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Hazard/Risk Assessment

Occurrence, Distribution, and Ecological Risk of Fluoroquinolones in Rivers and Wastewaters

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Abstract: The use of fluoroquinolones for the treatment of infections in humans and animals has increased in Argentina, and they can be found in large amounts in water bodies. The present study investigated the occurrence and associated ecological risk of 5 fluoroquinolones in rivers and farm wastewaters of San Luis, Santa Fe, Córdoba, Entre Ríos, and Buenos Aires provinces of Argentina by high-performance liquid chromatography coupled to fast-scanning fluorescence detection and ultra–high-performance liquid chromatography coupled to triple quadrupole mass spectrometry detection. The maximum concentrations of ciprofloxacin, enrofloxacin, ofloxacin, enoxacin, and difloxacin found in wastewater were 1.14, 11.9, 1.78, 22.1, and 14.2 $\mu g\ L^{-1}$, respectively. In the case of river samples, only enrofloxacin was found, at a concentration of 0.97 $\mu g\ L^{-1}$. The individual risk of aquatic organisms associated with water pollution due to fluoroquinolones was higher in bacteria, cyanobacteria, algae, plants, and anurans than in crustaceae and fish, with, in some cases, risk quotients >1. The proportion of samples classified as high risk was 87.5% for ofloxacin, 63.5% for enrofloxacin, 57.1% for ciprofloxacin, and 25% for enoxacin. Our results suggest that the prevalence of fluoroquinolones in water could be potentially risky for the aquatic ecosystem, and harmful to biodiversity. *Environ Toxicol Chem* 2019;38:2305–2313. © 2019 SETAC

Keywords: Fluoroquinolones; Environmental water; Risk assessment; High-performance liquid chromatography coupled to fast-scanning fluorescence detection; Ultra–high-performance liquid chromatography coupled to triple quadrupole mass spectrometry

INTRODUCTION

Over the last few years, concern about the occurrence of traces of pharmaceuticals in the environment has increased (Bottoni et al. 2010; Riaz et al. 2018). These emerging contaminants have impacted aquatic ecosystems and human health, affecting both target and nontarget organisms and generating antimicrobial resistance (Sim et al. 2011; Adachi et al. 2013; Munier et al. 2015; Ory et al. 2016; Riaz et al. 2017; Lin et al. 2018).

Huge numbers of drugs are used in human and veterinary medicine to prevent and treat diseases and also as weight promoters (Sim et al. 2011; Riaz et al. 2018). Antibiotics are one of the drug classes most frequently used to manage microbial infections (He et al. 2015). In the case of animals, antibiotics are delivered through feed, water, and other routes such as injection (Zhou et al. 2013). In this sense, fluoroquinolones are an important group of broad-spectrum antibiotics (Riaz et al. 2017), and represent the third largest class used in the world (Sukul and Spiteller 2007; Van Doorslaer et al. 2014; He et al. 2015). Because of their different structures and substituents, fluoroquinolones exhibit different antibacterial responses (Riaz et al. 2018), but in general they exert their action by inhibiting the DNA gyrase of microorganisms (Van Bambeke et al. 2005; Ramos Payán et al. 2011).

Depending on the path of administration and the metabolism of each species, fluoroquinolones can be excreted unmetabolized up to 70%. For this reason, and because of their extensive and continuous usage, fluoroquinolones can be found in animal manure (Zhou et al. 2013; Teglia et al. 2017; Riaz et al. 2018) and surface water sources (Ramos Payán et al. 2011; Van Doorslaer et al. 2014; Alcaraz et al. 2016; Du et al. 2017).

Because of their strong sorption properties and a degree of resistance to microbial degradation, fluoroquinolones can persist in environmental waters (Robinson et al. 2005; Ramos Payán et al. 2011). Rivers become contaminated with fluoroquinolones

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through the effluent of domestic, urban, hospital, and industrial wastewaters (Adachi et al. 2013; Zhou et al. 2013; Van Doorslaer et al. 2014). In addition, the rainfall runoff from agricultural fields fertilized with contaminated manure or sludge can contribute to the dispersion of fluoroquinolones into soil and water bodies; direct contamination from aquaculture applications also occurs (Sukul and Spiteller 2007; Zhou et al. 2013; Van Doorslaer et al. 2014; He et al. 2015). Fluoroquinolone residues can have negative effects on aquatic and terrestrial organisms, such as altering microbial activity and community composition in groundwater, and causing the development of antibiotic resistance (Zheng et al. 2012; Zhou et al. 2013; Ory et al. 2016; Riaz et al. 2017). The presence of these chemicals in the environment is alarming because they can appear not only individually, but as a complex mixture, which can lead to unwanted synergistic effects (Petrie et al. 2015; Liu et al. 2018). Moreover, contamination of water bodies can result in their bioaccumulation in aquatic ecosystems (Kim et al. 2017; Zhang et al. 2018, 2019; Zhao et al. 2018).

