



4th INTERNATIONAL CONFERENCE ON RISK ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT

Barcelona, 9 - 10 October 2023

Combination of **proteins, small-molecule** and **microbiological characterization** for a **holistic** understanding of wastewater based epidemiological studies.

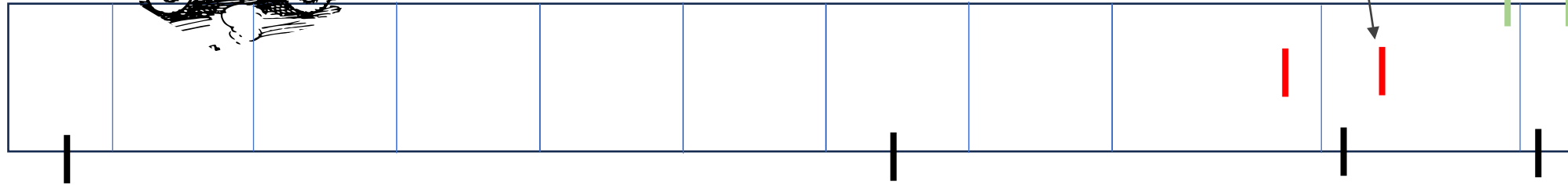
Gil-Solsona, R.; Subirats, J., Carrascal, M., Gago-Ferrero, P., Ginebreda, A., Barceló, D.



EXCELENCIA
SEVERO
OCHOA



WASTEWATER-BASED EPIDEMIOLOGY TRAVEL



1928: Wilson W.J. isolated typhosus from WW

1985: PCR was patented

2012: 1st study of antibiotic resistance genes in WW

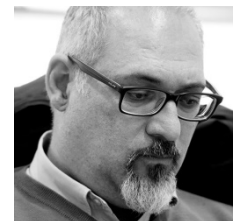
2020: COVID-19 spreading was studied using WWBE strategies around the globe

