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RESEARCH ARTICLE

Presence of veterinary antibiotics in livestock manure in two Southeastern Europe countries, Albania and Kosovo

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Abstract

Nowadays, veterinary drug application has become an integral practice in livestock farming. Veterinary antibiotics (VAs) are administered onto animals for therapeutic use; meanwhile, in some countries, they are used for growth promotion. To indicate the level of VAs use in livestock breeding in two countries, Albania and Kosovo, their presence was studied in the animal manure. In total, 38 manure samples, 22 from Kosovo and 16 samples from Albania, belonging to cattle, pig, and poultry, were collected and investigated for the presence of VAs. Seven VAs and 2 metabolites, from the groups of sulfonamides and tetracyclines, were identified by ultra-high pressure liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS). The detected antibiotics were sulfadiazine (SDZ), sulfathiazole (STZ), sulfamethazine (SMZ), oxytetracycline (OTC), tetracycline (TC), chlortetracycline (TC), and doxycycline (DOY). VAs were detected in 27% and 31.2% of the manure samples, from Kosovo and Albania, respectively, and the levels ranged from 0.04 to 10.1 mg kg^{-1}. VAs were widely detected (100%) in poultry manure from Kosovo, as well as poultry manure from Albania. The contamination rate ranged from pig manure (25%) to cow manure (66.6%). Sulfonamides were the most commonly detected VAs with maximum concentration of sulfadiazine (10.1 mg kg^{-1}) in poultry manure. Tetracyclines were most widely detected in poultry manure, as well as other animal manures. When it comes to the comparison between the two countries, VAs residues are more frequent per analyzed sample and higher in concentrations in the manure samples from Albania. Therefore, an environmental impact of VAs on both countries may be expected. These results indicate that VAs may enter the local ecosystem through manure application to agriculture and potentially may bring ecological risks.

Keywords Veterinary antibiotics · Tetracyclines · Sulfonamides · Livestock manure · Albania · Kosovo

Introduction

Application of veterinary pharmaceuticals has become an integral part of animal husbandry, with antibiotics the most widely spread class (Sarmah et al. 2006; Baynes et al. 2016). Veterinary antibiotics (VAs) have been applied for therapeutic use, as well as growth promoters in food animals (Chee-Sanford et al. 2009; Marshall and Levy 2011). In some parts of the world, they are used at sub-therapeutic levels, as additives to animal feed as growth promoters. In contrast, the use of antibiotics as growth promoters is banned in European Union (European Parliament and the Council 2003); meanwhile in the USA, since January 2017, the veterinary feed directive (VFD) has been expanded to antibiotics used in feed for prevention control (Baynes et al. 2016). As a result of a poor metabolism in the animal gut, their pharmacokinetics, and specific physical processes, the applied VAs and their metabolites reach the soil and water compartments via manure application (Boxall et al. 2004; Spielmeyer et al. 2015; Wohde et al. 2016; Hamscher and Bachour 2018). Significant amounts of VAs, their metabolites, or degradation products reach agroecosystems through their fertilization with antibiotic-contaminated manures, or irrigation with contaminated surface waters, and lesser proportion through their aerial transport (Wohde et al. 2016; Kim et al. 2011; Liu 2012), where they can persist for many years or even decades

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(Baguer et al. 2000; Spielmeyer 2018). Extensive use of VAs on intensive livestock farms has imposed large amounts to reach agricultural fields through manure fertilization practices (Jechalke et al. 2014).

More than 2000 manufactured veterinary pharmaceuticals, with approximately 400 active chemical ingredients, have been in use for over 60 years (Tasho and Cho 2016). The annual global application of antimicrobials in animal food production was estimated at 63,000 t, only during 2010. Published data project an impressive rise, by 67% or up to 106,000 t until 2030. This projection mainly will be driven by the growth in consumers' demand for animal proteins in middle-income countries, as well as the projected population growth in five major emerging economies (Van Boeckel et al. 2015). According to the European Surveillance Agency on Veterinary Antimicrobial Consumption (ESVAC), the overall antibiotics' sale in 30 European countries has reached 7860 t for food-producing animals, where tetracyclines, penicillins, and sulfonamides, together, account for 70% of the total VAs (ESVAC 2018). One study conducted in 25 European states, referring to the total sales of veterinary antimicrobial agents during 2011, accounted for tetracyclines and sulfonamides, 37% and 11%, respectively (Grave et al. 2014). The analysis of veterinary drug residues is not regulated in animal manure within the European Union while regulated in products of animal origin under Commission Decision 2002/657/EC (EU 2002). In the USA, there are approximately 122 tetracycline approvals, which include various salts of tetracycline, oxytetracycline, and chlortetracycline, with about 30 of these products approved for therapeutic use. The sulfonamides, one of the oldest groups of antimicrobials, have been used in food animal production for over 60 years (Baynes et al. 2016). The global average annual consumption of antibiotics per kilogram of animal produced was estimated to be 45 mg kg^{-1}, 148 mg kg^{-1}, and 172 mg kg^{-1} for cattle, chicken, and pigs, respectively (Van Boeckel et al. 2015). Continuous use of a wide variety of VAs has led to concern on the potential for the development of antibiotic-resistant bacteria (Kim et al. 2011). It is recognized as a global health problem by major health organizations and identified as one of the top health challenges in human and veterinary medicine in the twenty-first century (Marshall and Levy 2011).

