Are slaughterhouse effluents safe for water reuse?

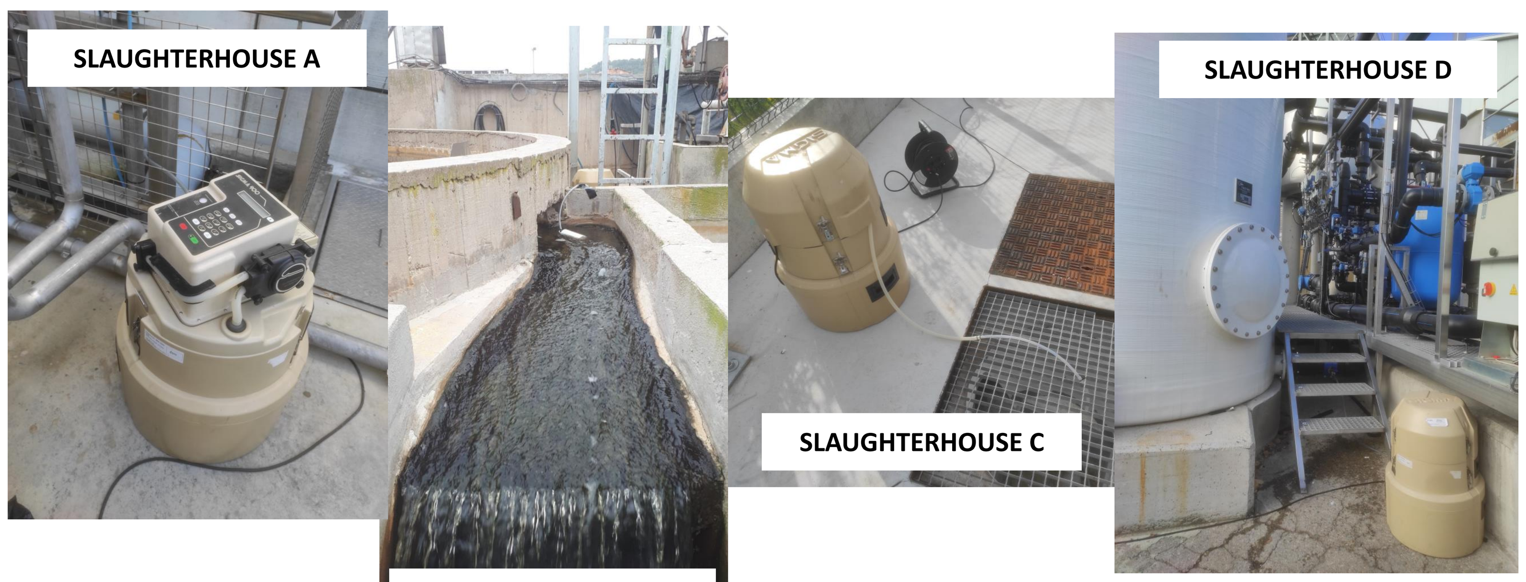
M. Gros[1,2], A. Sànchez-Melsió[1,2], J.L. Balcázar[1,2], C.M. Borrego[1,3], I. de Bona Muñoz[4], L. Paredes[4], E. Vega[4]

[1] Institut Català de Recerca de l'Aigua (ICRA), Girona, Spain. *ICRA is a CERCA Center*. e-mail: mgros@icra.cat
[2]University of Girona (UdG), Girona, Spain
[3]Group of Molecular Microbial Ecology, Institute of Aquatic Ecology, University of Girona (UdG), Girona, Spain
[4]BETA Tech Centre, TECNIO network University of Vic-Central University of Catalonia, Spain

