

Article

Long-Term Study of Antibiotic Presence in Ebro River Basin (Spain): Identification of the Emission Sources

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Supplementary Materials:

Table S1. Sulfadiazine quantitative concentration results in surface control points (ng/L). D = detected (LOD < D < LOQ), n/d = not detected (<LOD).

RIVER	SUB-BASIN	LOCATION	SULFADIAZINE (ng/L)					
			Spring 2018	Autumn 2018	Spring 2019	Autumn 2019	Autumn 2020	Spring 2021
Segre River	Segre	Torres de Segre	n/d	D	D	5	120	9
Noguera Ribagorzana River	Segre	Corbins	D	D	D	28	214	118
Clamor Amarga River	Cinca	Zaidín	300	19	D	3	227	16
Cinca River	Cinca	Fraga	n/d	D	D	3	325	4
Alcanadre River	Alcanadre	Sariñena	n/d	n/d	D	8	n/d	1
Flumen River	Alcanadre	Albalatillo	D	40	D	2	95	30
Gállego River	Gallego	San Mateo de Gállego	n/d	D	D	34	85	1
Arba de Ríquel River	Ebro	Ejea de los Caballeros	D	n/d	n/d	18	521	3
Aragon Subordan River	Aragón	Javierregay	n/d	n/d	n/d	n/d	50	n/d
Aragon River	Aragón	Caparroso	n/d	n/d	n/d	1	12	n/d
Irantzu River	Ega	Estella	n/d	21	n/d	4	144	27
Arakil River	Arga	Irañeta	n/d	23	D	20	13	2
Queiles River	Arga	Novallas	D	n/d	n/d	1.5	12	2
Alhama River	Alhama	Alfaro	n/d	n/d	n/d	2	13	3
Ega River	Ega	Estella	D	n/d	n/d	2	20	14
Ega River	Ega	Estella	n/d	n/d	D	n/d	11	n/d
Arga River	Arga	Arazuri	n/d	D	258	1.6	n/d	n/d
Arga River	Arga	Ororbía	D	16	D	2	n/d	7
Ebro River	Ebro	Fontellas	D	D	D	12	n/d	n/d
Ebro River	Ebro	Tudela	n/d	n/d	D	77	n/d	n/d

It should be taken in account that spring 2020 sampling campaign was postponed to autumn 2020 as a result of COVID-19.

Table S2. Enrofloxacin quantitative concentration results (ng/L) in surface control points (ng/L). D = detected (LOD < D < LOQ), n/d = not detected (<LOD).

RIVER	SUB-BASIN	LOCATION	ENROFLOXACIN (ng/L)					
			Spring 2018	Autumn 2018	Spring 2019	Autumn 2019	Autumn 2020	Spring 2021
Segre River	Segre	Torres de Segre	240	140	D	6	106	5
Noguera Ribagorzana River	Segre	Corbins	250	D	523	17	31	966
Clamor Amarga River	Cinca	Zaidín	350	D	D	32	41	5
Cinca River	Cinca	Fraga	380	D	D	5	28	2
Alcanadre River	Alcanadre	Sariñena	n/d	D	n/d	n/d	11	2
Flumen River	Alcanadre	Albalatillo	D	D	D	n/d	7	13
Gállego River	Gallego	San Mateo de Gállego	1,560	D	D	11	88	3
Arba de Ríquel River	Ebro	Ejea de los Caballeros	4,390	D	D	314	107	4
Aragon Subordan River	Aragón	Javierregay	240	n/d	n/d	n/d	8	2
Aragon River	Aragón	Caparroso	280	D	D	n/d	15	3
Irantzu River	Ega	Estella	240	n/d	n/d	n/d	11	15
Arakil River	Arga	Irañeta	260	n/d	n/d	n/d	n/d	7
Queiles River	Arga	Novallas	250	D	D	2	n/d	2
Alhama River	Alhama	Alfaro	350	D	n/d	3	18	7
Ega River	Ega	Estella	250	2,920	n/d	n/d	n/d	9
Ega River	Ega	Estella	240	330	n/d	1.5	15	2
Arga River	Arga	Arazuri	590	210	D	n/d	4	3
Arga River	Arga	Ororbia	650	D	D	n/d	n/d	13
Ebro River	Ebro	Fontellas	D	D	3,033	n/d	42	11
Ebro River	Ebro	Tudela	240	D	672	200	39	4