The VAs metabolites formed inside the animal during their metabolism are different from the transformation products developed, from excreted parent compounds and metabolites, in the environment (Wohde et al. 2016). Transformation rates of antibiotics are affected by different environmental conditions and medium matrix. A wide range of VAs half-lives are reported, varying from 3 days for chlortetracycline in composted manure in 53 to > 77 days for doxycycline in biosolids storage (Massé et al. 2014). Generally, it is accepted for faster degradation rates of VAs and their metabolites under aerobic compared with anaerobic conditions (Wohde et al. 2016). Plants cultivated on soils fertilized with animal manure might potentially take up antibiotics from the ground and translocate them in the plant or fruit (Kumar et al. 2005). Significant fractions of most VAs that enter the soil compartment via manure usually are retained in the surface (Kuppusamy et al. 2018), while a portion may leach into groundwater and surface water bodies by infiltration and runoff of agricultural fields (Kim et al. 2011; Pan and Cu 2017; Kivits et al. 2018).

There is a growing public health concern related to the increase in antimicrobial resistance. An essential contribution to this increase originates from antibiotics applied to husbandry animals. The animal manure, contaminated with VAs, applied to the agroecosystems may affect the soil microbial community by creating an ideal selective pressure to the bacterial strains carrying antibiotic-resistant genes (Gullberg et al. 2011). It is not the only way of spreading the bacteria carrying resistance genes. It occurs through direct or indirect contact, through the food, water, or animal excrements to the food (European Commission 2005; Marshall and Levy 2011; WHO (World Health Organization) 2014; Van Boeckel et al. 2015). Because of antimicrobial resistance concerns, FDA has also discouraged the use of antibiotics as growth promoters in livestock (Baynes et al. 2016).

Livestock breeding represents an integral part of the agriculture economy in both countries, with notable differences in livestock production. Albania features small cattle and pig farms, whereas intensive livestock farming plays the most crucial role in poultry production. Annual statistical reports covering the period from 2012 to 2017 indicate a decrease of 9.6% for the livestock's total number, including cattle (4.6%) and poultry (13.2%), respectively. In contrast, a reverse trend was evidenced in pigs and sheep/goat livestock, resulting in increased numbers by 13.2% and 9.2%, respectively (Supplementary Material, Table 1) (MARD 2018). Current data on livestock economy in Kosovo show that cattle and poultry are the main breeding species. Statistics for the period 2012–2017 indicate decreasing trends on the pig (21.9%), cattle (19.5%), and sheep and goat (11.5%) production, while a notable increase is considered in poultry economy (29.8%) leading to an overall rise of the country' livestock with 19.9% (Supplementary Material, Table 2) (FVA (Food and Veterinary Agency) 2019).

This study aims to describe the current situation regarding VAs residues in animal manure obtained from livestock breeding in two Southeastern European countries, Albania and Kosovo. Considered as developing countries, they are facing significant changes in their agricultural production, with livestock breeding as a vital economic resource. Data on VAs residue will allow for an assessment of the environmental impact produced by animal breeding and manures used as fertilizers in arable lands.

Material and methods

Sampling and field sites

The sampling procedure was accomplished during October 2018, by taking into consideration the regional distribution and livestock species in Albania and Kosovo. The sampling procedure was adopted, according to Martin and Beegle (2009). Briefly, a composite fresh manure sample of 10 kg was prepared from multiple subsamples, taken with clean steel shovels, and placed in a clean plastic bucket to mix thoroughly. A subsample of 100 g from the mixed composite samples was placed in plastic bottles. Samples were kept in the icebox and transferred in frozen conditions to the laboratory.

In total, 22 manure samples were collected from Kosovo: four from poultry, six from cattle/calf, and twelve from pig, respectively. From Albania, 16 samples were collected: ten from pig, two from chicken, three from cattle/calf, and one from sheep. The sampling sites strategy consisted of the selection of regions with the main contribution to intensive livestock breeding. In the case of Albania, these regions were Fieri, Durrësi, and Lezha, as well as Korça, an agricultural district in the Eastern part of the country (Fig. 1a). In the case of Kosovo, manure sampling was limited to the regions of Gjilani and Gjakova (Fig. 1b). Detailed information on manure samples regarding the type of animal and the region is provided in the Supplementary Material (Table 3).

Reagents and standards

The sulfonamides and tetracycline standards (96.1% purity) were purchased from Sigma-Aldrich. The sulfonamides used in our investigation were sulfathiazole (STZ), sulfamethiazine (SMZ), and sulfadiazine (SDZ), while for tetracycline, oxytetracycline (OTC), doxycycline (Doxy), chlortetracycline (CTC), iso-chlortetracycline (iso-CTC), and tetracycline (TC) were used. All chemicals used were of analytical and HPLC grades, while ultrapure water used in the analysis was produced from Milli-Q system (Millipore, Eschborn, Germany). The sulfonamides and tetracycline stock solutions were prepared by dissolving 10.0 ± 0.1 mg of each standard in 10 mL methanol resulting in 1 mg/mL stock solutions. Working dilutions were prepared freshly on the day of use while stored at − 80 °C until the day of analysis.

