an injection tracer solution (pentabromophenol; 10 mg/L) were then added. The mixtures were ultrasonicated for five minutes and then transferred to 2 mL glass vials. The 38 target compounds were analyzed by rapid resolution liquid chromatography coupled to tandem mass spectrometry (RRLC-MS/MS). The separation of the compounds was performed with an Agilent 1200 series system on an Xselect HSST3 column (2.1 mm x 100 mm; 2.5 µm; Waters). The column temperature was maintained at 40 °C. Mobile phase was composed of A) ultrapure water + 0.01% formic acid and B) acetonitrile + 0.01% formic acid and the flow rate was 0.4 mL/min. Initial gradient was composed of 87% (A) – 13% (B) during the first minute, followed by a linear gradient from 87% to 13% of A for one to ten minutes, then maintained at 13% (A) – 87% (B) for five minutes and finally returning linearly to the initial gradient of 87% (A) – 13% (B) for the last two minutes. Total run time was 17 minutes. Mass spectrometric

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detection was performed with a triple quadrupole mass spectrometer 6460 from Agilent Technologies equipped with an electrospray ion (ESI) source operating simultaneously in positive and negative modes during the analysis. The gas flow was nitrogen and its temperature was set to 200 °C for a flow of 5 mL/min. The sheath gas was also nitrogen with a temperature of 380 °C and a flow of 11 mL/min. Capillary voltage was 4000 V either in positive or negative mode and nozzle voltage was set at 500 V. Nebulizer was set at 45 psi. Quantification of each VPR of interest was performed in multiple reaction monitoring (MRM) mode. Masshunter software (Agilent) was used for instrument control, data acquisition and data analysis. The MS/MS parameters were optimized individually for each compound by Flow Injection Analysis, with standard solutions at 1 mg/L. Table S4 in Supplementary Information describes the optimal MS/MS conditions and internal standards.

2.5. Quality control/Quality assurance of the VPRs analysis method

For each series of analyses, one blank and eight calibration solutions prepared in Evian® water were extracted under the same conditions as the samples. An internal calibration curve was then established in quadratic mode including seven or eight points depending on the compound within the range: limit of quantification (LOQ) – 500 ng/L. Quadratic fit was used to compensate for the non-linearity of the instrument response over a wide working range. Correlation coefficients of the quadratic calibration curves had to be superior or equal to 0.99. The LOQ was determined as the minimum detectable amount of analyte with a signal-to-noise ratio of 10. LOQ was either 5 ng/L or 12.5 ng/L depending on the compound.

The MRM mode was used for the quantification. The most intensive and specific ion pairs, together with retention time, were used to identify the VPR of interest.

Quantitative analysis was based on peak area and was performed by internal standard calibration. A correction factor is applied to rectify the ionization (i.e. matrix effects). In order

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to do that, each sample was divided into two aliquots and one of them was spiked at 250 ng/L with the VPRs of interest to assess the matrix effects and to apply a correction factor.

The two MRM transitions were used for quantification: the two results were averaged, on condition that the difference between them was less than 10% in proportion to the lower result; the lower value was retained if the two results differed by more than 10%.

To compensate for the matrix effects that will differ greatly by water type the results of the standard addition are processed as follows and the following safety factors have been applied:

- If addition is found between 50 and 150% (i.e. between 125 and 375 ng/L), quantification is done by standard additions
- If addition is found between 20 and 50% (i.e. between 50 and 125 ng/L), quantification is done by standard additions and limit of quantification is multiplied by 2
- If addition is found between 10 and 20% (i.e. between 25 and 50 ng/L), quantification is done by standard additions and limit of quantification is multiplied by 4
- If addition is found below 10 % (below 25ng/L), the result is invalidated

Besides, the signals of the injection tracer (pentabromophenol) and the internal extraction standards are checked (these signals must be included between +/- 50% compared to the signals obtained in the calibration curve).

If VPR concentration levels were beyond the calibration range, the sample was diluted and re-analyzed, and a dilution coefficient was applied to obtain the initial concentration.

The VPR recoveries were assessed at three concentration levels (LOQ, 100 ng/L and 500 ng/L) in six different environmental matrices (two surface waters, one groundwater and three tap waters). Table S5 in supplementary information summarizes the LOQs, recoveries, accuracies, relative standard deviations and uncertainties obtained for all compounds with the transition with the lowest performance. All parameters have been calculated according to the guidelines of NF T90-210 (Afnor, 2018) and NF ISO 11352 (Afnor, 2013b). At the LOQs, all

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recoveries were between 69% and 187% except for 14 VPRs (amoxicillin, cefquinome, clorsulon, dicyclanil, florfenicol, flumequine, lincomycin, neospiramycin, spiramycin, sulfamethazine, tilmicosin, toltrazuril, triclabendazole sulfone, tylosin) which were subject to important matrix effects. At 100 ng/L and 500 ng/L, all recoveries were between 59% and 156% except for four VPRs (amoxicillin, lincomycin, neospiramycin, tylosin).

2.6. Stanol analysis

Fecal stanols were extracted from filtered (0.7 µm glass fiber) water samples (1 L) by solid phase extraction and quantified by gas chromatography coupled to mass spectrometry using an internal calibration as described by Jeanneau et al. (2011). The procedure is described in detail in S6 in Supplementary information.