To mitigate this problem, different technologies have been developed to remove fluoroquinolones from aquatic systems (Gros et al. 2010; Sim et al. 2011; Verlicchi et al. 2012; Van Doorslaer et al. 2014). Nevertheless, their removal is complex and they can be found in numerous water sources at concentrations ranging from nanograms to micrograms per liter (Pereira et al. 2015; Petrie et al. 2015; Riaz et al. 2018).

Thus environmental studies have focused on the presence of these types of analytes in surface and waste waters (He et al. 2015; Riaz et al. 2017). Table 1 summarizes the concentrations of fluoroquinolones found in different sources and countries in the period between 2011 and 2018. In Argentina, data are lacking concerning the occurrence and levels of antibiotics in wastewaters and rivers; until the present study, only the work of Alcaraz et al. (2016) and Valdés et al. (2014) has reported the presence of ciprofloxacin and enrofloxacin in these types of samples. The Argentinian rivers, one of the major sources of drinking water, receive sewage discharges daily from cities and farms, and their environmental monitoring is mandatory. Thus the areas we studied are situated in the Argentinian provinces that have major agricultural–livestock activity. The choice was based on the fact that both livestock and poultry farms can generate discharge of fluoroquinolones into the nearest rivers by

TABLE 1: Fluoroquinolone concentrations (μg L^{-1}) in different water sources

Location	Type	Ciprofloxacin	Enrofloxacin	Ofloxacin	Enoxacin	Difloxacin	Reference
Korea	M-WWTP	0.18–0.73	0.05–1.3				Sim et al. 2011
	L-WWTP	0.14	0.59				
	H-WWTP	2.0–3.1	0.14–0.22				
	P-WWTP	1.9–8.7	1.5–2.8				
Hebei, China	Lake	0–0.06	0–0.04	0.004–0.03			Li et al. 2012
Jiangsu, China	AWW	1.7–7.5	0.59–8.8				Wei et al. 2012
	AFE	1.1–3.4	0.27–1.1				
	River	0.89–5.9	0.21–4.4				
	PW	1.2–2.1	0.50				
Beijing, China	WWTP	0.003–0.15		0.15–3.1			Gao et al. 2012
Beijing, China	WWTP	0.002–0.12	0–0.012	0.02–4.6		0–0.01	Li et al. 2013
Osaka, Japan	River	0.003–0.04		0–0.51	0–0.02		Adachi et al. 2013
Guangxi, China	AWW		0.06	0.06–0.16			Zhou et al. 2013
Córdoba, Argentine	River	0.02					Valdés et al. 2014
Wangyang, China	River	0.24–0.55	0.24–0.98	0.67–11.7			Jiang et al. 2014
	GW	0–0.03	0–0.18	0.03–0.38			
Romania	River	0.006					Chitescu et al. 2015
China	RW and GW	0–0.10	0–0.05	0.008–1.1		0.0004–0.02	Ma et al. 2015
Vietnam	AQW	0.06–0.25	0.05–0.68				Andrieu et al. 2015
China	Lake		0–0.08	0–0.18		0–0.01	Tang et al. 2015
Portugal	River and AQW	0–0.02	0–0.02				Pereira et al. 2015
Maryland, USA	Raw M-WWTP	0.50–1.3		0.29–0.57			He et al. 2015
	E-M-WWTP	0.12–0.16		0.10–0.15			
	D-M-WWTP	0.007–0.03		0.009–0.04			
China	SW	0.005–0.19	0.002–0.06	0.0008–0.01			Chen et al. 2015
Khyber Pakhtunkhwa, Pakistan	IWW	15.8–83	20–89				Riaz et al. 2017
Islamabad, Paksistan	WW	17–341	11–260				
India	River	6.6–5528	2.6–123	1.6–318		0.47–38	Gothwal and Thatikonda, 2017
Lahore, Pakistan	PWW	0–2.2		1.2–80			Ashfaq et al. 2017
China	SW		0–0.121	0–0.50	0–0.10		Du et al. 2017
Hunan, China	M-WWTP			0.01–0.41			Lin et al. 2018
Argentina	River and AWW	0.74–7.7	0.50–11.9	0.71–1.78	14.8–22.1	0.57–14.2	This work

WW = wastewater; WWTP = wastewater treatment plant; M-WWTP = municipal wastewater treatment plant; L-WWTP = livestock wastewater treatment plant; H-WWTP = hospital wastewater treatment plant; P-WWTP = pharmaceutical wastewater treatment plant; AWW = animal wastewater; AFE = animal farm effluent; PW = pond water; GW = groundwater; RW = reclaimed water; AQW = aquaculture water; IWW = industrial wastewater; SW = seawater; PWW = pharmaceutical wastewater; E-M-WWTP = effluent from municipal wastewater treatment plant; D-M-WWTP = downstream from municipal wastewater treatment plant.