2008: E. Zuccato et al analyze drugs in WW

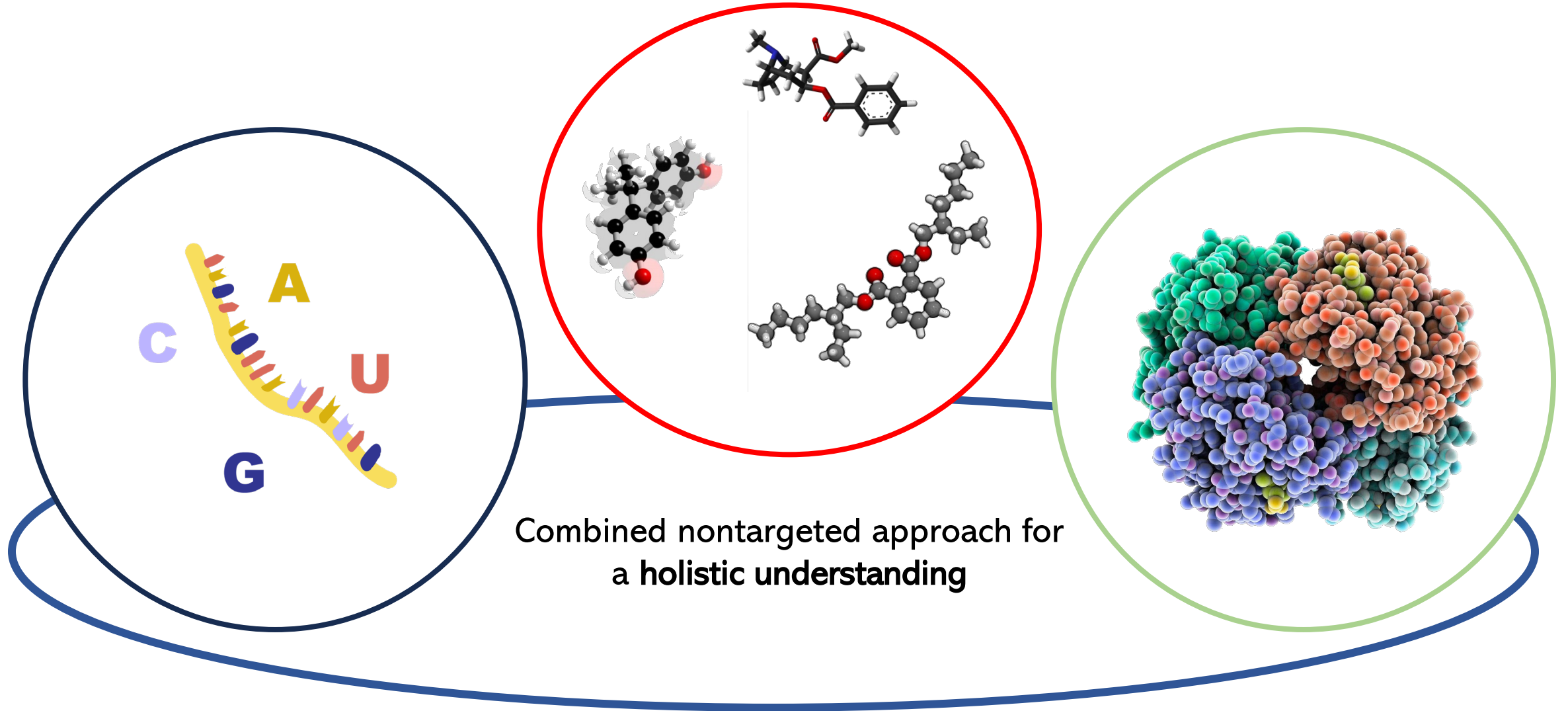
2014: D.A. Devaul et al included PhACs

2019: J. Rice & B. Kasprzyk-Hordern firstly publish about proteins & WWBE

2023: M. Carrascal performed proteomics analysis with WW



WASTEWATER-BASED EPIDEMIOLOGY DIRECTION



OBJECTIVES

OBJECTIVE: Evaluate the potential of combining different analysis to highlight trends or relationships

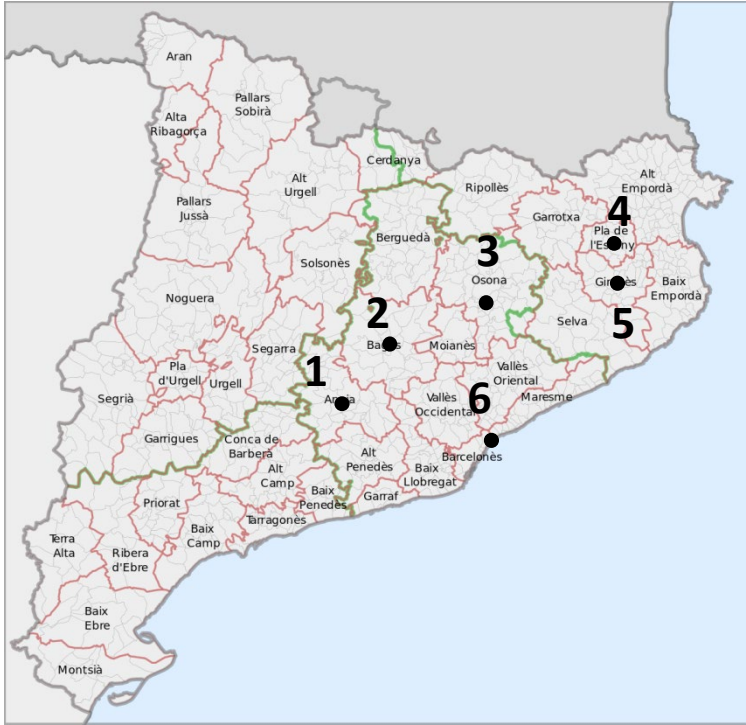
1: Characterize the profile of small-molecule (low molecular weight chemicals) present in WW samples in a target (antibiotics) and a nontarget way.

2: Characterize the presence of some specific Antibiotic Resistance Genes (ARGs) in WW samples.