INTRODUCTION & OBJECTIVES

The food industry constitutes one of the major water consumers in Europe, accounting for the 12% of the total water used in the industrial sector. Among industries, the meat processing sector is one of the biggest water users, with an estimated average water consumption in Spanish slaughterhouses between 500-1000 and 250-500 litres per animal in the cattle and swine production, respectively. Slaughterhouse effluents are characterized by a high organic matter and nutrient content, with high loads of veterinary pharmaceuticals, antibiotic resistance genes (ARGs) and microbial pathogens. Accordingly, most slaughterhouses usually have their own wastewater treatment, with an outlet connected to the municipal sewer to further treat these waters in the urban wastewater treatment systems. This study shows the results of the chemical and microbiological characterization of slaughterhouse effluents, performed within the frame of the REAQUA project, funded by the Catalan Department of Climate Action, Food and Rural agenda (DACC) and within the Cooperació per a la Innovació program. The main goal of the project is to demonstrate the feasibility of reusing treated slaughterhouse effluents, by using advanced tertiary treatments such as membrane technologies (e.g. ultrafiltration, reverse osmosis, electrodialysis) and advanced oxidation processes (e.g. ozonation and UV), to use the produced water in external activities to the production process. Effluent wastewaters were collected and analysed for pharmaceuticals, antibiotics, ARGs concentrations and microbial pathogens to evaluate the potential chemical and microbiological risks associated with slaughterhouse wastewater reuse.

SAMPLING



- **Four major slaughterhouses** in north-Eastern Catalonia (Spain), an area with intensive livestock production, were sampled
- **Effluent wastewaters** derived from the conventional treatments applied in the slaughterhouses, were **collected for four weeks**, in the period from October 2022 to January 2023
- **Veterinary pharmaceuticals**, **ARGs and microbial pathogens** were analysed in the samples

METHODOLOGY

PHARMACEUTICALS ANALYSIS (30 compounds)

Pre-treatment
- Sample filtration (GFF 1 µm; PVDF 0.45 µm)
- Addition of IS mix
- Addition of Na_2EDTA 0.1M solution

Pre-concentration
Solid Phase Extraction (SPE)
Oasis HLB (200 mg, 6mL)
50 mL sample

Elution
- Elution with MeOH (4x 2mL)
- Extract evaporation (N_2)
- Reconstitution 1mL $MeOH/H_2O$ (50:50, v/v)

Instrumental analysis
UPLC-MS/MS (QqLIT)