2.7. Escherichia coli (*E. coli) analysis*

Microbial fecal indicator *E. coli* in surface water samples was counted using microplate methods (EN ISO 9308-3 (Anonymous, 1999)) with a detection limit of 15 most probable number (MPN) per 100 mL of water sample. In groundwater, *E. coli* was counted using membrane methods (EN ISO 9308-1 (Anonymous, 2000)) with a detection limit of 1 colony forming unit (CFU) per 100 mL of water sample.

2.8. Dissolved organic carbon (DOC) analysis, daily flow rates and rainfall

The samples were filtered at 0.45 µm and the analysis was carried out by chemical oxidation according to standard NF EN-1484 (1997) on a Total Organic Carbon analyzer TOC-VWP (Shimadzu).

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Daily flowrates at the closest nested gauged watershed were collected on the website of the hydrological data bank (http://hydro.eaufrance.fr/). Daily flow rate in the ungauged sampling point were calculated with the surface ratio.

Rainfall in the 72 hours before sampling was collected after sampling on the website Meteo France (www.meteofrance.com/). The closest weather station to the water resources was chosen to gather the data.

3. RESULTS AND DISCUSSION

3.1 Veterinary pharmaceutical residues occurrence in water resources

In 32% of water resources samples at least one VPR was quantified (i.e. 34 out of the 105 samples analyzed), comprising 2 groundwaters and 32 surface waters. Quantified VPRs and their concentration levels are shown in Figure 1.

[Figure 1]

Seventeen out of 38 VPRs were quantified. Nine antibiotics were quantified (florfenicol, flumequine, lincomycin, neospiramycin, oxytetracycline, sulfadiazine, sulfamethazine, tilmicosin, trimethoprim), six antiparasitic drugs (eprinomectin, ivermectin, levamisole, triclabendazole, triclabendazole sulfone, triclabendazole sulfoxide) and two anti-inflammatory drugs (flunixin, ketoprofen). Among these compounds, eight are mixed-use compounds (flumequine, ketoprofen, levamisole, lincomycin, neospiramycin, oxytetracycline, sulfadiazine, trimethoprim) and nine are only used in veterinary medicine (eprinomectin, florfenicol, flunixin, ivermectin, sulfamethazine, tilmicosin, triclabendazole, triclabendazole sulfone, triclabendazole sulfoxide). Some of the VPRs were quantified in water resources at concentration levels with significant differences in their recoveries, and high expanded relative uncertainties, such as flumequine lincomycin, neospiramycin, tilmicosin, eprinomectin and ivermectin (see Table S5). On the other hand, the identification of

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compounds is ensured by the method's quality controls. In end, considering that this work is a first exploratory study in the region of Brittany, the data for these compounds was retained, however the quantitative results should be considered with caution, especially when close to the limit of quantification.

21 VPRs were never quantified in water resources during this study (13 antibiotics: amoxicillin, ampicillin, cefquinome, chlortetracycline, doxycycline, enrofloxacin, erythromycin, marbofloxacin, oxalinic acid, sulfadimethoxine, spiramycin, tylosin; four antiparasitic drugs: clorsulon, dicyclanil, diazinon, flubendazole; the anticoccidian toltrazuril and its metabolites toltrazuril sulfone and toltrazuril sulfoxide; two anti-inflammatory drugs: dexamethasone, meloxicam). Among these 21 compounds not detected, some may have been degraded by freezing. As samples were frozen before analysis in sampling campaign (SC)1 and SC2, the conservation of VPRs during freezing was tested on two water samples (one surface water sample and one tap water sample) spiked with all compounds. At -20 °C, penicillins (amoxicillin and ampicillin) were completely degraded in less than two weeks in both matrices (data not shown). Tetracyclines (chlortetracycline, doxycycline and oxytetracycline) were completely degraded in tap water matrix within 48 hours (data not shown). According to Mompelat et al. (Mompelat et al., 2013) florfenicol, sulfadiazine and trimethoprim are stable in surface water samples when stored at – 20°C during 56 days, while oxolinic acid is stable during 124 days. Another publication (Fedorova et al., 2014) studied the impact of storage of effluents of wastewater treatment plants at – 18°C. Florfenicol and sulfadiazine remained stable after 120 days of storage, which is in agreement with Mompelat et al. (Mompelat et al., 2013). On the other hand, oxolinic acid was stable until 60 days of storage only, and so were enrofloxacin, erythromycin, flumequine, oxytetracycline, sulfadimethoxine, sulfamethazine and trimethoprim. Doxycycline and ketoprofen remained stable for 7 days. Finally, Llorca et al. (Llorca et al., 2014) studied the impact of storage of

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purified water samples spiked with pharmaceuticals at -20°C. Erythromycin, tilmicosin, spiramycin, tylosin, chlortetracycline, oxytetracycline, doxycycline, flumequine, ampicillin, lincomycin, sulfadiazine were unstable after one week of storage. Enrofloxacin, marbofloxacin and oxolinic acid remained stable after 1 week of storage but were unstable (concentration levels <80%) after 2 weeks of storage. Sulfadimethoxine remained stable after 2 weeks of storage but were unstable (concentration levels <80%) after 12 weeks of storage. Surprisingly, amoxicillin remained stable after 12 weeks of storage while β-lactams are known to be highly unstable in aquatic environment, and rapidly subjected to hydrolysis (Braschi et al., 2013; Mitchell et al., 2014). Supplementary experiments are required to validate the conservation of compounds during freezing. Overall, concentration levels may have been underestimated.