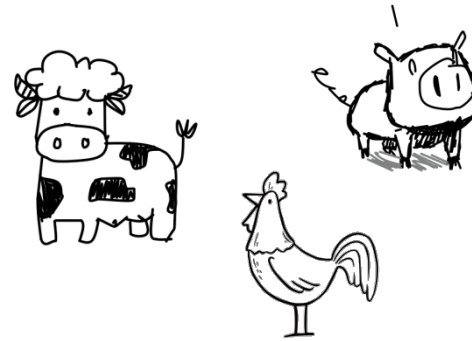
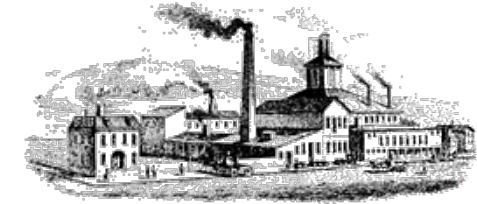
3: Characterize the profile of proteins (high molecular weight chemicals) present in WW samples.

4: Use uni/multivariate statistical analysis to disentangle relationships between all collected data for its use as **early-warning system**.

STUDY AREA



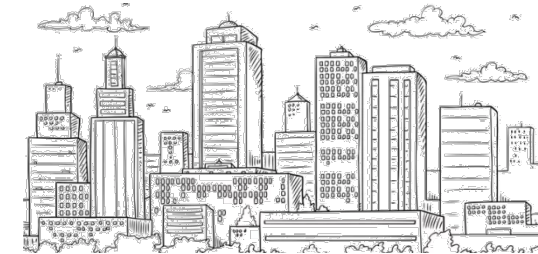
1 and 2
Cities with a strong industrial sector



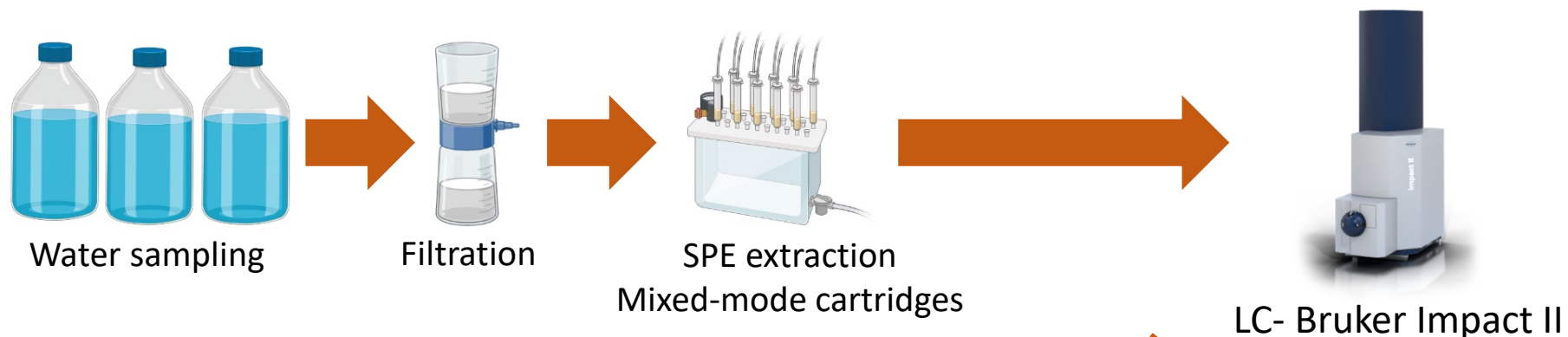
3 and 4
Cities with a strong primary sector (Cow, Pig and Chick farms)

A total of **6 WWTP** (influent wastewater) were collected during **4 weeks** (September – October 2022) n=24 samples