It should be taken in account that spring 2020 sampling campaign was postponed to autumn 2020 as a result of COVID-19.

Table S3. Azithromycin quantitative concentration results (ng/L) in surface control points (ng/L). D = detected (LOD < D < LOQ), n/d = not detected (<LOD).

RIVER	SUB-BASIN	LOCATION	AZITHROMYCIN (ng/L)					
			Spring 2018	Autumn 2018	Spring 2019	Autumn 2019	Autumn 2020	Spring 2021
Segre River	Segre	Torres de Segre	n/d	n/d	D	n/d	22	2
Noguera Ribagorzana River	Segre	Corbins	n/d	n/d	n/d	n/d	n/d	4
Clamor Amarga River	Cinca	Zaidín	n/d	n/d	n/d	n/d	n/d	4
Cinca River	Cinca	Fraga	n/d	n/d	D	n/d	n/d	n/d
Alcanadre River	Alcanadre	Sariñena	n/d	n/d	D	n/d	n/d	n/d
Flumen River	Alcanadre	Albalatillo	n/d	n/d	D	n/d	n/d	1
Gállego River	Gallego	San Mateo de Gállego	n/d	n/d	n/d	n/d	n/d	1
Arba de Ríquel River	Ebro	Ejea de los Caballeros	n/d	n/d	n/d	n/d	n/d	n/d

Aragon Subordan River	Aragón	Javierregay	n/d	n/d	n/d	n/d	n/d	n/d
Aragon River	Aragón	Caparroso	n/d	n/d	n/d	n/d	n/d	n/d
Irantzu River	Ega	Estella	n/d	n/d	D	n/d	n/d	1
Arakil River	Arga	Irañeta	n/d	n/d	n/d	n/d	n/d	n/d
Queiles River	Arga	Novallas	n/d	n/d	D	22	n/d	1
Alhama River	Alhama	Alfaro	n/d	n/d	n/d	4	n/d	5
Ega River	Ega	Estella	n/d	n/d	n/d	3	n/d	1
Ega River	Ega	Estella	n/d	n/d	D	n/d	12	2
Arga River	Arga	Arazuri	n/d	n/d	D	n/d	n/d	1
Arga River	Arga	Ororbia	n/d	739	D	n/d	n/d	0
Ebro River	Ebro	Fontellas	n/d	n/d	n/d	n/d	n/d	5
Ebro River	Ebro	Tudela	n/d	n/d	n/d	n/d	n/d	1

It should be taken in account that spring 2020 sampling campaign was postponed to autumn 2020 as a result of COVID-19.

Table S4. Trimethoprim quantitative concentration results (ng/L) in surface control points. D = detected (LOD<D<LOQ), n/d = not detected (<LOD).