In addition, VPRs consumption and VPRs behaviors in the environment have to be considered to interpret the results. The antiparasitic drugs dicyclanil and flubendazole were reported to be the least prescribed of the VPRs studied in Brittany, which may explain why they were not detected. Flubendazole is also reported to be subjected to hydrolysis (Horvat et al., 2012). Other VPRs, such as the β-lactams amoxicillin, ampicillin and cefquinome may have been hydrolyzed in the aquatic (Braschi et al., 2013; Jiang et al., 2010; Li et al., 2011; Mitchell et al., 2014). Besides, this study focuses on water phase, while VPRs are found in different all fractions of the aquatic environment (i.e. soluble fraction, colloidal fraction, particular fraction or sediment) (Cheng et al., 2014; Li et al., 2016; Zhou et al., 2016). Some antibiotics such as fluoroquinolones, macrolides and tetracyclines and also antiparasitic drugs as avermectins are known to have a strong binding capacity to sediment (Dong et al., 2016; Li et al., 2017; Liebig et al., 2010; Luo et al., 2011; Yang et al., 2010; Zhou et al., 2011). Thus, VPRs belonging to these chemical families may occur in suspended particulate matter or sediment. Sediment constitutes an important sink but also a potential secondary source for

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VPRs, which can be released in water through sorption-desorption and re-suspension processes (Cheng et al., 2014; Yang et al., 2010; Zhou et al., 2011, 2016). Other VPRs such as sulfonamides, trimethoprim, lincomycin and florfenicol, which were quantified during this study, tend to be distributed only in the aqueous phase phase (Li et al., 2016; Zhou et al., 2011).

Individual detection frequencies of VPRs in water resources were below 10% for all compounds (Figure 1). 82% of the quantified compounds had concentrations below 80 ng/L, 16% of VPR concentrations were between 100 ng/L and 1 µg/L and only the antibiotic sulfadiazine was quantified at a concentration above 1 µg/L. Among the five metabolites investigated, three were detected (neospiramycin, triclabendazole sulfone, triclabendazole sulfoxide). Among the 17 VPRs quantified in water resources in this study, 11 have been previously found in natural waters at similar concentrations (florfenicol, flumequine, flunixin, levamisole, ivermectin, oxytetracycline, sulfadiazine, sulfamethazine, tilmicosin, trimethoprim, triclabendazole) (Charuaud et al., 2019; Sinclair et al., 2007). For example, sulfadiazine (from 508 ng/L to 2946 ng/L in this study) was quantified in surface water up to 2313 ng/L in the Llobregat River in Spain (Iglesias et al., 2014) and florfenicol (from 7 ng/L to 930 ng/L in this study) was quantified in South Korea up to 340 ng/L (Kim et al., 2016) and in China up to 930 ng/L (Zhou et al., 2016). On the other hand, sulfadiazine and trimethoprim were quantified at lower concentrations in the other study performed in Brittany (Jaffrézic et al., 2017), with sulfadiazine ranging from 15 ng/L to 35 ng/L and trimethoprim ranging from 3 ng/L to 23 ng/L. However, flunixin (from 35 to 1450 ng/L), flumequine (from 1 ng/L to 143 ng/L) and lincomycin (from 6 ng/L to 163 ng/L) concentrations were higher in Jaffrézic et al. (Jaffrézic et al., 2017).

To our knowledge, this study is the first to provide data on the occurrence in natural waters of the antiparasitic drug eprinomectin (7 ng/L to 45 ng/L) and of the following three metabolites:

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neospiramycin (24 ng/L), metabolite of the antibiotic spiramycin, triclabendazole sulfone (9 ng/L) and triclabendazole sulfoxide (6 ng/L to 8 ng/L) which are metabolites of the antiparasitic drug triclabendazole.

Table S8 in Supplementary Information provides the VPRs quantified per site, as well as the corresponding concentration ranges. No VPRs were quantified in seven of the 25 sites studied. The largest number of VPRs quantified on a single site was nine (site L). One or two VPRs were quantified in 11 sites, but VPRs exclusively used in veterinary medicine were quantified in only three sites. These three sites (B, I and U) have very different characteristics: two are surface waters (one river and one dam) and the other is a 20 meter depth groundwater. The main livestock is poultry on two sites (B and I) and cattle on the other site (U). Tilmicosin and flunixin were quantified on site U, which is consistent with the main livestock as these compounds can be administered to cattle. Eprinomectin, ivermectin and triclabendazole sulfoxide were quantified on site I, although these VPRs are not administered to poultry. Nevertheless, swine and cattle can be found in the watershed even if poultry is the major livestock. Sites U and B showed the presence of VPRs in only one sampling campaign, respectively SC2 (spreading time) and SC3 (low-water period). On site I, VPRs were quantified during both SC2 and SC3.

3.2. Associated fecal contamination in water resources

3.2.1. Fecal contamination in water resources

Among the 34 natural water samples with quantified VPRs, 88% presented associated fecal contamination, consistent with *E. coli*.