ARGS ANALYSIS

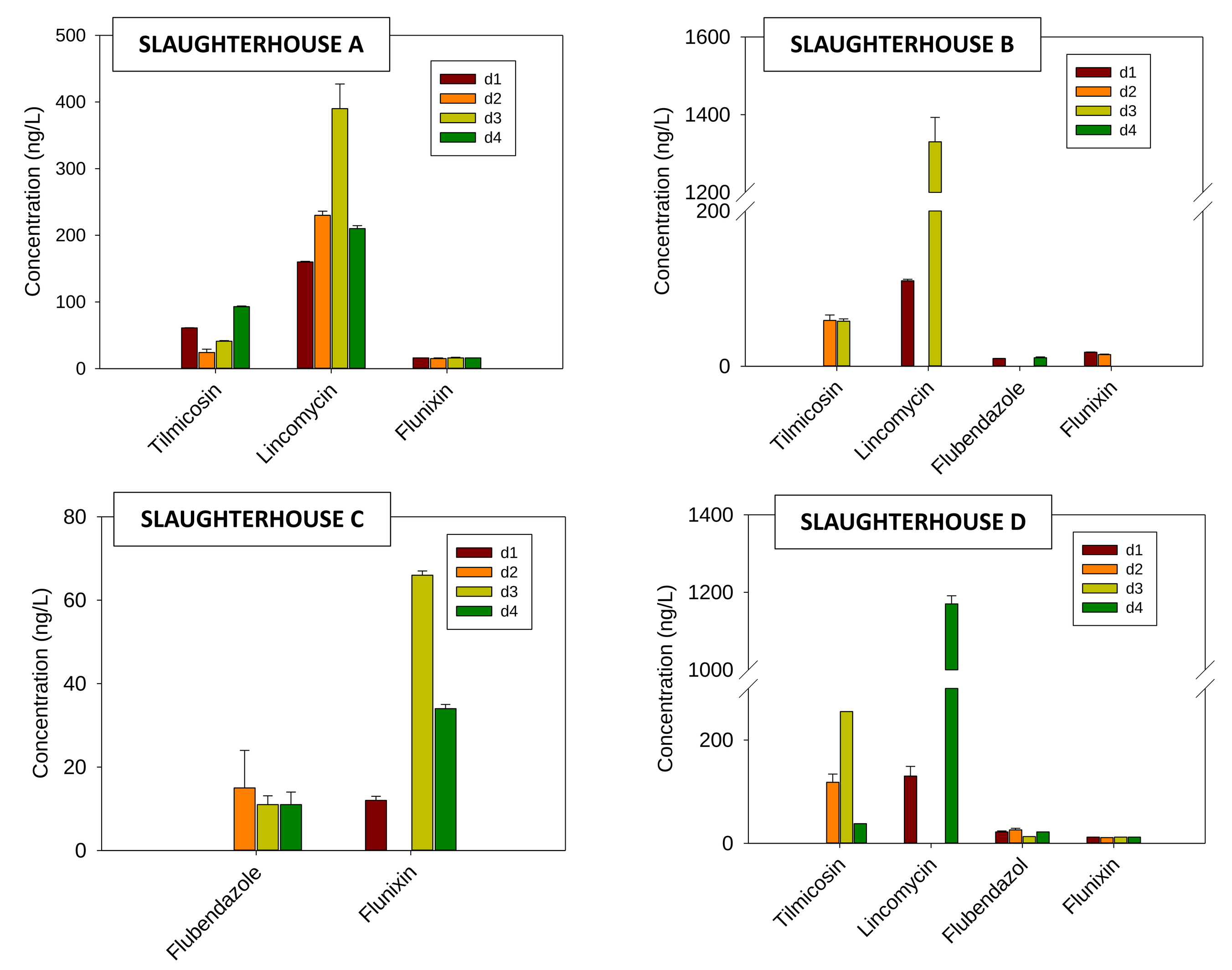
Sample filtration
Membrane filters 0.22 µm
50-200 mL sample (n=3)