Liquid chromatography-tandem mass spectrometry and quantification

Samples were prepared and analyzed according to Spielmeyer et al. (2014). In brief, 1 g sample material was extracted with methanol/ethanol/dichloromethane (1:3:1, v/v/v). Analysis of the VAs extract was performed in the multiple reaction monitoring modes by UHPLC-MS/MS using two transitions per

Fig. 1 Region locations of collected manure samples in (**a**) Albania and (**b**) Kosovo (http://www.encyclopediabritannica.com)

compound. Due to a new MS system (QTrap 3200, ABSciex, Darmstadt, Germany), lower limits of detection (LOD) and limit of quantifications (LOQ) were obtained (Table 1). LODs for veterinary antibiotics belonging to the sulfonamides group were below or equal to 10 g kg^{-1}, while the LOD values for VAs to tetracyclines group were higher, with LOD of doxycycline 75 g kg^{-1}. This method is able to screen the presence of 14 VAs and one metabolite from two groups: sulfonamides and tetracyclines.

Mass spectrometry was carried out using a LCQ ion trap with an electrospray ionization source (Finnigan Mat, San Jose, CA). Standard compounds (10 ng/μL) were infused through an integrated syringe pump at a flow rate of

Table 1 Limits of detection (LOD), limits of quantifications (LOQ), recoveries, and RSD of the 14 investigated antibiotics and a metabolite of chlortetracycline in manure

Substance	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Recovery (&)	RSD (%)
Sulfonamides				
Sulfachlorpyridazine	0.008	0.025	101.5	9.4
Sulfadiazine	0.008	0.025	98.1	8.1
Sulfadimethoxine	0.003	0.008	84.6	7.8
Sulfaguanidine	0.010	0.050	85.6	8.9
Sulfamerazine	0.005	0.025	78.1	6.1
Sulfamethazine	0.008	0.025	98.4	3.2
Sulfamethoxazole	0.010	0.025	96.1	4.5
Sulfamethoxypyridazine	0.005	0.025	88.1	6.6
Sulfapyridine	0.005	0.025	90.5	8.9
Sulfathiazole	0.010	0.075	88.7	9.1
Tetracyclines				
Chlortetracycline	0.025	0.100	102.3	3.2
Doxycycline	0.075	0.300	99.3	7.8
Iso-chlortetracycline	0.010	0.050	89.6	5.6
Oxytetracycline	0.050	0.150	97.7	9.8
Tetracycline	0.010	0.100	96.5	6.7

Values refer to mg per kg fresh weight; due to missing standard compound, values cannot be provided for the metabolite 4-hydroxy-sulfadiazine

10 µL/min for tuning the mass spectrometer and optimizing capillary temperature, sheath gas, and auxiliary gas flow rates. The polarity source was set positive for all compounds; the spray needle voltage was 5 kV. Drying gas was nitrogen generated from pressurized air in an Ecoinert 2 ESP nitrogen generator (DWT-GmbH, Gelsenkirchen, Germany). The optimized conditions were as follows: sheath gas flow was set at 100 units, the auxiliary gas was turned off, and the capillary temperature was 150 °C. LOD, LOQ, recovery, and RSD are summarized in Table 1. MS(3) is a technique that can be exclusively performed on ion trap mass spectrometers. The HPLC system employed was a gradient system consisting of a Thermoquest P4000 pump, an AS3000 autosampler (San Jose, CA) and a Puresil C18 column (5 µm, 4.6 × 150 mm, Waters Corp., Milford, MA) operated at 23 °C. The flow of 1 mL/min was split 1:10 before entrance into the mass spectrometer. The mobile phase consisted of 0.5% formic acid in water with 1 mM ammonium acetate (solvent A, pH 2.5) and acetonitrile (solvent B). The gradient run was 0–50% B in 9 min and then held at 50% B for 1 min. After each run, the column was rinsed for 3 min with 99% acetonitrile and re-equilibrated for 12 min with solvent A. The injection volume was 1–2 µL. After each injection, the autosampler was rinsed with 3 mL of 90% methanol/10% 10 mM oxalic acid in water. Calibration curves constructed ranged from 0.1 to 10 ng per injection and were linear, with $r^2 > 0.99$ for the MS-MS procedure. The quantification procedure was obtained by comparing the peak areas with external calibration curves, and all data were corrected for recovery.

Results and discussion

Antibiotic use in the animal husbandry in Albania and Kosovo

A report from the World Organization for Animal Health (OIE) concluded that this practice of using antibiotics as growth promoters is banned in many parts of the world. However, it has found that 45 countries out of 155 are still supplying them as a growth promoter (OIE 2019). According to the literature review, there is no published information on statistics for antibiotic use in livestock farming in Albania and Kosovo. An estimation over the VAs use was calculated through extrapolation on each country's meat production data and the average Veterinary Pharmaceutical Consumption Factor (VPCFa) calculated by Kools et al. (2008) based on the reported European animal meat production. Differences that exist between the livestock economies in the EU countries and two countries in our study might not present the exact values of the VAs application in animal husbandry in two states, referring to the VPCFa approach. A combination of the VPCFa (135 mg antibiotics kg^{-1} meat) and the country's annual meat production for 2017 is said to be 161,200 t; eventually, an amount of 21.8 t VAs was estimated to have been used in Albania, while for Kosovo, this value was 15.1 t.