The origin of the fecal contamination identified with fecal stanols was combined with the source of fecal contamination obtained from pharmaceutical residues to obtain the overall sources of fecal contamination of the sample. Among the 34 samples of water resources with

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quantified VPRs, 62% of fecal contaminations had a mixed origin, i.e. both human and animal; 35% were of animal origin and 3% were of human origin only. Thus, 97% of the samples showed fecal contamination totally or partly attributed to an animal source.

3.2.2. Determination of origin of mixed-use compounds in water resources

Mixed-use pharmaceuticals were quantified in twelve samples of water resources (35% of the samples with VPRs quantified). The sources of fecal contamination of these 12 samples are described in Table 2.

[Table 2]

Nine samples had a mixed overall fecal contamination, thus the source of contamination for the mixed-use VPR in the samples could not be differentiated either with fecal stanols or pharmaceuticals. The antiparasitic drug levamisole was once linked to a human origin due to the presence of carbamazepine in the sample. The VPR contamination of two samples was attributed to an animal origin and more specifically to cattle. Oxytetracycline and trimethoprim were quantified in sample A. Sulfadiazine, trimethoprim and neospiramycin (spiramycin metabolite) were quantified in sample G. The bovine origin of the contamination is plausible as all these compounds are administered to cattle. Primary livestock is aquaculture on site G and both aquaculture and poultry on site A. Sulfadiazine, oxytetracycline and trimethoprim are compounds that are administered in aquaculture. Spiramycin (the parent compound of neospiramycin) is administered to swine, cattle and poultry. In addition to aquaculture activities, there are also cattle, pig and poultry farms near to site G, thus the quantification of neospiramycin is consistent with the livestock types.

The results obtained with the two kinds of marker in this study (i.e. stanols and pharmaceuticals) were classified into the following groups according to Jardé et al. (Jardé et al., 2018):

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Group 1: both markers had the same source of fecal contamination (either a single source or a combination of sources)

Group 2: one marker assigned a source of fecal contamination (either a single source or a combination of sources) while the second marker did not provide a source of fecal contamination

Group 3: the two markers gave different assignments (no common source attributed between stanols and pharmaceuticals)

Group 4: none of the markers was detected or quantified.

Among the 12 samples of water resources in which mixed-used VPRs were quantified, half were attributed to group 1 (n=6) and the other half to group 2 (n=6). No samples with quantified VPRs were attributed to group 3 or group 4, with the result that 100% of the fecal contamination of the samples was assumed to be properly assigned.

Within group 1, three scenarios were observed. In the first, pharmaceuticals showed a mixed source of fecal contamination of the samples and stanols showed an animal source of fecal contamination. This was observed in three samples. In the second scenario, both markers showed a mixed fecal contamination of the sample (n=2). In the last scenario, stanols showed a mixed source of contamination and pharmaceuticals an animal source of fecal contamination (n=1). In these six samples, *E. coli* concentrations were between 77/100 mL and 490/100 mL.

Within group 2, the absence of source was mostly due to pharmaceuticals (four samples out of six). Samples from site A (SC1), G (SC3) and Z (SC1) were surface water. The quantified *E. coli* concentrations were respectively 260/100 mL, 210/100 mL and 30/100 mL. Sample from site O (SC5) was a groundwater and *E. coli* was quantified at 20 CFU/100 mL. Pharmaceutical markers were composed of two compounds exclusively used in human medicine (carbamazepine, diclofenac) and of 14 compounds exclusively used in veterinary

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medicine in France. However, other pharmaceutical residues not analyzed in this study may have been present in these samples. In the two other samples (R during SC1 and Y during SC2), fecal stanols did not provide a source of fecal contamination. In these samples the associated *E. coli* concentrations were 15/100 mL (R in SC1) and 292 /100 mL (Y in SC2). The low concentration level of *E. coli* in sample R could be linked to an old fecal contamination, explaining the difficulty in identifying the origin of fecal contamination. In addition, site Y has poultry and swine as primary livestock types and the fecal stanol fingerprinting used in this study cannot identify fecal contamination from poultry.

3.3. Seasonal variations and factors influencing occurrence in water resources

Table 3 summarizes the results of VPR occurrence during the five sampling campaigns. It also summarizes the minimum and maximum DOC concentrations, *E. coli* concentrations, and daily flow rates.

[Table 3]

The two campaigns performed during spreading periods are those with both the highest (SC2; 53%) and lowest (SC5; 19%) percentage of samples with at least one quantified VPR. The highest diversity of compounds was observed during SC3, the low-water period campaign with ten VPRs quantified. On the other hand, the lowest diversity of VPRs was observed during SC5, with three VPRs quantified. VPRs were quantified in site Y in all sampling campaigns (four sampling campaigns were performed on this site). VPRs were quantified in site L in four out of five sampling campaigns and in site G in three out of the five sampling campaigns. VPRs occurred in eight sites in two out of the five sampling campaigns and in six sites in one of the five sampling campaigns.

Figure 2 presents the results of fecal contamination parameters (*E. coli*, enterococci) as well as DOC, daily flow rate and rainfall 72 hours before sampling over the five sampling

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campaigns, on all sites. The daily flow rate was unavailable for some dams and for the two groundwaters.