DNA extraction

qPCR
sulI, qnrS, tetW, ermB, blaKPC, mcr1, intI1, 16SrRNA

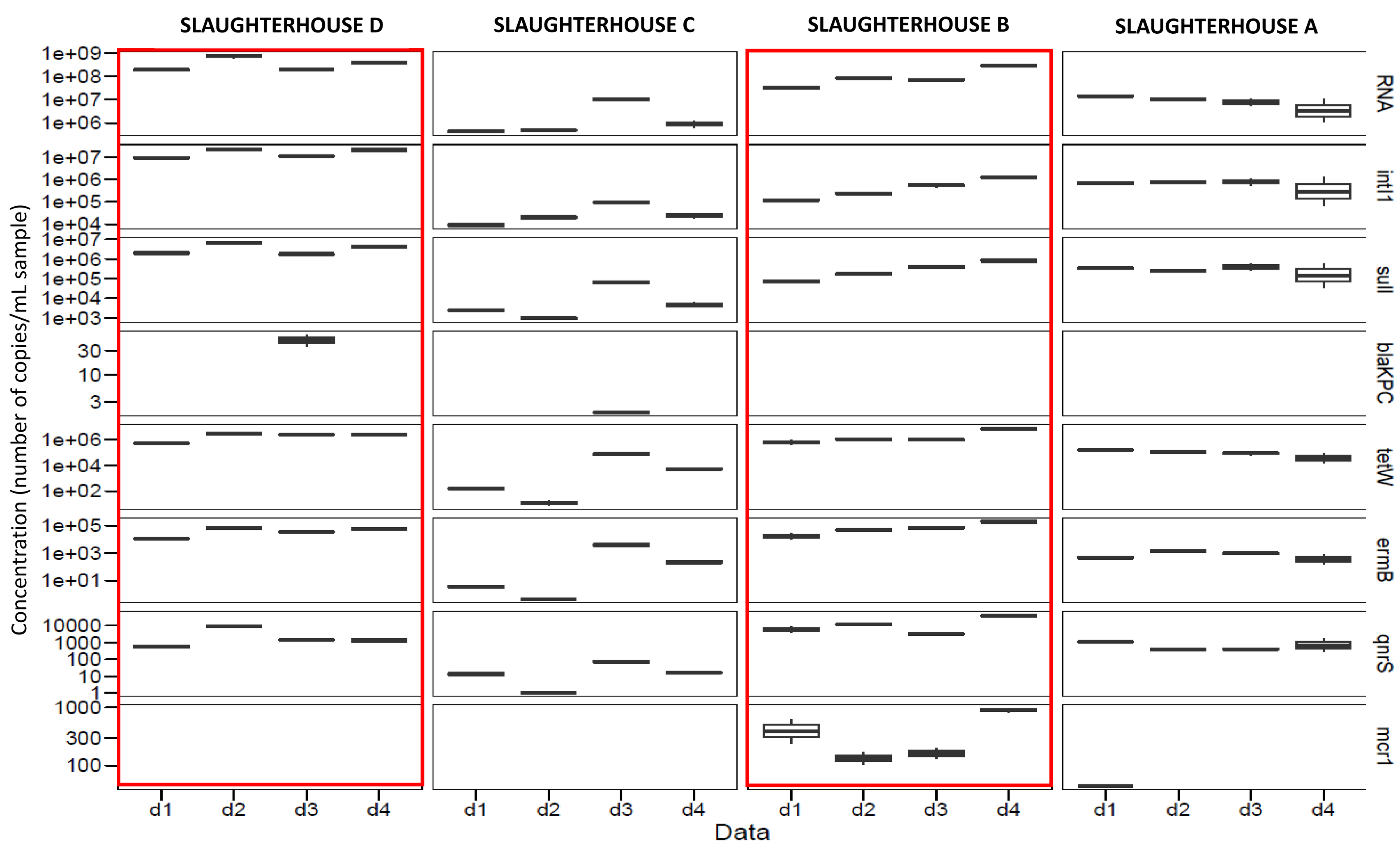
RESULTS

1. PHARMACEUTICALS AND ANTIBIOTICS DETECTED



Representative pharmaceuticals and antibiotics detected in the slaughterhouse effluents and their concentrations along the four sampling days. Tilmicosin and Lincomycin are macrolide and lincosamide antibiotics, respectively, while flubendazole is an anti-helmintic drug and flunixin an analgesic and anti-inflammatory of wide use in veterinary medicine

2. ANTIBIOTIC RESISTANCE GENES (ARGs)



Absolute concentrations (number of copies/mL) of the ARGs detected in all the samples. ARGs measured confer resistance to different antibiotic classes, such as sulfonamides (*sul1*), ß-lactams (*blaKPC*), tetracyclines (*tetW*), macrolides (*ermB*), fluoroquinolones (*qnrS*) and colistin (*mcr1*). Slaughterhouses with the highest ARGs detections are highlighted in red

3. MICROBIAL PATHOGENS: microorganisms analyzed and the parameters tested

SLAUGHTERHOUSE A

Parameter	Unit	d1	d2	d3	d4
Microorganisms 22ºC	CFU/1mL	7,6 x 10 4	1,4 x 10 5	4,6 x 10 4	2,4 x 10 4
Enterococcus	CFU/1mL	< 1	3,9 x 10 3	1,6 x 10 3	1,9 x 10 3
Escherichia coli	CFU/100mL	< 100	< 100	2 x 10 3	1,2 x 10 4
Samonella	Presence	Yes	NO	NO	NO
Listeria	CFU/1 mL	< 1	< 1	< 1	< 1
Trichina	Presence	NO	NO	NO	NO
Nematodes	Eggs / 10L	0	0	0	0
Legionella	CFU/1 L	< 100	< 100	< 100	< 100