VA occurrence in livestock manure in countries of the study

This study presents the occurrence of VAs in the livestock manures in Albania and Kosovo, two countries situated in Southeastern Europe. The employed analytical method screened the presence of 14 VAs and one metabolite belonging to the sulfonamides and tetracyclines classes (Table 2). One or more antibiotics were simultaneously detected in 11 out of the 38 manure samples or resulting in 28.9% positive manure samples. The concentration levels varied from 0.04 to 10.1 mg kg^{-1}. The VAs levels for both countries are presented in Table 2. The VAs belonging to the tetracyclines class were the most common in ten out of 11 positive manure samples investigated with VAs presence. Referring to the total manure samples, the incidence rate of tetracyclines was 26.3%. The highest value belonged to poultry manure, with 3 out of 5 samples, and co-occurrence of 3 or more tetracyclines. The second group with tetracyclines co-occurrence belonged to the cattle manure samples with residual levels variation of 0.10–3.91 mg kg^{-1}. From 4 tetracyclines and one metabolite, the tetracycline (TC) was the most common antibiotic, even though chlortetracycline (CTC) was detected in the highest concentration (3.91 mg kg^{-1}), in the range of approximately 10–15 times higher compared with TC or OTC. Tetracyclines' presence rates were analyzed in the following sequence order: TC > OTC = CTC = Iso-CTC > DOXY.

The sulfonamides co-occurrence in livestock manure was found less frequent compared with tetracyclines. It is in line with the use of tetracyclines in animal feeding operation, which is not always familiar with sulfonamides for operators. Meanwhile, the concentration levels showed a variation factor of 250. Regarding three sulfonamides and one metabolite, their presence rates were less common in four out of 11 positive manure samples investigated with VA presence, which means 10.5% to overall analyzed manure samples. However, the highest residue levels of the VA belonged to sulfonamides. The detectable levels to sulfonamides ranged 0.04–10.1 mg kg^{-1} in the poultry manure. Sulfadiazine was the principal antibiotic both in residue level and incidence rate. Their presence rate was similar among different types of animal manure.

Antibiotics in manure samples from Kosovo

In the analyzed manure samples from Kosovo, in total, six VAs and one metabolite were detected in 6 out of 22 manure samples, marking a presence rate of 27.3%. VAs present in manure samples belonged to tetracyclines, which include oxytetracycline (OTC), tetracycline (TC), chlortetracycline (CTC), and doxycycline (DOXY). Besides, iso-chlortetracycline (iso-CTC) was detected as the primary CTC metabolite, and the second group of antibiotics, sulfonamides, includes sulfadiazine (SDZ) and sulfathiazole (STZ). The highest concentration was found in a poultry manure sample (3.91 mg CTC kg^{-1} fresh weight). In the same manure sample, two other antibiotics were detected (0.31 mg OTC kg^{-1} wet weight, 0.25 mg TC kg^{-1} wet weight) accompanied by the metabolite iso-CTC. In three poultry manure samples, all VAs detected were below the LOQ. Low levels, together

Table 2 Co-occurrence and detected concentrations of seven VAs and two metabolites in animal manure from both countries

VA	Occurrence (%)			Range (mg kg^{-1})		
	Cattle ($n = 9$)	Pig ($n = 22$)	Poultry ($n = 6$)	Cattle ($n = 9$)	Pig ($n = 22$)	Poultry ($n = 6$)
SDZ	11.1	4.54	16.6	<LOQ	0.04–0.04	10.1–10.1
SDZ-OH	–	–	16.6	–	–	(1.4)*
STZ	–	4.54	–	–	0.11–0.11	–
SMZ	11.1	–	–	<LOQ	–	–
OTC	11.1	9.09	33.3	1.17–1.17	<LOQ-0.47	<LOQ–0.31
TC	11.1	4.54	83.3	<LOQ	<LOQ	<LOQ–0.25
CTC	11.1	4.54	50.0	0.10–0.10	0.68	0.12–3.91
Iso-CTC	11.1	4.54	50.0	<LOQ	0.25–0.25	<LOQ–0.73
DOXY	22.2	4.54	16.6	<LOQ–0.49	0.30–0.30	<LOQ
ΣSuls[a]				n.d–<LOQ	n.d – 0.15	n.d–11.5
ΣTCs[a]				n.d–1.76	n.d – 1.40	<LOQ–5.10
ΣAntibiotics[a]				<LOQ-1.76	0.15–1.40	<LOQ–13.44

SDZ sulfadiazine, *SDZ-OH* 4-hydroxy sulfadiazine, *STZ* sulfathiazole, *SMZ* sulfamethazine, *iso-CTC* iso-chlortetracycline, *CTC* chlortetracycline, *DOXY* doxycycline, *OTC* oxytetracycline, *TC* tetracycline. *SDZ-OH: semi-quantitative as no standard substance available; *LOQ* limit of quantification; *n.d* none detected. All values are given in mg kg^{-1} fresh weight, *ΣSuls* sum of sulfonamides, *ΣTCs* sum of tetracyclines, *Σantibiotics* sum of antibiotics, [a] per positive samples with low detection frequencies, were found for manure samples belonging to cattle and pig. All in all, the detection rates for at least one VA were 100% in poultry manures, 8.3% in pig manures, and 16.7% for cattle manure samples.