[Figure 2]

With regard to fecal contamination, the campaigns conducted during spreading periods (SC2 and SC5) have higher mean values of *E. coli* concentrations. However, the samples in which VPRs were quantified are not necessarily samples with the highest *E. coli* concentrations. As the contribution of fecal contamination in the water resources is expressed with *E. coli* concentrations, it can be concluded that, in this study, there is no evident relationship between recent fecal contamination and the occurrence of VPRs. The amounts of DOC are relatively similar between the five sampling campaigns. Mean values are between 4.7 mg/L and 5.8 mg/L. The samples in which VPRs were identified are distributed relatively homogeneously with respect to the average DOC values. A trend only emerges in SC2, with a majority of positive samples having lower DOC amounts below the average DOC value. This may be explained by a transfer of VPRs from soil to water during the first post-spreading rainfall events before the increase in organic matter concentrations in water resources. Daily flow rates of water resources showed variations between the sampling campaigns. SC4 has the highest daily flow rates and SC3 has the lowest. This is in accordance with the hydrological periods of sampling, i.e. high water for SC4 and low water for SC3. The differences in VPR concentration levels between sampling campaigns shown in Table 3 may be explained in part by daily flow rates. High daily flow rates in SC4 correspond to a high water regime that may have caused a dilution phenomenon and the low daily flow rate in SC3 corresponds to low water that may have led to a concentration phenomenon.

Finally, rainfall events in the 72 hours before sampling were observed as these events can indicate an easier soil-to-water transfer by runoff. Overall, higher rainfall was observed for samples collected during the spreading periods (SC2 and SC5) with mean rainfall values two

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or three times superior to mean rainfalls during SC1, SC3 and SC4. These two campaigns (i.e. SC2 and SC5) seem to have been conducted under suitable conditions for transfer processes. However, unlike SC2, SC5 exhibited a low occurrence of VPRs. This difference can be explained in part by daily flow rates, as the mean daily flow rate in SC5 was 3.5 times higher than that during SC2, resulting in an increased dilution phenomenon in SC5. When considering the samples in which VPRs were quantified, most of them were collected after rainfall events superior to the mean rainfall values during both SC2 and SC3. Although SC3 was carried out at a different time from spreading, heavy rains can remobilize VPRs previously stored in the soils or in the sediments. In the context of this large-scale study with catchments of various characteristics and because of the low-frequency sampling strategy, it appears difficult to determine precisely the factors impacting the occurrence of VPRs.

These conclusions must be considered with caution as the sampling strategy consists in grab sampling. Indeed, concentrations in the water phase fluctuate over time with varying amplitudes and frequencies. A grab sampling strategy does not take these fluctuations into account and it is therefore possible to miss a concentration peak or, on the contrary, to overestimate the levels of concentrations over a period of time. Nevertheless, for this first exploratory study, point sampling was chosen for logistical considerations and to allow sampling of a larger number of sites. Next steps in the study should include other sampling strategies such as average sampling (i.e. with an accumulation of successive samples at defined frequencies over a defined period of time in order to obtain a composite sample representative of this period) or passive sampling which allow in situ and integrative sampling of contaminants.

3.4. Veterinary pharmaceutical residues occurrence in tap water

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At least one VPR was quantified in 20% of tap water samples (i.e. 19 samples). On all data expected, 6.4% of the data were invalidated for non-compliance with the quality assurance criteria. Figure 1 shows the quantified VPRs and their concentration levels in tap waters.

Twelve VPRs were quantified in tap waters, including ten compounds exclusively used in veterinary medicine and two mixed-use compounds: the antibiotic sulfadiazine and the anti-inflammatory drug ketoprofen. Within the ten veterinary specific compounds, seven are antiparasitic drugs (eprinomectin, ivermectin, toltrazuril, toltrazuril sulfone, triclabendazole, triclabendazole sulfone and triclabendazole sulfoxide) including three metabolites (toltrazuril sulfone, triclabendazole sulfone and triclabendazole sulfoxide), and two are antibiotics (florfenicol and tylosin). All detection frequencies are below 10%, ranging from 1% (toltrazuril sulfone, tylosin, ketoprofen) to 8% for triclabendazole sulfoxide. Concentration levels are inferior to 40 ng/L for all compounds, with the exception of florfenicol which was quantified at 159 ng/L and 211 ng/L.

To our knowledge, only four studies have reported VPR occurrence in tap water as mentioned in Charuaud et al. (2019). Florfenicol was detected (<50 ng/L) in a French national study (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, 2011), while it is reported in this study at significant concentrations (from 18 to 211 ng/L). These concentrations demonstrate that florfenicol is probably not completely removed in the treatment processes used in the studied DWTPs, whereas a previous study on a full-scale DWTP showed a removal rate of more than 90% after the chlorination and filtration steps, and in the end an absence of the molecule in the water of the distribution system (Azzouz and Ballesteros, 2013). This difference is unexpected as the tap waters with florfenicol at concentrations superior to 100 ng/L come from two DWTPs (G and J) both equipped with advanced treatments while the DWTP studied by Azzouz and Ballesteros (2013) was composed of a pre-oxidation followed by classical clarification and disinfection steps. DWTP

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G was composed of a clarification process combined with powder activated carbon, ultrafiltration and disinfection. DWTP J was composed of a pre-ozonation step followed by clarification with granular activated carbon filtration, post-ozonation and disinfection. Thus, advanced processes such as ozonation, activated carbon and ultrafiltration are not always effective in achieving complete removal of florfenicol. The antibiotic tylosin was quantified in three studies with concentrations ranging between 4 and 20 ng/L (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail, 2011; Leung et al., 2013; Ye et al., 2007) and the anti-inflammatory drug ketoprofen was quantified at 3 ng/L in another French study (Togola and Budzinski, 2008). The concentration levels found in this study were similar to those in the literature. The antiparasitic drugs (eprinomectin, ivermectin, toltrazuril, toltrazuril sulfone, triclabendazole, triclabendazole sulfone and triclabendazole sulfoxide) quantified in this study have never been reported before in the literature.