rainfall runoff. In this context, we need to develop an efficient method to determine the presence of fluoroquinolones in natural environments and matrices of different complexities. The method developed by Alcaraz et al. (2016), based on high-performance liquid chromatography (HPLC) coupled to fluorescence detection, allowed the sensitive and robust determination of 7 fluoroquinolones in different environmental water samples, without extensive sample pretreatment. We therefore chose that method to monitor 5 fluoroquinolones, ciprofloxacin, ofloxacin, difloxacin, enrofloxacin, and enoxacin, in rivers and wastewaters located near farms in 5 Argentinean provinces. The samples were also analyzed by ultra UHPLC coupled to triple quadrupole mass spectrometry (MS) detection.

In addition, ecological risk assessments were conducted for 7 aquatic species—bacteria, cyanobacteria, algae, plant, crustaceae, anurans, and fish—to estimate their potential risk due to the presence of the analytes detected in the samples. Among the native species of the aquatic systems, prokaryotes are likely the most sensitive environmental organisms to antibiotics because antimicrobial agents are efficient inhibitors of bacterial growth (Välitalo et al. 2017). At the same time, algae and cyanobacteria are a vital part of the food chain, and small changes in the populations could affect the balance of the whole ecosystem (Välitalo et al. 2017). Moreover, cyanobacteria are probably more sensitive to fluoroquinolones because they are prokaryotic, making them structurally similar to bacteria and therefore more susceptible to the fluoroquinolone mode of action (Robinson et al. 2005). As for green algae, the toxic effects may be related to interference or inhibition of the pathways related to chloroplast metabolism, especially the photosynthetic apparatus, which finally affects cell growth (Välitalo et al. 2017).

MATERIALS AND METHODS

Chemical, reagents, and solutions

All standards were of analytical grade. Enoxacin and ofloxacin were provided by Sigma-Aldrich. Ciprofloxacin, difloxacin, and enoxacin were purchased from Fluka. The HPLC-grade acetonitrile and methanol (MeOH) were obtained from Merck, and Milli-Q water was obtained from Millipore. Glacial acetic acid (HAc) was purchased from Merck, and sodium acetate trihydrate (NaAc) was provided by Anedra. Yttrium (III) nitrate hexahydrate ($Y(NO_3)_3{\cdot}6H_2O$) was purchased from Sigma-Aldrich.

To perform UHPLC–MS/MS, Optima®-grade water, acetonitrile, and formic acid (98% purity) were purchased from Fisher Scientific.

Stock standard solutions of each fluoroquinolone and yttrium (Y^{3+}) were prepared following the method of Alcaraz et al. (2016) and stored at 4 °C in the dark. A 6-point calibration curve for each fluoroquinolone was prepared in triplicate in ultrapure water with a final Y^{3+} concentration of 0.1 mmol L^{-1}. Standard and sample solutions were filtered through syringe 0.22-μm nylon membranes before injection into the chromatographic system.

A 0.02 mol L^{-1} HAc/NaAc buffer solution (AcYB) of pH 4.0 containing a final Y^{3+} concentration of 0.1 mmol L^{-1} was prepared in ultrapure water. Solutions and solvents for the mobile phase were always filtered through 0.45-μm nylon membranes.

Instrumentation, procedure, and software

HPLC coupled to fast-scanning fluorescence detection. All experiments were performed on an Agilent 1100 series liquid chromatography instrument equipped with a quaternary pump, membrane degasser, thermostated column compartment, autosampler, fast-scanning fluorescence detector (FSFD), and the ChemStation software package (all from Agilent Technologies) to control the instrument, the data acquisition, and the data analysis. The separation was performed on a Zorbax Eclipse XDB-C18 column (4.6 × 75 mm, 3.5-μm particle size; Agilent Technologies) in isocratic mode at 2.20 mL min^{-1} flow rate and 45 °C. The mobile phase consisted of a mixture of 0.02 mol L^{-1} AcYB and acetonitrile (91:1; Alcaraz et al. 2016). The time-emission fluorescence data matrices were registered in the emission spectral range between 380 nm and 510 nm, with the excitation wavelength fixed at 280 nm, at an elution time of 0.0 to 16.0 min. Data processing and multivariate curve resolution–alternating least squares (MCR-ALS) analysis were performed in MATLAB 7.10 (The MathWorks 2010). The MCR-ALS algorithms are available online (Multivariate Curve Resolution–Alternating Least Squares 2015).