5 and 6
Highly populated cities, with prevalence of tertiary sector



CHARACTERIZE THE PROFILE OF SMALL-MOLECULE



10.1016/j.jhazmat.2023.130876



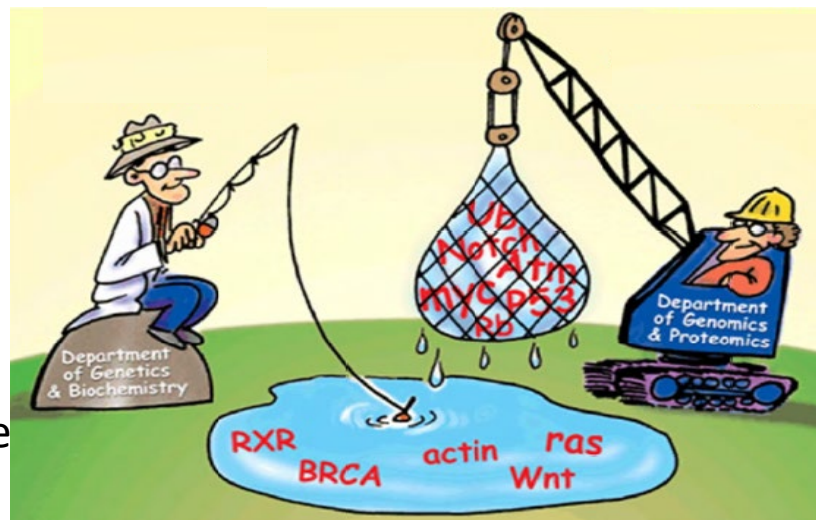
Chemicals of emerging concern in coastal aquifers: Assessment along the land-ocean interface

Daniel Gutiérrez-Martín^a, Rubén Gil-Solsona^{a,*}, Maarten W. Saaltink^{b,c}, Valentí Rodellas^d, Rebeca López-Serna^{e,f}, Albert Folch^{b,c}, Jesús Carrera^{a,b}, Pablo Gago-Ferrero^{a,*}

Suspect Analysis:

NORMAN SLE S6

Contains 676 different antibiotics.
Further manual refinement to find the ones present in our samples.



Nontarget Analysis:

Peak-picking process with all collected data

Further statistical analysis for select identification efforts

CHARACTERIZE THE PRESENCE OF ARGs



qPCR assay



DNA
extraction



DNA

High-quality treated wastewater causes remarkable changes in natural microbial communities and *int11* gene abundance

Jèssica Subirats^{a,*}, Andrea Di Cesare^b, Saulo Varela della Giustina^a,
Antonino Fiorentino^b, Ester M. Eckert^b, Sara Rodríguez-Mozaz^a, Carles M. Borrego^{a,c},
Gianluca Corno^b

Abundance and diversity of Antibiotic Resistant Genes (ARGs)

sul1 -> marker of sulfonamide resistance

tetM -> marker of tetracycline resistance

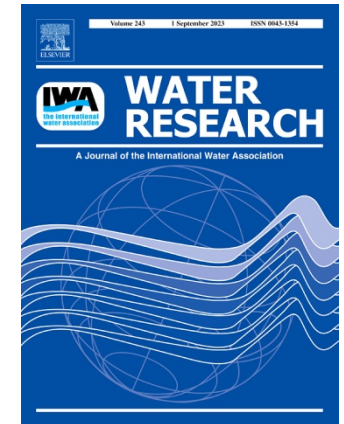
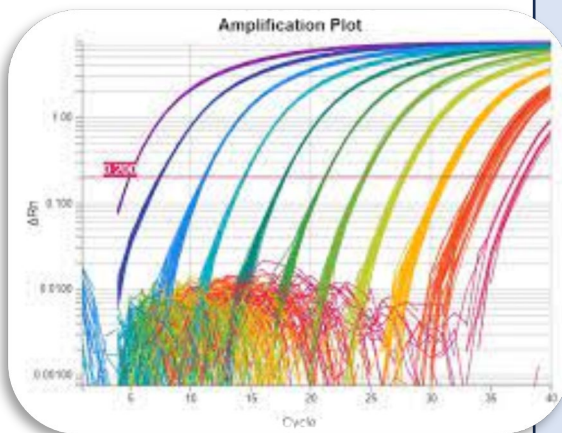
blaTEM, *blaOXA*, *blaCTX*, *mecA* -> markers of β -lactamic
ABs resistance

qnrS1 -> marker of quinolone resistance

Abundance and diversity of Mobile Genetic Elements (MGEs)