Among the 23 DWTPs studied, nine (i.e. 39%) showed at least one occurrence of VPR in tap water. VPRs were quantified in the tap samples originating from DWTP L in three sampling campaigns out of five (SC1, SC2 and SC4), with a total of seven VPRs quantified (eprinomectin, ketoprofen, toltrazuril, triclabendazole, triclabendazole sulfone and triclabendazole sulfoxide twice). DWTP L was composed of a clarification process (coagulation flocculation and filtration) with occasional use of powder activated carbon if needed, followed by ozonation and disinfection by chloration. The higher occurrence in the DWTP L may be related to the treatment processes, as activated carbon is not used constantly but only as a crisis reagent to deal with specific events of contamination. Besides, this occurrence may also be due to the location of the DWTP. DWTP L is located next to a pig farm, as well as cattle in pastures. In addition, the watercourse concerned is subjected to fish farming activities. Site L was also the site with the higher occurrence of VPRs in the resources.

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For SC1, SC3, SC4 and SC5, VPRs were quantified in less than 20% of tap water samples. However, in SC2, VPRs occurred in 65% of tap water samples. Fewer VPRs were quantified in tap water than in water resources and at lower concentrations thanks to the treatments applied in the DWTPs.

Antibiotics had lower detection frequencies in tap water than in water resources. The processes applied in DWTPs allow a partial removal of VPRs, as has already been observed in the literature (Boleda et al., 2011; Liu et al., 2016; Stackelberg et al., 2007). However, the opposite phenomenon was observed for the antiparasitic drugs toltrazuril and triclabendazole and also for their metabolites. These metabolites may have been generated during the treatment processes from the parent compound. For example, triclabendazole sulfoxide and triclabendazole sulfone result from oxidative reactions occurring in the animal (Moreno et al., 2014). Oxidation processes are also used in water treatment for example with ozonation, and especially during pre-oxidation (with higher concentration of ozone and longer time contact). Thus, triclabendazole sulfoxide and sulfone metabolite may have been produced during the ozonation step. As this is the first data on antiparasitic drugs in tap water, further investigation in the future is required in order to understand this phenomenon.

CONCLUSION

On the basis of a prioritization work (Charuaud et al., 2016), a specific method of analysis was developed to analyze the selected VPRs in water. Five sampling campaigns were conducted between 2017 and 2018. It should be noted that 2017 and 2018 were drier-than-average years in Brittany. According to the results of this study, Brittany's water resources are subject to contamination by residues of veterinary drugs (31% of the water resources samples contained at least one VPR). In addition, VPRs were quantified in 20% of the tap water samples collected from DWTPs. The population of Brittany may therefore be exposed to

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these contaminants through tap water. These observations should be compared with the detection frequencies per compound which are all below 10% in both water resources and tap water.

Nevertheless, some of the results deserve to be considered for future work. One example is the exclusively veterinary antibiotic florfenicol, which was found in tap water at concentrations above 100 ng/L and shows certain persistence against the advanced treatment processes applied in drinking water treatment plants. Another example is antiparasitic drugs, which were also found despite the literature containing no data on their occurrence in tap water. Although the concentrations are lower than that of florfenicol, more interest should be given to this subject in the future.

The combined use of fecal stanols and specific human or specific veterinary pharmaceutical residues established a proper fecal contamination source for 70% of the water resource samples. However, a majority of the samples had a mixed overall fecal contamination, thus the individual source of contamination for the mixed-use VPR(s) in the samples could not be identified with the Toolbox.

Finally, this is a first study conducted at the regional scale of Brittany and understanding the factors influencing water resource contamination by VPRs requires further investigation. Future studies should work on a more restricted scale with a high-frequency sampling strategy to take better account of the specificities of the site(s). Indeed, the catchments present great disparities (resources on rivers, on reservoirs with medium or long storage times, etc.) that require different approaches.

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Figure 1: Concentration levels of VPRs quantified in water resources and tap water (all sampling campaigns considered)

fWR: detection frequency in water resources

fTW: detection frequency in tap water

Figure 2: Results of *E.coli*, DOC, daily flow rate and rainfall during 72 hours before sampling over the five sampling campaigns (SC), all sites combined. Values below LQ were considered as zero. These zero values are not reported on the graphics expressed in logarithm.

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Table 1: Sampling periods and characteristics of the sampling campaigns

Sampling campaign	**Sampling Period**	**Characteristics**
1	march 2nd -april 6th 2017	Before animal manure spreading times
2	may 2nd – june 13th 2017	During manure and slurry spreading times
3	july 10th – october 12th 2017	Low-water period
4	january 4th – february 10th 2018	High-water period
5	may 2nd – june 13th 2018	During manure and slurry spreading times

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Table 2: Sources of fecal contamination in the samples with mixed-use compounds.