UHPLC coupled to triple quadrupole MS detection. The UHPLC was performed using an ACQUITY UPLC™ System (Waters) coupled to a triple quadrupole MS (Micromass TQ Detector from Waters) through an orthogonal-Z-spray ionization source. Separations were achieved using an ACQUITY UPLC® BEH C18 RP Shield (2.1 × 100 mm, 1.7-μm particle size) column from Waters. The chromatographic and MS detection conditions are described in the Supplemental Data (Section SM1 and Table SM1).

Environmental water samples

Water samples were collected from 36 sampling sites in San Luis, Santa Fe, Córdoba, Entre Ríos, and Buenos Aires provinces of Argentina, between March and August 2017 (Figure 1). The selected zones correspond to wastewater near livestock, poultry farm, and urban zones, as well as river courses (see the Supplemental Data, Table SM2 for more details). The samples were collected in 500-mL light-resistant glass bottles without added preservatives, transported to the laboratory, and processed immediately after arrival or stored at 4 °C until all assays were performed.

To remove sediments, the samples were centrifuged at 4000 rpm for 10 min. Before injection into the chromatographic system, 5.0 μL of Y^{3+} solution were transferred into 5.0-mL volumetric flasks, which were then filled up with each sample. Each sample solution was prepared in triplicate.

To verify the feasibility of the HPLC–FSFD method to accurately quantitate fluoroquinolones in complex samples at low concentration levels, the samples were also analyzed by the reference method UHPLC–MS/MS, and, subsequently, the percentage ratios (R%) were calculated according to $R\% = \frac{\text{Concentration}_{\text{HPLC–FSFD}}}{\text{Concentration}_{\text{UHPLC–MS/MS}}} \times 100\%$. The method was considered adequate if the average percentage ratios were between 85 and 115% (European Medicines Agency 2012).

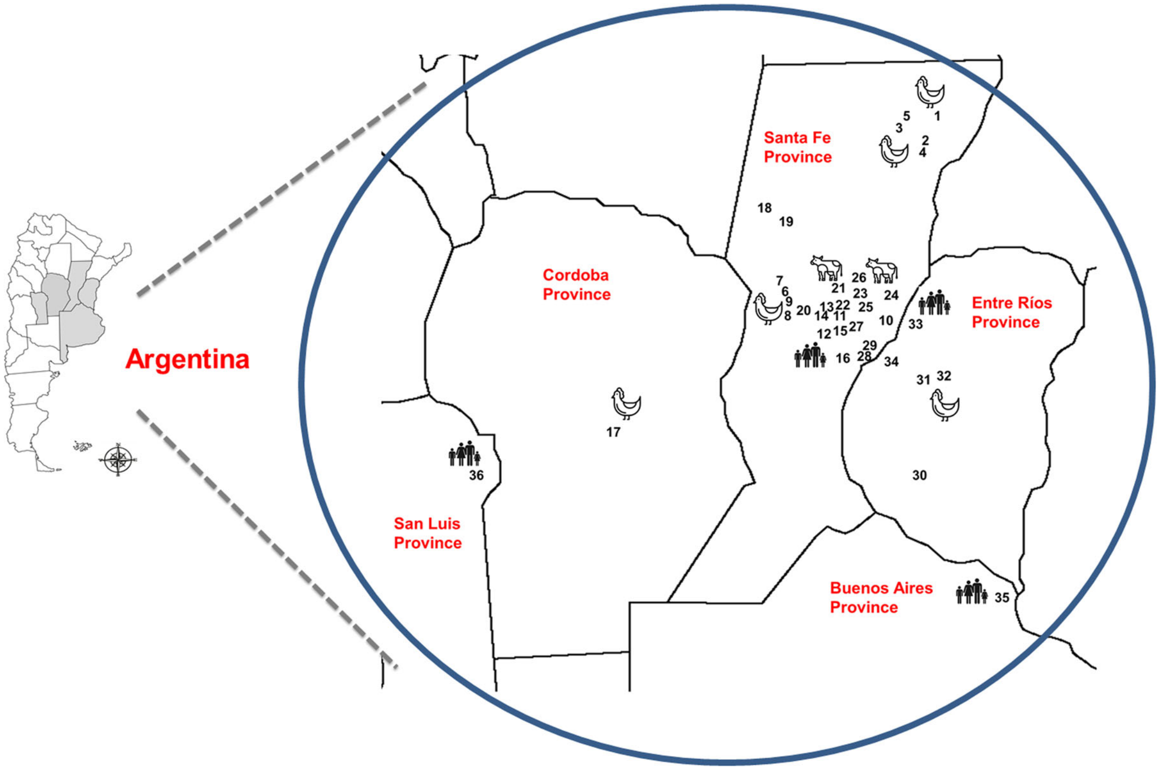

FIGURE 1: Map of the studied area and sampling locations. = poultry farm; = livestock farm; = urban site.