The VAs distribution pattern in poultry manure samples from two countries shows a non-significant difference regarding the antibiotic's occurrence in Kosovo poultry manure samples with three VAs and one metabolite, compared with three VAs and two metabolites found in poultry manure from Albania. The maximum value in poultry manure from Kosovo belonged to CTC (3.91 mg CTC kg^{-1} fresh weight) compared with SDZ (10.1 mg kg^{-1} fresh weight) in poultry manure from Albania.

In poultry manure, four tetracyclines and one metabolite analyzed, the antibiotics levels were generally lower compared with VAs concentrations in samples from China, except the CTC (Zhao et al. 2010; Ji et al. 2012). The CTC levels in samples from Kosovo were found in much higher manure concentration (3.91 mg kg^{-1}) compared with data from two studies in poultry manure from China. This pattern was different when compared with CTC (1.09 mg kg^{-1}) in poultry manure from Albania. The OTC presence in one poultry manure sample from Kosovo (0.31 mg kg^{-1}) was considerably lower compared with data published in both studies, respectively, 1.6 mg kg^{-1} (Zhao et al. 2010) and 21.96 mg kg^{-1} (Ji et al. 2012). Meanwhile, OTC was not present in samples from Albania. Two other tetracyclines, TC and DOXY, were found in significant differences compared with poultry manure samples from China. So, TC (10.31 mg kg^{-1}) according to (Ji et al. 2012), and DOXY (3.4 mg kg^{-1}) according to (Zhao et al. 2010) occurred in much higher values compared to TC concentration (0.25 mg kg^{-1}) and (0.12 mg kg^{-1}) in samples from Kosovo and Albania, respectively, while the DOXY presence was not observed in this samples. The VAs concentrations in poultry manure from both countries in the study were found in higher levels when compared with data from Austria (Martínez-Carballo et al. 2007). Sulfonamide analysis covered 10 different antibiotics for their presence in poultry manure samples in both countries. Only SDZ was detected in one sample from Albania (10.1 mg kg^{-1}). The SDZ concentration was much lower compared with SDZ levels (51.0 mg kg^{-1}) in poultry manure samples from Austria (Martínez-Carballo et al. 2007) while similar to data from China (8.03 mg kg^{-1}) according to Ji et al. (2012).

Antibiotic in manure samples from Albania

In the manure samples, six different VAs and two metabolites were found in five out of sixteen samples or 31.2% (Table 2). Two VAs belonged to the class of sulfonamides (SDZ and SMZ) while the other four VAs to tetracyclines (OTC, TC, CTC, and DOXY). Also, two metabolites—4-hydroxy sulfadiazine and iso-chlortetracycline—were as well detected. The maximum concentration of studied VAs was detected in poultry manure, the sulfonamide SDZ (10.1 mg kg^{-1}). Comparison of manure samples concerning their animal species origin reveals that cattle manures are a source of five different VAs, OTC (1.17 mg kg^{-1}), CTC (0.1 mg kg^{-1}), and DOXY (0.49 mg kg^{-1}), together with SMZ and TC under LOQ levels. Three VAs and one metabolite were detected in pig manure, at very low concentrations. Altogether, 31.2% of investigated manure samples contained at least one VA.

The antibiotics spectrum included seven VAs, which belonged to the sulfonamides and tetracyclines classes. Referring to VAsr co-occurrence and concentration levels, these data are in good accordance with investigations from different countries, such as Austria, Belgium, and the Netherlands (Table 3) (Martínez-Carballo et al. 2007; Berendsen et al. 2015; Van den Meersche et al. 2016; Widyasari-Mehta et al. 2016), but much lower compared with VAs in manure samples from Germany and China (Zhao et al. 2010; Ji et al. 2012; Spielmeyer et al. 2014).

The antibiotics' concentrations in pig manure, from both countries in the study, were generally similar with those found in pig manure in the Netherlands (Berendsen et al. 2015) but much lower compared with the data published in studies from Germany (Widyasari-Mehta et al. 2016), Belgium (Van den Meersche et al. 2016), and China (Ji et al. 2012). The CTC (0.68 mg kg^{-1}) was detected in samples from Albania and Germany (Widyasari-Mehta et al. 2016), but not in samples from Kosovo, the Netherlands (Berendsen et al. 2015), and China (Ji et al. 2012). The second antibiotic, from the tetracycline class, studied for its presence, DOXY, was not detected in pig manure samples in both countries, similar with data published for swine manure from China (Ji et al. 2012), while found in high levels in samples from Germany (Widyasari-Mehta et al. 2016) and Belgium (Van den Meersche et al. 2016), 27. 4 mg kg^{-1} and 22.8 mg kg^{-1}, respectively. The same pattern is present in the case of OTC, where concentrations found in samples from Albania and Kosovo resulted much lower compared with data from Germany (Widyasari-Mehta et al. 2016), Belgium (Van den Meersche et al. 2016), and China (Ji et al. 2012), while TC was not detected in our study, presenting a similar situation with the Netherlands (Berendsen et al. 2015) and Belgium (Van den Meersche et al. 2016). The concentrations of sulfonamides resulted much lower compared with data from Germany (Widyasari-Mehta et al. 2016), Belgium (Van den Meersche et al. 2016), and China (Ji et al. 2012) while similar with those presenting results from the Netherlands (Berendsen et al. 2015).