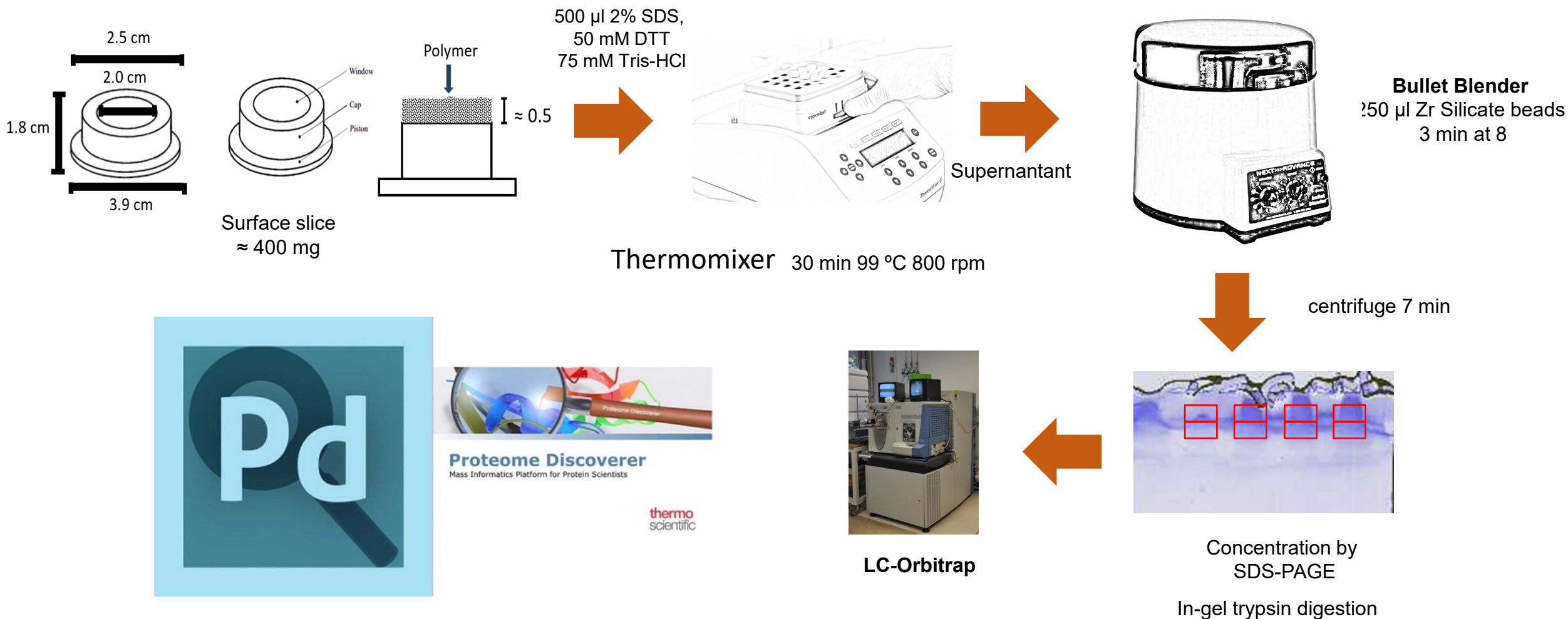
int11 -> mobile genetic element

Abundance of total bacterial loads 16S rRNA



10.1016/j.watres.2019.114895

CHARACTERIZE THE PROFILE OF PROTEINS



RESULTS (Characterize the profile of small-molecule)

NONTARGET ANALYSIS:

A total of 8,824 features were found

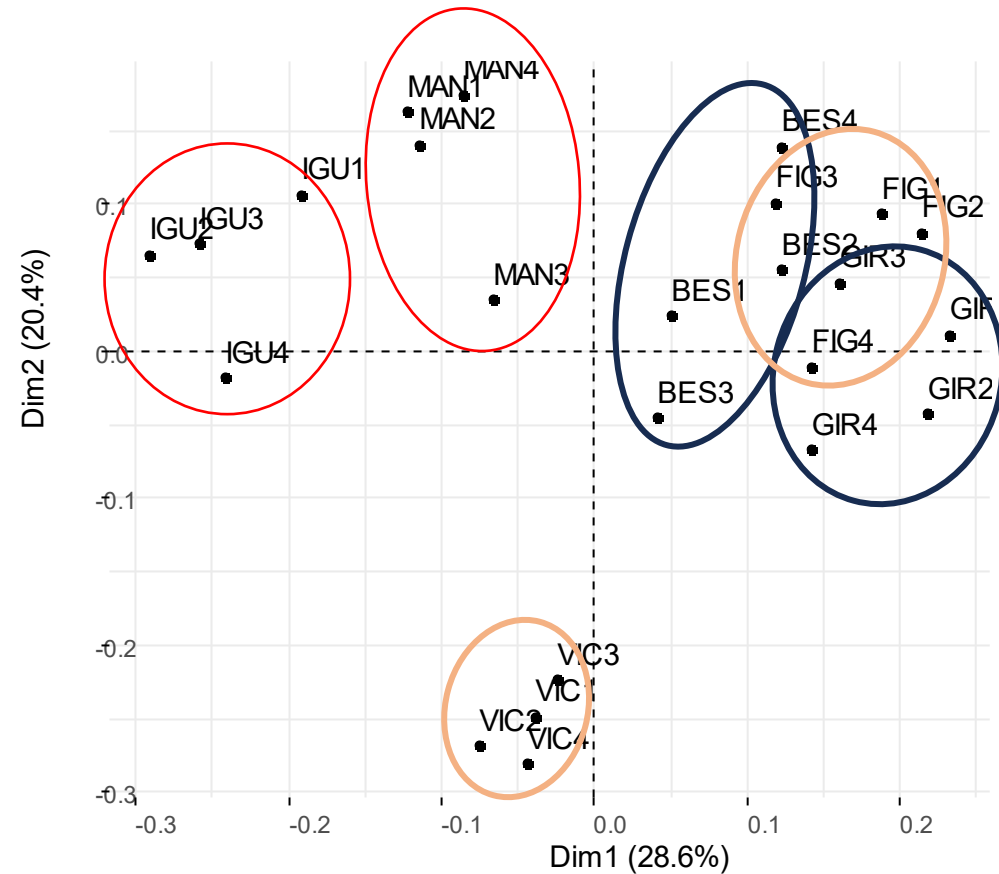
A clear differentiation was obtained from PCA



Possible to find same patterns that in ARGs or proteins



Candidates which comes from good correlations can be elucidated with software (e.g. SIRUIS 5)



RESULTS (Characterize the profile of small-molecule)

SUSPECT ANALYSIS:

16 of them tentatively found:

Penicillin-like: Cyclacillin

Macrolides: Rifaximin, Clarithromycin, Azithromycin, Spectinomycin

Sulfonamides: Sulfamethoxazole, Sulfadiazine

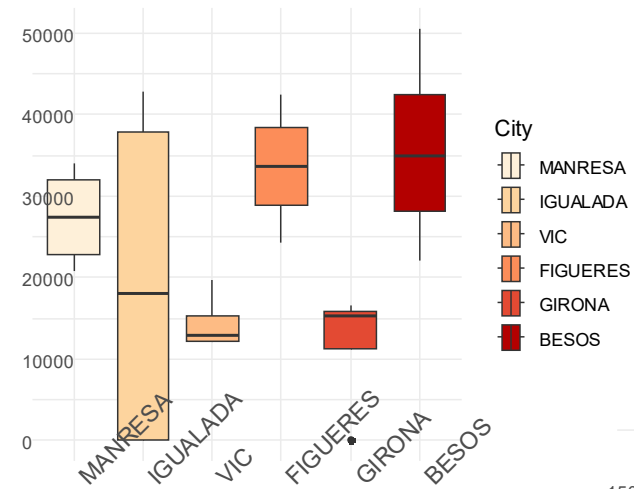
Antivirals: Acyclovir

Antiseptics: Xibornol, 8-Hydroxychinolin, Thymol

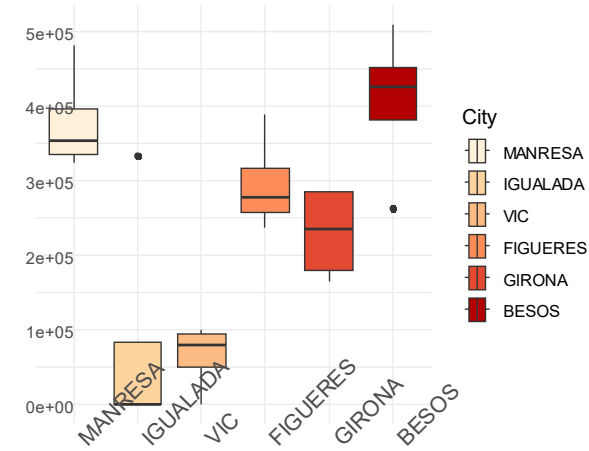
Antiprotozoal: 1,2-Dimethyl-5-nitroimidazole