Risk assessment

The impact on the aquatic environment of pollution due to fluoroquinolones was determined through risk assessment measurements. Risk assessment is generally expressed in terms of the risk quotient, according to:

$$\text{Risk quotient} = \frac{\text{MEC}}{\text{PNEC}} \tag{1}$$

where MEC is the measured environmental concentration of the analyte and PNEC is the predicted no-effect concentration for the analyte in relation to the species under consideration. A risk quotient >1 indicates high risk to the aquatic community, and a risk quotient <1 indicates medium or no risk (European Medicines Agency 2006; Grung et al. 2008). In the present study, the MEC values were obtained from each sample by HPLC–FSFD, and the PNEC values of each fluoroquinolone for bacteria, cyanobacteria, algae, plant, crustaceae, anurans, and fish were collected from the literature and are detailed in the Supplemental Data, Table SM3 (Grung et al. 2008; Iatrou et al. 2014; Andrieu et al. 2015).

RESULTS AND DISCUSSION

Assessment of the HPLC–FSFD method performance

Before quantitation of the fluoroquinolones, linearity assessment and calculation of the limits of detection and quantification (LOQ) for the HPLC–FSFD and the UHPLC–MS/MS methods were computed according to Bauza et al. (2012) and Currie (1999), respectively. It is important to highlight that the HPLC–FSFD method is more sensitive than the UHPLC–MS/MS method for all the analytes, except for enoxacin (see the Supplemental Data, Table SM4). Moreover, the method is simple and fast, and does not include preconcentration steps, which are tedious and require the use of solvents harmful to the environment.

To further assess the performance of the HPLC–FSFD method, the percentage ratio values were calculated between the fluoroquinolone concentrations found in each sample by the HPLC–FSFD and UHPLC–MS/MS methods. Because the percentage ratio values were between 82.7 and 118.9%, with an average of 98.3%, the HPLC–FSFD method proved to be suitable to quantitate these analytes in the samples (European Medicines Agency 2012).

Fluoroquinolone determination in environmental water samples

As can be seen in Table 2, several fluoroquinolones were already found and could be quantitated in different environmental water samples. The low LOQs obtained with the HPLC–FSFD method allowed the accurate quantitation of ciprofloxacin in S10, enrofloxacin in S1, S7, S9, S12, and S15, ofloxacin in S3, and difloxacin in S7 and S14, which could not be performed with the UHPLC–MS/MS method. The rest of the analyzed samples, which are not included in Table 2, did not show detectable fluoroquinolone levels.

TABLE 2: Results for the samples found to contain detectable levels of fluoroquinolones[a]

		CPF		ENF		OFL		ENO		DIF	
Sample[b]	Type	HPLC–FSFD	UHPLC–MS/MS	HPLC–FSFD	UHPLC–MS/MS	HPLC–FSFD	UHPLC–MS/MS	HPLC–FSFD	UHPLC–MS/MS	HPLC–FSFD	UHPLC–MS/MS
S1				0.53 ± 0.05	D/NQ						
S3		—	—	—	—	1.04 ± 0.08	NQ	—	—	—	—
S6		7.7 ± 0.3	6.45 ± 0.08	1.24 ± 0.08	1.50 ± 0.02	1.78 ± 0.02	1.97 ± 0.04	22.1 ± 0.2	23.5 ± 0.4	14.2 ± 0.8	14.7 ± 0.2
S7		—	—	0.50 ± 0.09	D/NQ	—	—	—	—	0.57 ± 0.03	D/NQ
S8		1.14 ± 0.04	1.33 ± 0.02	11.9 ± 0.9	11.1 ± 0.5	—	—	—	—	—	—
S9	AWW	—	—	0.70 ± 0.01	D/NQ	—	—	—	—	—	—
S10		0.74 ± 0.08	D/NQ	—	—	—	—	—	—	—	—
S12		—	—	0.7 ± 0.1	D/NQ	—	—	—	—	—	—
S13		—	—	—	—	1.23 ± 0.01	1.29 ± 0.03	14.8 ± 0.7	13.5 ± 0.2	—	—
S14		—	—	1.30 ± 0.06	1.34 ± 0.05	—	—	15.3 ± 0.5	16.2 ± 0.3	0.89 ± 0.04	D/NQ
S15		—	—	0.65 ± 0.06	D/NQ	0.71 ± 0.05	D/NQ	—	—	—	—
S20	River	—	—	0.97 ± 0.02	1.15 ± 0.03	—	—	—	—	—	—

[a]The analytes under study were not detected in AAW samples 2, 4,5,11,16 and 17, neither in river samples 18 to 19 and 21 to 36.
CPF = ciprofloxacin; ENF = enrofloxacin; OFL = ofloxacin; DIF = difloxacin; HPLC-FSFD = high-performance liquid liquid chromatography-fast-scanning fluorescence detector; UHPLC-MS/MS = ultra-high-performance liquid chromatograph-tandem mass spectrometry; D/NQ = detectable but no quantifiable; value below the limit of quantification of the method; AWW = animal wastewater.
[b]The information regarding sampling date and site for the samples is described in Table SM2.