Evaluation of VAs concentrations in cattle manure samples revealed a better situation compared with data from recent studies worldwide (Ji et al. 2012; Spielmeyer et al. 2014). The co-occurrence of the analyzed antibiotics in samples from

Table 3 Comparison of antibiotic concentrations present in various manure samples worldwide with our results (mg kg^{-1})

Country	Matrix	CTC	DOXY	OTC	TC	Iso-CTC	SDZ	SMZ	STZ	Reference
Netherlands	Swine feces	n.d	1.9	0.16	n.d	–	0.13	n.d	–	Berendsen et al. (2015)
Belgium	Swine manure[a]	n.d	22.8	2.0	n.d	–	3.0	n.d	–	Van den Meersche et al. (2016)
Germany	Pig manure[b]	37.4	27.4	13.6	152	–	7.3	2.2	–	Widyasari-Mehta et al. (2016)
China	Swine manure	–	–	18.7	12.27	–	4.87	7.56	–	Ji et al. (2012)
Albania	*Pig manure*	*0.68*	*n.d*	*0.47*	*<LOQ*	*n.d*	*n.d*	*n.d*	–	
Kosovo	*Pig manure*	*n.d*	*n.d*	*<LOQ*	*n.d*	*0.25*	*0.04*	–	*0.11*	
Netherlands	Cattle feces[a]	n.d	0.04	8.0	0.04	n.d	0.02	0.02	–	Berendsen et al. (2015)
China	Cattle manure	–	–	*21.36*	*12.01*	–	*4.57*	*9.36*	–	Ji et al. (2012)
Albania	*Cattle manure*	*0.10*	*0.49*	*1.17*	*<LOQ*	*<LOQ*	*n.d*	*<LOQ*	–	
Kosovo	*Cattle manure*	*n.d*	*n.d*	*n.d*	*n.d*	*n.d*	*<LOQ*	*n.d*	*n.d*	
Germany	Manure[b]	36.5	n.d	n.d	6.6	–	1.1	201	–	Spielmeyer et al. (2014)
Austria	Chicken dung[b]	n.d	n.d	n.d	n.d	–	51	n.d	–	Martínez-Carballo et al. (2007)
China	Chicken dung[b]	1.1	3.4	1.6	n.d	–	0.15	0.43	–	Zhao et al. (2010)
	Chicken dung	–	–	21.96	10.31	–	8.03	8.62	–	Ji et al. (2012)
Albania	*Chicken dung*	*1.09*	*n.d*	*n.d*	*0.12*	*0.73*	*10.1*	*n.d*	–	
Kosovo	*Chicken dung*	*3.91*	*n.d*	*0.31*	*0.25*	*0.63*	*n.d*	*n.d*	*n.d*	

CTC chlortetracycline, *DC* doxycycline, *OTC* oxytetracycline, *TC* tetracycline, *SDZ* sulfadiazine, *SMX* sulfamethoxazole, *SMZ* sulfamethazine; [a] mg kg^{-1} dryweight; [b] mg kg^{-1} fresh weight

Albania was higher compared with cattle manure samples from Kosovo, 5 VAs and one metabolite, compared with one antibiotic. Overall, VAs from the tetracyclines class were present in the cattle manure samples from Albania, but not in samples from Kosovo. Also, antibiotics belonging to sulfonamides class were found below LOQ for SMZ in one sample from Albania and in the case of SDZ for one sample from Kosovo. VAs concentrations were much higher in studies from Germany (Spielmeyer et al. 2014), the Netherlands (Berendsen et al. 2015), and China (Ji et al. 2012). The antibiotics' concentration in manure samples from Germany was CTC (36.5 mg kg^{-1}) and SMZ (201.0 mg kg^{-1}) (Spielmeyer et al. 2014). In China, the highest concentrations belonged to OTC (21.36 mg kg^{-1}) and TC (12.01 mg kg^{-1}). VAs concentrations and their co-occurrence in cattle manure found in positive samples from Albania show similar patterns with data presented in positive samples from the Netherlands (Berendsen et al. 2015).