Site	Mixed-use VPR	Source of fecal contamination determined by stanols	Source of fecal contamination determined by pharmaceuticals	Source of global faecal contamination
A (SC3)	Oxytetracycline Trimethoprim	Cattle	nd [b]	Animal (bovine)
G (SC3)	Neospiramycin Sulfadiazine, Trimethoprim	Cattle	nd	Animal (bovine)
J (SC3)	Sulfadiazine, Trimethoprim	Cattle (61%)/ Swine (35%)	Mixed	Mixed
L (SC3)	Oxytetracycline, Trimethoprim	Swine (65%)/ Cattle (20%)	Mixed	Mixed
O (SC5)	Ketoprofen	Cattle (44%)/Human (30%)	nd	Mixed
Q (SC1)	Levamisole	Swine (46%) / Human (42%) / Cattle (12%)	Mixed	Mixed
R (SC1)	Levamisole	- [a]	Human	Human
R (SC4)	Lincomycin	Cattle (33%) /Swine (20%) /Human (47%)	Human	Mixed
Y (SC1)	Flumequine	Swine (56%)/ Cattle (29%)/ Human (15%)	Mixed	Mixed
Y (SC2)	Flumequine	-	Mixed	Mixed
Y (SC3)	Flumequine	Cattle	Mixed	Mixed
Z (SC1)	Lincomycin	Swine (54%) / Cattle (31%) / Human (15%)	nd	Mixed

[a] The source of fecal contamination could not be attributed by the distribution of the stanols

[b] Not detected

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Table 3: Summary of sampling campaign results

Sampling campaign	**Percentages of samples with quantified VPRs**	**Number of different VPRs quantified in water resources (/38)**	**Concentration minimum (ng.L^{-1}) (VPR)**	**Concentration maximum (ng.L^{-1}) (VPR)**	**DOC min –max (mg/L) (surface waters)**	**E.coli min – max (/100mL) (surface waters)**	**Daily flow rate min-max (m^3/s) (surface waters)**
1	32% (frozen samples)	6	6 (LIN)	35 (SMZ)	2.4 – 9.1	15 - 457	0.25 – 7.78
2	53% (frozen samples)	9	5 (FLX; TRI)	436 (FF)	3.0 – 10.7	15 - 5712	0.07 – 6.18
3	36%	10	5 (TIL)	2946 (SDZ)	2.6 – 10.0	15 - 800	0.003 – 2.15
4	21%	5	5 (LIN)	16 (EPR)	2.9 – 9.5	38 - 2500	0.12 – 42.90
5	19%	3	13 (IVER)	287 (FF)	2.8 – 9.9	38 - 21000	0.30 – 19.4

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Conflict of interest

All authors confirm that they have no conflicts of interest

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HIGHLIGHTS

- High husbandry pressure impacts quality of natural waters and tap waters
- No clear impact of the seasonal variations on the occurrence of pharmaceuticals
- Concentrations in water resources were up to 400 times higher than in tap waters
- Specific attention should be given to the antibiotic florfenicol in tap water
- First data on antiparasitic drugs in tap water