The present study (results summarized in Table 1) sheds light on the prevalence of this type of emerging contaminant in waste and natural waters, which were all located in Santa Fe province. One or more fluoroquinolones were found in 64% of the wastewater samples, and only one in a river sample (S20). Moreover, enrofloxacin had a higher frequency, appearing in 25% of the total samples (wastewater and rivers). It is important to highlight that enrofloxacin was present in 75% of the samples that were found to have detectable fluoroquinolone concentrations, followed by ofloxacin (33%).

In general, the fluoroquinolone concentrations found in the present study are in accordance with the levels reported in the literature (Table 1). However, the presence of enoxacin at concentrations higher than those found by Adachi et al. (2013) and Du et al. (2017) constitutes an important cause for concern; this persistence is probably due to enoxacin's slower degradation process (Andreozzi et al. 2003; Speltini et al. 2010; Sturini et al. 2015). In the present study, the summed concentrations of the detected fluoroquinolones measured in wastewater ranged from 0.50 to 22.1 $\mu g\ L^{-1}$. Santa Fe province is an important producer of meat, and in an enormous part of its territory, poultry and cattle farms are in continuous production. We would thus expect to find fluoroquinolones in this area. In this situation, the risk of environmental contamination caused by intensive livestock and poultry production might be increased because farms and rivers are so close to each other. Moreover, the high rainfall characteristic of this zone can accelerate the runoff and favor the bioaccumulation of fluoroquinolones in aquatic organisms.

Ciproflaxin and enrofloxacin were previously measured by Alcaraz et al. (2016) in a river sample collected at the same place as S20, and their concentrations were found to be 0.4 and 3.6 $\mu g\ L^{-1}$, respectively. Currently, the presence of 0.97 $\mu g\ L^{-1}$ of enrofloxacin shows the continuous existence of fluoroquinolones in this water source. Many studies have noted the ubiquity of enrofloxacin worldwide. With respect to the presence of this fluoroquinolone in river waters, the highest reported concentration (due to huge discharges of domestic and industrial wastewaters [123 $\mu g\ L^{-1}$]) was found in the Musi River in India (Gothwal and Thatikonda, 2017). In addition, enrofloxacin has been found in river waters directly contaminated by large-scale animal farms at a level of 4.42 $\mu g\ L^{-1}$ (Wei et al. 2012). Another 2 instances that deserve mention are a Chinese river (Jiang et al. 2014) and courses at the end of aquaculture systems in Portugal (Pereira et al. 2015), which were found to contain maximum enrofloxacin concentrations of 0.979 $\mu g\ L^{-1}$ and 0.0232 $\mu g\ L^{-1}$, respectively. The presence of enrofloxacin in this kind of natural water course constitutes a huge environmental problem related to the generation of harmful effects on native flora and fauna, and on the population as well. In this regard, Peltzer et al. (2017) have demonstrated the negative effects that enrofloxacin and ciprofloxacin have on the development of the South American common toad *Rhinella arenarum* (Anura: Bufonidae). In their in vitro study of biological endpoints (mainly development, growth, and antioxidant enzyme activities), impairments were observed after exposure to enrofloxacin and ciproflaxin; the authors concluded that continuous exposure to these substances can lead

TABLE 3: Calculated risk quotients for different taxonomic groups according to their exposure to fluoroquinolones (FQs) found in environmental aqueous samples (concentrations and PNECs in μg L^{-1})