The co-occurrence of VAs in positive samples was evaluated as the sum of tetracyclines (ΣTCs), the sum of sulfonamides (ΣSulfs), and the sum of antibiotics. In the cattle manure samples, the ΣSulfs was detected below LOQ and the ΣTCs with the maximum value of 1.76 mg kg^{-1} wet weight, which means that tetracyclines were the only contributors to the contaminated cattle manure samples. In pig manure, the ΣSulfs reached the maximum of 0.15 mg kg^{-1} wet weight, the ΣTCs reached the peak (1.40 mg kg^{-1} wet weight), and calculated as the sum of antibiotics was found in the interval 0.15 to 1.40 mg kg^{-1} wet weight. Finally, the VAs concentration in poultry manure samples was calculated as the ΣSulfs, ΣTCs, and the sum of antibiotics: 11.5 mg kg^{-1}, 5.10 mg kg^{-1}, and 13.44 mg kg^{-1} wet weight, respectively. These values are considerably lower compared with the data on VAs occurrence in livestock manure in China (Ji et al. 2012). Ji et al. (2012) found ΣTCs (30.97 mg kg^{-1} wet weight) in pig manure, 33.37 mg kg^{-1} in cattle manure, and 32.27 mg kg^{-1} in poultry manure. The sum of sulfonamides in pig manure was 18.59 mg kg^{-1} wet weight, in cattle manure 20.31 mg kg^{-1}, and in poultry manure 24.66 mg kg^{-1}, while the sum of antibiotics in pig manure was 60.57 mg kg^{-1} wet weight, in cattle manure 66.58 mg kg^{-1}, and poultry manure 74.78 mg kg^{-1}.

Kim et al. (2011) concluded that proper composting practices application in livestock manures will eliminate the excreted VAs and their metabolites on a scale of more than 90%. In the case of Albania, the livestock manure composting practices consist mainly of the composting process. To the best of our knowledge, there is no one intensive farm that has implemented the anaerobic treatment of animal manure for both countries. However, referring to the efficacy, such treatment practice should not overestimate (Hamscher and Bachour 2018). The environmental issue arising from the application of contaminated animal manure with antibiotics and other veterinary drugs, both to arable fields, as well as dumping to surface waters, or wastewater systems, in both of these countries, urges for significant intervention to the treatment practices of livestock manure.

Conclusions

Seven VAs and two metabolites were detected in the livestock manure of three different animal species. Antibiotics belonged to sulfonamides and tetracyclines classes. Based on this study, we conclude that the assessed VAs in manure samples belong to the two most used antibiotic groups. Tetracyclines were most present antibiotics, especially in poultry manure. TC, OTC, CTC, and DOXY were the most typical antibiotics found in animal manure. Six VAs accompanied by two metabolites were found in manure samples from Albania while six VAs and one metabolite in Kosovo manure samples. VAs present in manure samples from Albania resulted in higher levels compared with manure samples from Kosovo. The maximum content was found in poultry manures for both countries, SDZ (10.1 mg kg^{-1}) from Albania and CTC (3.91 mg kg^{-1}) from Kosovo, respectively. Low levels of VAs indicate that the environmental impact originating from livestock farming in both countries is considered not an emergent situation. Lack of national programs to address the issue of manure disposal and implementation of anaerobic treatment procedures to livestock farms indicates that the actual situation in both countries needs to address this issue. Further studying the presence of antibiotics and other veterinary drugs in manure samples, arable land, and surface waters in both countries, including other neighboring countries, will give information on ecological risks accompanying the livestock manure applications for the entire region of Southeastern Europe.

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References

Baguer AJ, Jensen J, Krogh PH (2000) Effects of the antibiotics oxytetracycline and tylosin on soil fauna. Chemosphere 40:751–757

Baynes RE, Dedonder K, Kissell L, Mzyk D, Marmulak T, Smith G, Tell L, Gehring R, Davis J, Riviere JE (2016) Health concerns and management of select veterinary drug residues. Food Chem Toxicol 88: 112–122

Berendsen BA, Wegh RS, Memelink J et al (2015) The analysis of animal faeces as a tool to monitor antibiotic usage. Talanta 132:258–268

Boxall AB, Fogg LA, Blackwell PA et al (2004) Veterinary medicines in the environment. Rev Environ Contam T 180:1–91

Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC, Maxwell S, Aminov RI (2009) Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. J Environ Qual 38:1086–1108

ESVAC (European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption) (2018) 'Sales of veterinary antimicrobial agents in 30 European countries in 2016′ (EMA/275982/2018)

EU (2002) Commission Decision 2002/657/EC Implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Communities L 221/20:29

European Commission (2005) Ban on antibiotics as growth promoters in animal feed enters into effect. Press Release Database, IP/05/1687. Brussels. http://europa.eu/rapid/press-release_IP-05-1687_en.html (Accessed February 28th 2019)

European Parliament and the Council (2003) Regulation (EC) No 1831/2003 of 22 September 2003 on additives for use in animal nutrition. Off J Eur Union L 268:29–43

FVA (Food and Veterinary Agency) (2019) Data on Veterinary antibiotics used during 2018 in husbandry. (http://www.auv-ks.net/). (Accessed on February 10th, 2019)

Grave K, Torren-Edo J, Muller A, Greko C, Moulin G, Mackay D, on behalf of the ESVAC Group, Fuchs K, Laurier L, Iliev D, Pokludova L, Genakritis M, Jacobsen E, Kurvits K, Kivilahti-Mantyla K, Wallmann J, Kovacs J, Lenharthsson JM, Beechinor JG, Perrella A, Mi ule G, Zymantaite U, Meijering A, Prokopiak D, Ponte MH, Svetlin A, Hederova J, Madero CM, Girma K, Eckford S (2014) Variations in the sales and sales patterns of veterinary antimicrobial agents in 24 European countries. J Antimicrob Chemother 69(8):2284–2291