SLAUGHTERHOUSE B

Parameter	Unit	d1	d2	d3	d4
Microorganisms 22ºC	CFU/1 mL	5,6 x 10 3	5,5 x 10 4	1,7 x 10 4	1,7 x 10 5
Enterococcus	CFU/1 mL	< 1	8,7 x 10 3	8,6 x 10 2	2,9 x 10 4
Escherichia coli	CFU/100 mL	< 100	1,9 x 10 5	1,7 x 10 3	< 100
Samonella	Presence	NO	NO	NO	NO
Listeria	CFU/1 mL	< 1	< 1	< 1	< 1
Trichina	Presence	NO	NO	NO	NO
Nematodes	Eggs / 10L	0	0	0	0
Legionella	CFU/1 L	< 100	< 100	< 100	< 100

SLAUGHTERHOUSE C

Parameter	Unit	d1	d2	d3	d4
Microorganisms 22ºC	CFU/1 mL	4,6 x 10 4	6,9 x 10 3	9,8 x 10 3	9,8 x 10 4
Enterococcus	CFU/1 mL	1,5 x 10 1	1,9 x 10 1	8,6 x 10 1	1,6 x 10 1
Escherichia coli	CFU/100 mL	< 100	< 100	< 100	< 100
Salmonella	Presence	NO	NO	NO	NO
Listeria	CFU/1 mL	< 100	< 100	< 100	< 100
Trichina	Presence	NO	NO	NO	NO
Nematodes	Eggs / 10L	0	0	0	0
Legionella	CFU/1 L	< 100	< 100	< 100	< 100

SLAUGHTERHOUSE D

Parameter	Unit	d1	d2	d3	d4
Microorganisms 22ºC	CFU/1 mL	1,9 x 10 4	1,2 x 10 3	1,3 x 10 3	8,7 x 10 4
Enterococcus	CFU/1 mL	2,7 x 10 3	< 1	< 1	7,6 x 10 2
Escherichia coli	CFU/100 mL	< 100	< 100	< 100	8 x 10 3
Salmonella	Presence	NO	NO	NO	NO
Listeria	CFU/1 mL	< 1	< 1	< 1	< 1
Trichina	Presence	NO	NO	NO	NO
Nematodes	Eggs / 10L	0	0	0	0
Legionella	CFU/1 L	< 100	< 100	< 100	< 100

The values highlighted in red are those exceeding the maximum admissible values according to the national regulations (RD 1620/2007) for wastewater reuse for agricultural, urban and industrial purposes.

CONCLUSIONS

- The most representative compounds, in terms of frequency of detection and concentration, identified in the samples were antibiotics, anti-helmintics and anti-inflammatories of major use in veterinary medicines, such as tilmicosin, lincomycin, flubendazole and flunixin. Other antibiotics, such as tiamulin, sulfamethoxazole, sulfamethazine, oxytetracycline, enrofloxacin and marbofloxacin, were detected in a limited number of samples.
- For ARGs, *sul1* (resistance to sulfonamides), and *tetW* (resistance to tetracyclines) were the most prevalent, together with *intl1* gene, with concentrations ranging from 10^5 to 10^8 gene copies/mL. The other genes, *ermB*, *qnrS* and *mcr1* (resistance to macrolides, fluoroquinolones and colistin, respectively), were less represented.
- Some pathogens, such as *Escherichia coli* (*E. coli*) and *Salmonella* were present in some effluents, while *Listeria* and *Trichina* were not detected. The values for *E. coli* and *Salmonella* exceeded the maximum admissible values included in the Spanish national regulations (RD 1620/2007) concerning wastewater reuse for agricultural, urban and industrial purposes in some samples.
- These results suggest that the implementation of advanced treatment technologies is recommended to ensure a safe water reuse in the meat industry.

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