RIVER	SUB-BASIN	LOCATION	TRIMETHOPRIM (ng/L)					
			Spring 2018	Autumn 2018	Spring 2019	Autumn 2019	Autumn 2020	Spring 2021
Segre River	Segre	Torres de Segre	n/d	D	D	n/d	138	12
Noguera Ribagorçana River	Segre	Corbins	n/d	n/d	D	30	246	106
Clamor Amarga River	Cinca	Zaidín	D	n/d	D	n/d	153	4
Cinca River	Cinca	Fraga	n/d	D	D	n/d	n/d	1
Alcanadre River	Alcanadre	Sariñena	n/d	n/d	D	5	n/d	n/d
Flumen River	Alcanadre	Albalatillo	D	n/d	D	n/d	n/d	1
Gállego River	Gallego	San Mateo de Gállego	n/d	D	D	6	n/d	n/d
Arba de Ríquel River	Ebro	Ejea de los Caballeros	n/d	D	n/d	4	n/d	n/d
Aragon Subordan River	Aragón	Javierregay	n/d	n/d	n/d	n/d	n/d	1
Aragon River	Aragón	Caparroso	n/d	n/d	n/d	n/d	n/d	1
Irantzu River	Ega	Estella	n/d	D	D	n/d	n/d	1
Arakil River	Arga	Irañeta	n/d	D	n/d	n/d	n/d	6
Queiles River	Arga	Novallas	D	D	D	3	n/d	4
Alhama River	Alhama	Alfaro	D	n/d	D	n/d	n/d	2
Ega River	Ega	Estella	D	n/d	n/d	n/d	n/d	1
Ega River	Ega	Estella	n/d	D	D	n/d	n/d	1
Arga River	Arga	Arazuri	n/d	D	D	n/d	n/d	1
Arga River	Arga	Ororbia	10	D	189		n/d	16
Ebro River	Ebro	Fontellas	D	D	n/d	n/d	n/d	96
Ebro River	Ebro	Tudela	n/d	D	n/d	4	n/d	23

It should be taken in account that spring 2020 sampling campaign was postponed to autumn 2020 as a result of COVID-19.

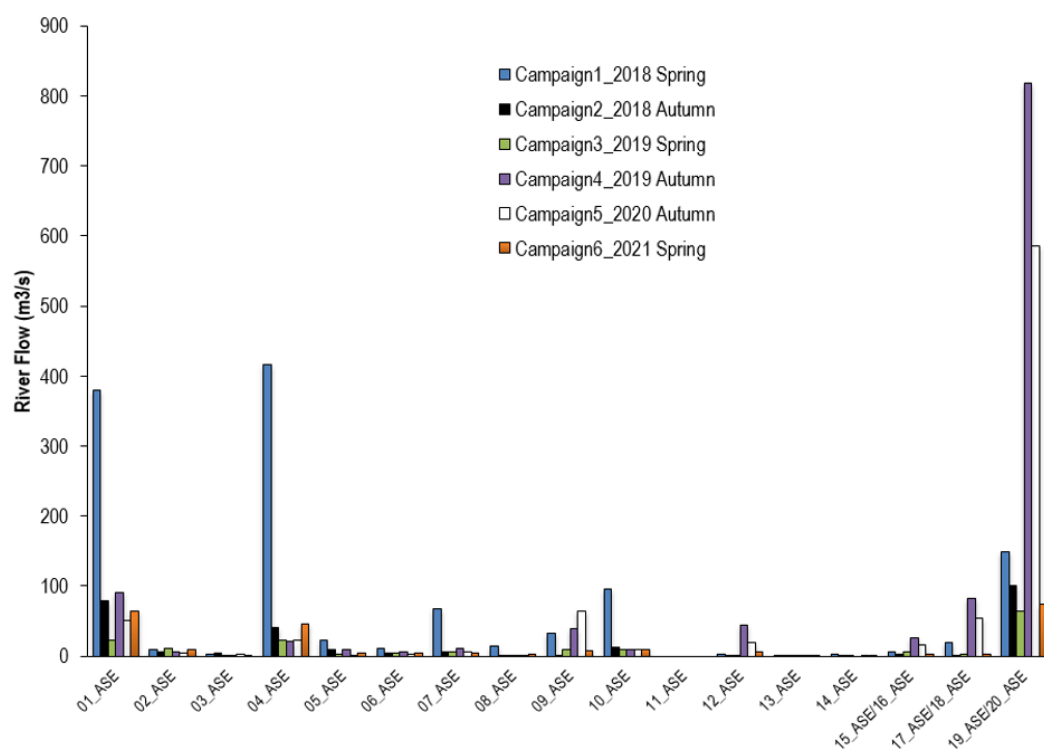


Figure S1. River flow during the six sampling campaigns 2018–2021.