			Bacteria		Cyanobacteria		Algae		Plants		Crustaceae		Anurans		Fish	
FQ	Sample	Concentrations (MEC)	PNEC	RQ	PNEC	RQ	PNEC	RQ	PNEC	RQ	PNEC	RQ	PNEC	RQ	PNEC	RQ
CPF	6	7.7	120[a]	0.07	0.005[b]	1500	0.007[c]	1100	0.20[d]	40	1.2[e]	6.4	1.0[f]	7.7	60[e]	0.13
	8	1.14		0.01		230		170		5.6		0.95		1.1		0.02
	10	0.74		0.006		150		110		3.6		0.62		0.74		0.01
ENF	1	0.53	0.03[g]	20	0.05[h]	10	0.05[c]	10	0.11[b]	4.9	11.5[a]	0.05	1.0[f]	0.56	79.5[e]	<0.01
	6	1.24		40		30		30		10		0.11		1.2		0.07
	7	0.50		20		10		10		4.4		0.04		0.50		<0.01
	8	11.9		410		240		240		100		1.0		>10		0.15
	9	0.70		20		10		10		6.1		0.06		0.70		0.01
	12	0.7		20		10		10		6.1		0.06		0.70		<0.01
	14	1.30		50		30		30		10		0.11		1.3		0.07
	15	0.65		20		10		10		5.7		0.06		0.65		<0.01
	20	0.97		30		20		20		8.5		0.08		0.97		0.01
OFL	3	1.04	0.001[b]	1000	0.02[h]	50	0.01[b]	100	0.13[a]	8.3	1.4[b,i]	0.72			0.53[b,i]	2.0
	6	1.78		1800		90		160		10		1.2				3.4
	13	1.23		1200		60		110		10		0.85				2.3
	15	0.71		700		30		70		5.6		0.49				1.3
ENO	6	22.1	0.003[j]	7700			8100[h]	<0.01			8100[h]	<0.01			1000[a]	0.02
	13	14.5		5000				<0.01				<0.01				0.02
	14	15.3		5000				<0.01				<0.01				0.02
DIF	6	14.2					242[c]	0.06			2500[c]	<0.01			2300[c]	<0.01
	7	0.57						<0.01				<0.01				<0.01
	14	0.89						<0.01				<0.01				<0.01

[a] Van Doorslaer et al. 2014.
[b] Ashfaq et al. 2017.
[c] Pereira et al. 2015.
[d] Robinson et al. 2005.
[e] Andrieu et al. 2015.
[f] Peltzer et al. 2017.
[g] Jiang et al. 2014.
[h] Du et al. 2017.
[i] Iatrou et al. 2014.
[j] Orias and Perrodin 2013.
CPF = ciproflaxin; ENF = enroflaxin; OFL = ofloflaxin; ENO = enoxacin; DIF = difloxacin; MEC = measured environmental concentration; PNEC = predicted no-effect concentration; RQ = risk quotient.

to potential damage to long-term maintenance of *R. arenarum* natural larvae populations.

It is important to note that, although the Argentinian government has set maximum residue limits for veterinary pharmaceuticals in animal foodstuff (Argentine National Food Safety and Quality Service 2011), no regulations have been issued to control the presence of these analytes in the discharge of livestock wastewater. Therefore, study of the environmental contamination constitutes a highly important step with an impact on the development of appropriate regulations.

Environmental risk assessment of fluoroquinolones

The risk of fluoroquinolones to different aquatic species in the investigated areas was classified into 4 levels, as follows: no risk for risk quotients <0.01, low risk for risk quotients between 0.01 and 0.1, medium risk for risk quotients between 0.1 and 1, and high risk for risk quotients >1 (Andrieu et al. 2015; Ashfaq et al. 2017). Table 3 summarizes the ecological risk assessment performed in the samples containing detectable fluoroquinolones levels. It should be mentioned that in some cases the risk quotients were not calculated because the PNEC values were not available. In-depth analysis of the results achieved by the implementation of this simple, screening-level estimate allowed us to discriminate between high- or low-risk situations (see Figure 2 for enrofloxacin and the Supplemental Data, Figure SM1, for the other target fluoroquinolones). In general terms, the fluoroquinolone levels found in both river and wastewater samples pose at least medium risk to the aquatic organism. In the case of ofloxacin and enrofloxacin, high and medium risks prevail for all the species, with the same holding true ciprofloxacin, except for bacteria. In contrast, high risk was observed for bacteria due to the presence of enoxacin. In conclusion, the actual concentrations of ciprofloxacin, enrofloxacin, and ofloxacin in all sampling sites pose a threat to several species. With respect to algae, cyanobacteria, and invertebrates, similar results for ciprofloxacin, ofloxacin, and enrofloxacin were also reported in wastewater from Pakistan (Riaz et al. 2017), in the Mekong River Delta, Vietnam (Andrieu et al. 2015), in the Wangyang River in China (Jiang et al. 2014), and in Lake Chaohu, China (Tang et al. 2015).