Gullberg E, Cao S, Berg OG, Ilbäck C, Sandegren L, Hughes D, Andersson DI (2011) Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog 7(7):e1002158. https://doi.org/10.1371/journal.ppat.1002158

Hamscher G, Bachour G (2018) Veterinary drugs in the environment: current knowledge and challenges for the future. J Agr Food Chem 66:751–752

Jechalke S, Heuer H, Siemens J, Amelung W, Smalla K (2014) Fate and effects of veterinary antibiotics in soil. Trends Microbiol 22:536–545

Ji X, Shen Q, Liu F et al (2012) Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animal manures and agricultural soils adjacent to feedlots in Shanghai. China J Hazard Mater 235–236:178–185

Kim KR, Owens G, Kwon SI, So KH, Lee DB, Ok YS (2011) Occurrence and environmental fate of veterinary antibiotics in the terrestrial environment. Water Air Soil Pollut 214:163–117

Kivits T, Broers HP, Beeltje H, van Vliet M, Griffioen J (2018) Presence and fate of veterinary antibiotics in age-dated groundwater in areas with intensive livestock farming. Environ Pollut 241:988–998

Kools SAE, Moltmann JF, Knacker T (2008) Estimating the use of veterinary medicines in the European Union. Regul Toxicol Pharmacol 50:59–65

Kumar K, Gupta SC, Baidoo SK, Chander Y, Rosen CJ (2005) Antibiotic uptake by plants from soil fertilized with animal manure. J Environ Qual 34:2082–2085

Kuppusamy S, Kakarla D, Venkaterswarlu K et al (2018) Veterinary antibiotics (VAs) contamination as a global agro-ecological issue: a critical view. Agric Ecosyst Environ 257:47–59

Liu D (2012) Occurrence, fate, and ecotoxicity of antibiotics in agro-ecosystems. A review. Agronomy for Sustainable Development, Springer Verlag/EDP Sciences/INRA, 32:309–327

MARD (2018) Statistical annual of livestock breeding. http://www.instat.gov.al/media/4412/vjetari-statistikor-i-bujq%C3%ABsis%C3%AB-2017-blegtoria_final.xlsx. (Accessed January 14th, 2019)

Marshall BM, Levy SB (2011) Food animals and antimicrobials: impacts on human health. Clin Microbiol Rev 24:718–733

Martin J, Beegle D (2009) Manure sampling for nutrient management planning. https://extension.psu.edu/programs/nutrient-management/educational/manure-storage-and-handling/manure-sampling-for-nutrient-management-planning/extension_publication_file. (Accessed on October 2nd, 2018)

Martínez-Carballo E, González-Barreiro C, Scharf S, Gans O (2007) Environmental monitoring study of selected veterinary antibiotics in animal manure and soils in Austria. Environ Pollut 148:570–579
Massé D, Saady N, Gilbert Y (2014) Potential of biological processes to eliminate antibiotics in livestock manure: an overview. Animals 4(2):146–163
OIE (World Organization for Animal Health) (2019) The Third OIE Annual report on the use of antimicrobial agents in animals
Pan M, Cu LM (2017) Leaching behavior of veterinary antibiotics in animal manure-applied soils. Sci Total Environ 579:466–473
Sarmah K, Meyer MT, Boxall ABA (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate, and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65:725–759
Spielmeyer A (2018) Occurrence and fate of antibiotics in manure during manure treatments: a short review. Sustain Chem Pharmacy 9:76–86
Spielmeyer A, Ahlborn J, Hamscher G (2014) Simultaneous determination of 14 sulfonamides and tetracyclines in biogas plants by liquid-liquid-extraction and liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem 406:2513–2524
Spielmeyer A, Breier B, Groißmeier K, Hamscher G (2015) Elimination patterns of worldwide used sulfonamides and tetracyclines during anaerobic fermentation. Bioresour Technol 193:307–314
Tasho RP, Cho JG (2016) Veterinary antibiotics in animal waste, its distribution in soil and uptake by plants: a review. Sci Total Environ 563–564:366–376
Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Laxminarayan R (2015) Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A 112:5649–5654
Van den Meersche T, Van Pamel E, Van Poucke C, Herman L, Heyndrickx M, Rasschaert G, Daeseleire E (2016) Development, validation, and application of an ultra-high performance liquid chromatographic-tandem mass spectrometric method for the simultaneous detection and quantification of five different classes of veterinary antibiotics in swine manure. J Chromatogr A 1429:248–257
WHO (World Health Organization) (2014) Antimicrobial resistance: global report on surveillance 2014, WHO
Widyasari-Mehta A, Hartung S, Kreuzig R (2016) From the application of antibiotics to antibiotic residues in liquid manure and digestates: a screening study in one European Centre of conventional pig husbandry. J Environ Manag 177:129–137
Wohde M, Berkner S, Junker T, Konradi S, Schwarz L, Düring RA (2016) Occurrence and transformation of veterinary pharmaceuticals and biocides in manure: a literature review. Environ Sci Eur 28:23. https://doi.org/10.1186/s12302-016-0091-8
Zhao L, Dong YH, Wang H (2010) Residues of veterinary antibiotics in manures from feedlot livestock in eight provinces of China. Sci Total Environ 408:1069–1075