Table S5. One-way ANOVA test of antibiotics concentration in high-livestock-pressure points.

Sources of Variation	Sum of Squares	Degree of Freedom	Mean Square	Factor F	p-Value
Between antibiotics	88,463	3	294,876,667	F = 4.41669	0.009 *
Within antibiotics	2,670,569,091	40	66,764,227		
Total	3,555,199,091	43			

* significant at $p < 0.05$.**Table S6.** Tukey's honestly significant difference test results in high-livestock pressure points.

Pairwise Comparisons	HSD.05 = 93.3888 HSD.01 = 115.6699		Q.05 = 3.790 Q.01 = 4.695
SDZ:ENR	M1 = 25.91 M2 = 114.09	88.18	Q = 3.58 (p -value = 0.070) **
SDZ:TMT	M1 = 25.91 M3 = 10.73	15.18	Q = 0.62 (p -value = 0.971)
SDZ:AZI	M1 = 25.91 M4 = 1.09	24.82	Q = 1.01 (p -value = 0.892)
ENR:TMT	M2 = 114.09 M3 = 10.73	103.36	Q = 4.20 (p -value = 0.025) **
ENR:AZI	M2 = 114.09 M4 = 1.09	113.00	Q = 4.59 (p -value = 0.012) **
AZI:TMT	M3 = 10.73 M4 = 1.09	9.64	Q = 0.39 (p -value = 0.992)

** significant at $p < 0.05$. M = mean, Q = Studentized range distribution statistic, HSD = honestly significant difference**Table S7.** One-way ANOVA test of sulfadiazine concentration between the reference point ASE19 and the points exposed to high and medium livestock pressure.

	Sum of Squares	Degree of Freedom	Mean Square	Factor F	p-Value
Between-treatments	82,990,139	1	82,990,139	F = 5.24636	0.032 *
Within-treatments	348,009,444	22	15,818,611		
Total	430,999,583	23			

* significant at $p < 0.05$.**Table S8.** One-way ANOVA test of antibiotics concentration in WWTPs.

Sources of Variation	Sum of Squares	Degree of Freedom	Mean Square	Factor F	p-Value
Between antibiotics	353,593,698,419	3	11,786,456,614	F = 9.49363	0.009 *
Within antibiotics	434,529,413,889	35	12,415,126,111		
Total	788,123,112,308	38			

* significant at $p < 0.05$.

Table S9. Tukey's honestly significant difference test results in WWTPs.

Pairwise Comparisons		HSD.05 = 1,362.4039	Q.05 = 3.8140
		HSD.01 = 1,692.9315	Q.01 = 4.7393
SDZ:ENR	M ₁ = 181.20	186.30	Q = 0.52 ($p = 0.98259$)
	M ₂ = 367.50		
SDZ:TMT	M ₁ = 181.20	602.91	Q = 1.69 ($p = 0.63495$)
	M ₃ = 784.11		
SDZ:AZI	M ₁ = 181.20	2,376.20	Q = 6.65 ($p = 0.00022$) *
	M ₄ = 2,557.40		
ENR:TMT	M ₂ = 367.50	416.61	Q = 1.17 ($p = 0.84239$)
	M ₃ = 784.11		
ENR:AZI	M ₂ = 367.50	2,189.90	Q = 6.13 ($p = 0.00065$) *
	M ₄ = 2,557.40		
AZI:TMT	M ₃ = 784.11	1,773.29	Q = 4.96 ($p = 0.00656$) *
	M ₄ = 2,557.40		

* significant at $p < 0.05$. M = mean, Q = Studentized range distribution statistic, HSD= honestly significant difference.