Taking into account the calculated risk quotient and the species analyzed, 87.5% of the samples in which ofloxacin was quantitated was found to present a high risk for the species under study. With respect to enrofloxacin, ciprofloxacin, and enoxacin, 63.5, 57.1, and 25.0% of the samples in which they were quantitated, respectively, presented a high risk for the analyzed species. In addition, between 2 and 6% of the samples were found to imply a medium risk in relation to ciprofloxacin, enrofloxacin, and ofloxacin. Moreover, a previous study carried out on a sample collected at the same location as S20 (Alcaraz et al. 2016) showed high and medium risks for cyanobacteria, algae, plants, crustaceae, and anurans because of ciprofloxacin, and high and medium risks in bacteria, cyanobacteria, algae, plants, crustaceae, and anurans due to enrofloxacin. These values suggest that the presence of fluoroquinolones in rivers and wastewaters of the studied region constitutes a potential risk factor for the aquatic ecosystem that could be injurious to biodiversity and to the ecosystems of aquatic organisms. Future efforts should gather additional experimental data related to the chronic effects fluoroquinolones may have on aquatic organisms, focusing on an estimation of the toxicity of fluoroquinolone mixtures and on a clarification of their mode of action in these organisms.

CONCLUSIONS

In recent years, fluoroquinolones have appeared as a new class of emerging contaminant in the aquatic environment of many countries. The presence of fluoroquinolones may compromise drinking water quality and aquatic ecosystems. The present study investigated the presence of 5 fluoroquinolones in wastewaters from farms and rivers of 5 provinces in Argentina.

In the target sources, fluoroquinolones were only found in waters localized in Santa Fe province. In terms of the wastewaters analyzed, most of them (70%) had at least one type of fluoroquinolone, whereas others were found to have 2 or more fluoroquinolones simultaneously; this association may promote drug interactions that may increase their concomitant adverse effects on nontarget hosts. Specifically, the evidence suggests

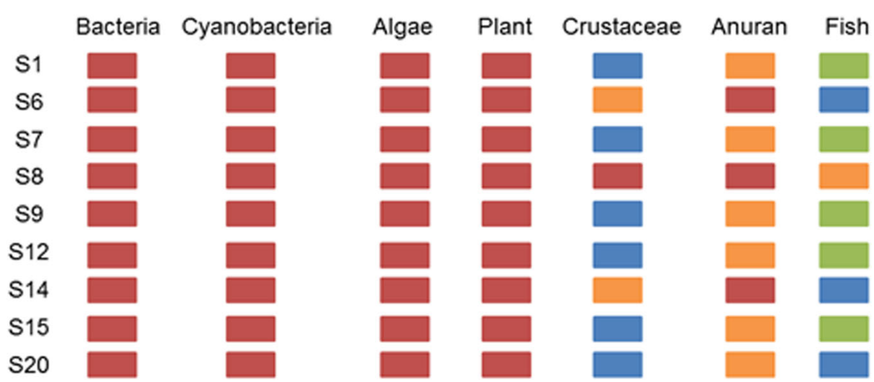

FIGURE 2: Diagrams based on the calculated risk quotients for enrofloxacin detected in river and wastewater. S1, 6, 7, 8, 9, 12, 14, and 15 correspond to wastewater samples and S20 to a river sample. The colors correspond to: high risk (red), medium risk (orange), low risk (blue), and risk quotient <0.01 (green).

that enrofloxacin and ofloxacin were the fluoroquinolones of choice for livestock treatment across the studied area. The detection of enrofloxacin in the farm wastewaters was not unexpected given that it has been extensively used in multiple treatments of different animal species since its introduction in livestock operations, especially in intensively stabled facilities. Moreover, the presence of ofloxacin suggests that this fluoroquinolone is currently used in livestock operations in Santa Fe in single treatments or in combination with enrofloxacin.

In addition, the environmental risk assessment of fluoroquinolones was carried out for different types of species. The results suggest that enrofloxacin levels in wastewater are potentially risky for bacteria, cyanobacteria, algae, and plants. The calculated value for the South American common toad *R. arenarum* has been found to be >1, showing a negative effect on native fauna (Peltzer et al. 2017). The risk quotient values for ofloxacin were >1 for bacteria, cyanobacteria, algae, plants, and fish. In most cases, the risk quotient values were >1 for the majority of the species, suggesting that risk of damage is especially due to the presence of enrofloxacin and ofloxacin. Efforts need to be made to control their potential environmental risk by taking action to diminish the discharge of these emerging contaminants in water courses mainly in Santa Fe province, which is an important producer of meat, poultry, and cattle.

Finally, more research focused on the ecotoxicological risks associated with fluoroquinolone pollution is needed, especially to assess chronic effects on environmental habitats. The scientific findings should help policy makers to decide which compounds pose a threat to the aquatic environment and need to be included in the priority substance lists defined in legislative frameworks, such as that issued by the Argentine National Food Safety and Quality Service (2011). Moreover, ecotoxicological data can be used to set environmental quality standards for monitoring of ambient water, sediments, and biota, especially taking into account the native flora and fauna.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4532.

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Data Accessibility—Data, associated metadata, and calculation tools are available from the corresponding author (mculzoni@fbcb.unl.edu.ar).

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