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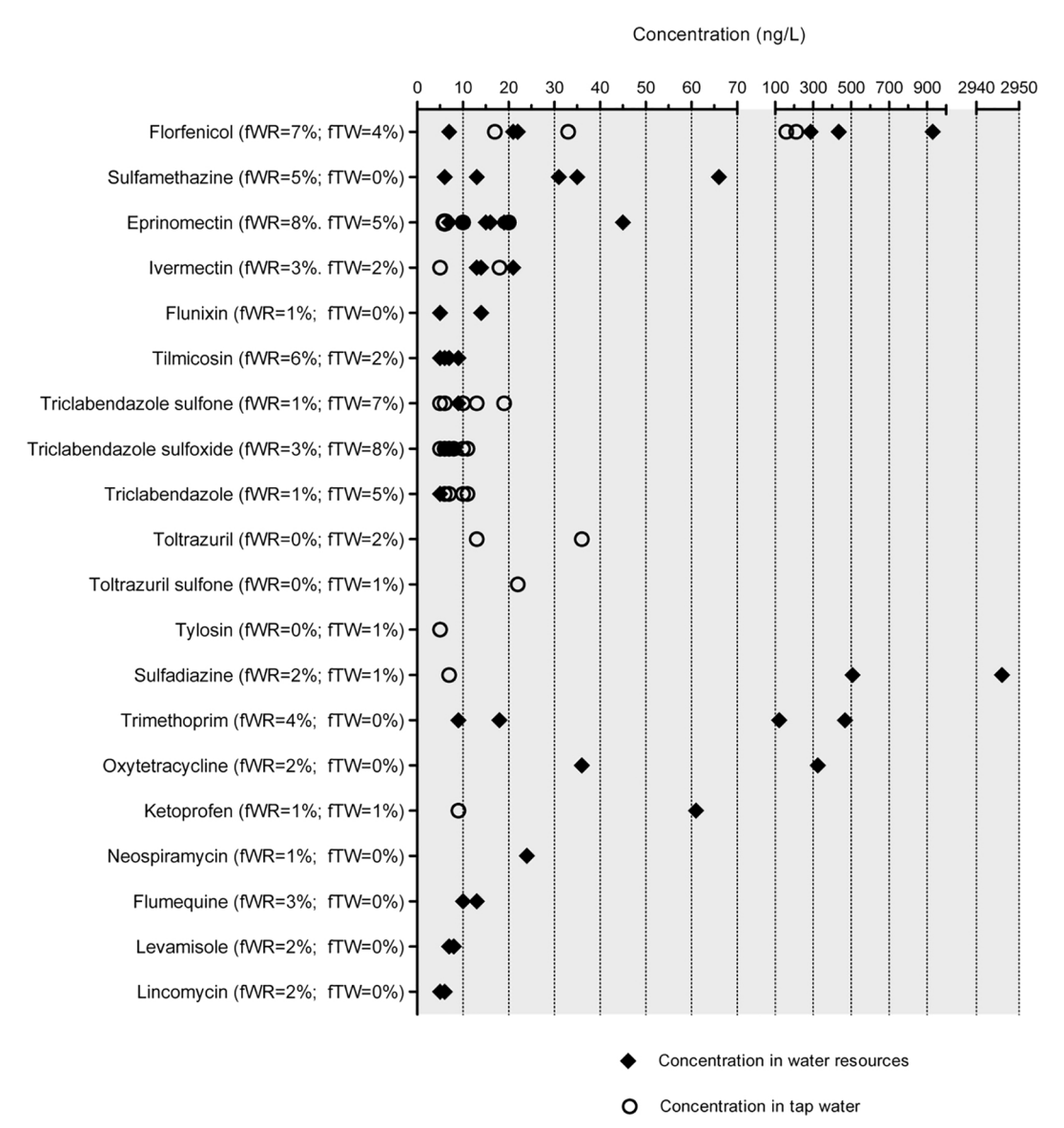
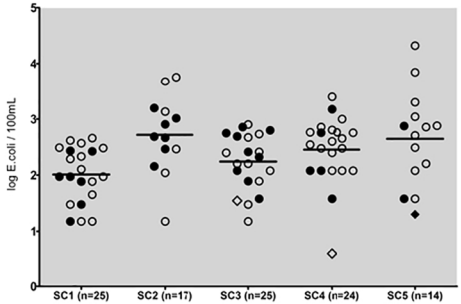
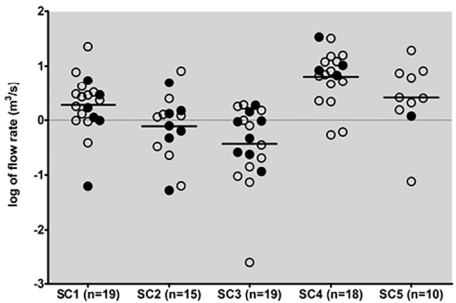

Concentration (ng/L)
0
10
20
30
40
50
60
70
100
300
500
700
900
2940
2950
Florfenicol (fWR=7%; fTW=4%)
Sulfamethazine (fWR=5%; fTW=0%)
Eprinomectin (fWR=8%. fTW=5%)
Ivermectin (fWR=3%. fTW=2%)
Flunixin (fWR=1%; fTW=0%)
Tilmicosin (fWR=6%; fTW=2%)
Triclabendazole sulfone (fWR=1%; fTW=7%)
Triclabendazole sulfoxide (fWR=3%; fTW=8%)
Triclabendazole (fWR=1%; fTW=5%)
Toltrazuril (fWR=0%; fTW=2%)
Toltrazuril sulfone (fWR=0%; fTW=1%)
Tylosin (fWR=0%; fTW=1%)
Sulfadiazine (fWR=2%; fTW=1%)
Trimethoprim (fWR=4%; fTW=0%)
Oxytetracycline (fWR=2%; fTW=0%)
Ketoprofen (fWR=1%; fTW=1%)
Neospiramycin (fWR=1%; fTW=0%)
Flumequine (fWR=3%; fTW=0%)
Levamisole (fWR=2%; fTW=0%)
Lincomycin (fWR=2%; fTW=0%)
Concentration in water resources
Concentration in tap water

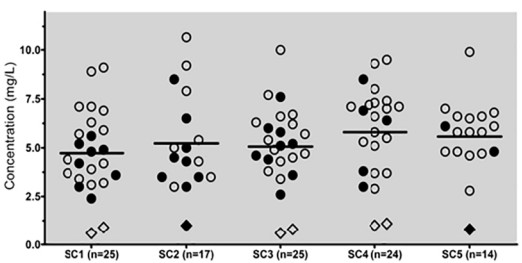
Figure 1



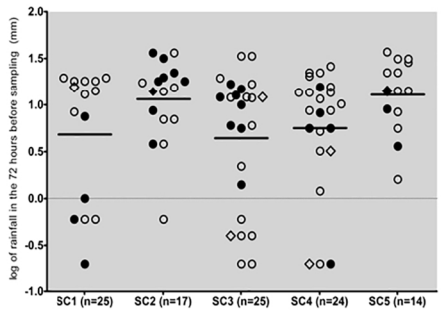
E.coli



Flow rate



DOC



Rainfall during the 72 hours before sampling

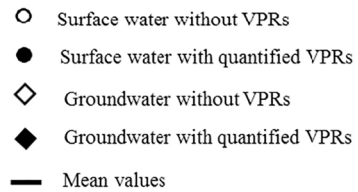


Figure 2