Others: Gliorosein, Dehydroepiandrosterone, Furasic acid, Indirubin

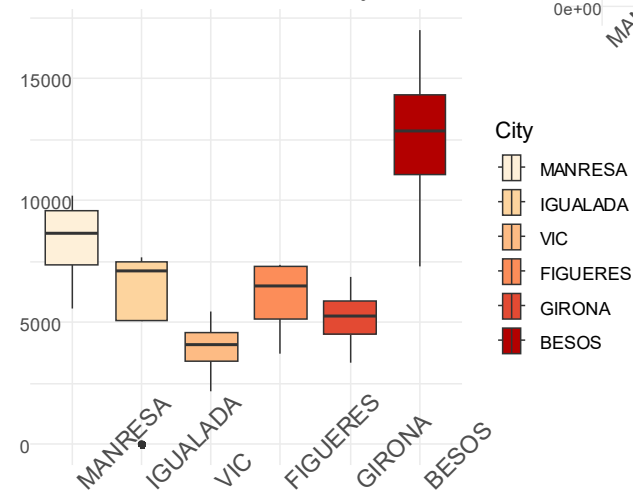
Sulfamethoxazole levels



Cyclacillin levels



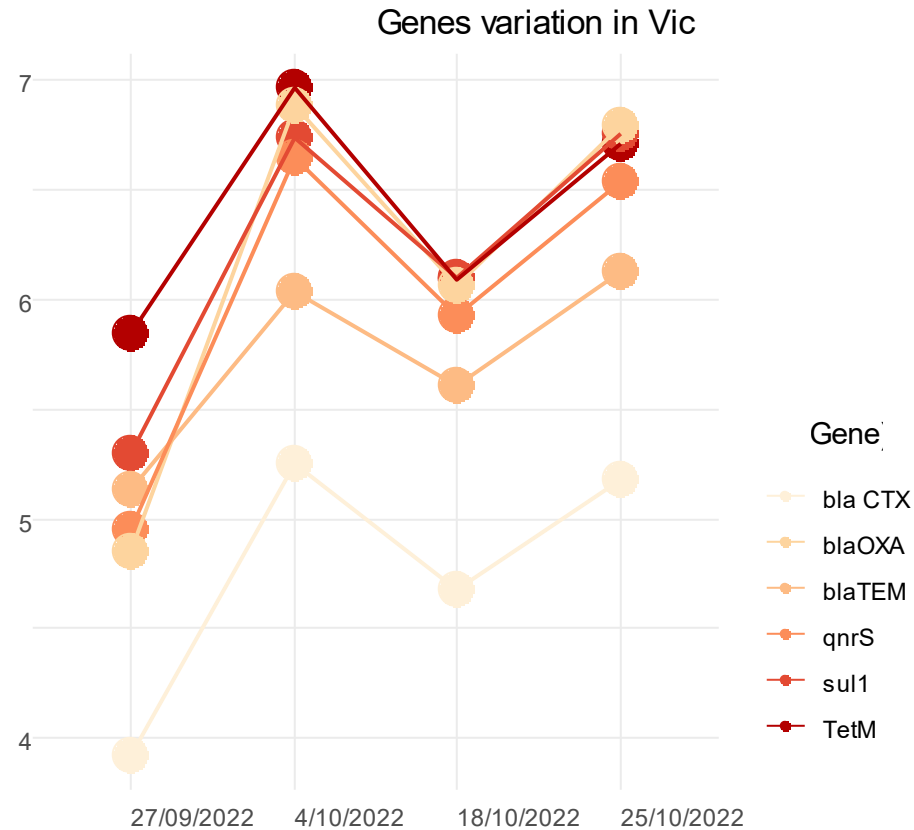
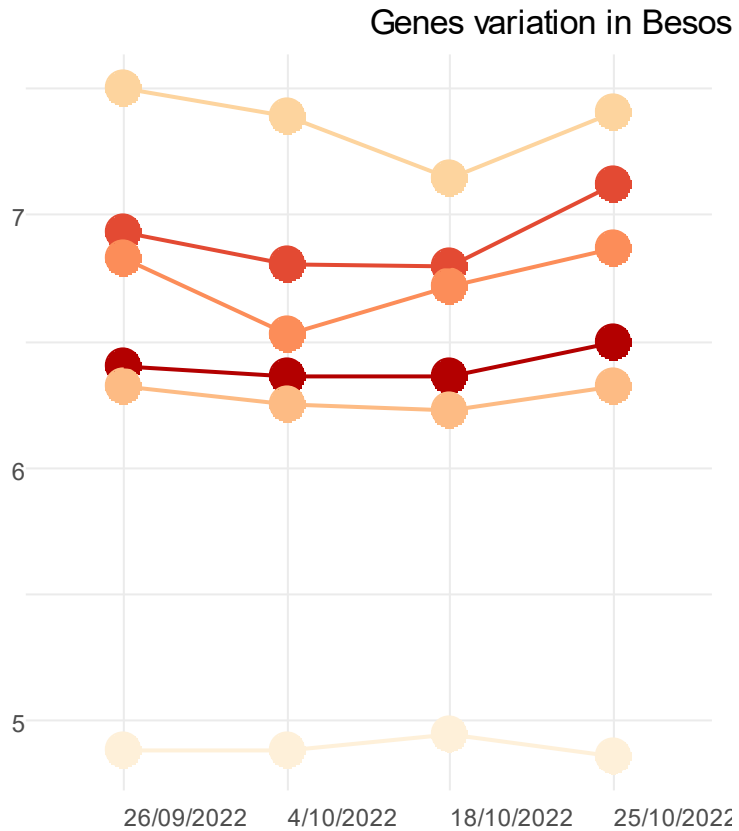
Azithromycin levels



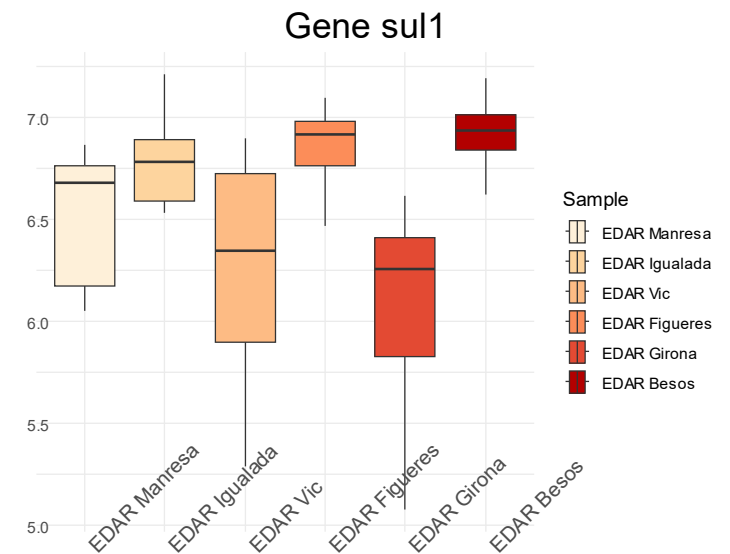
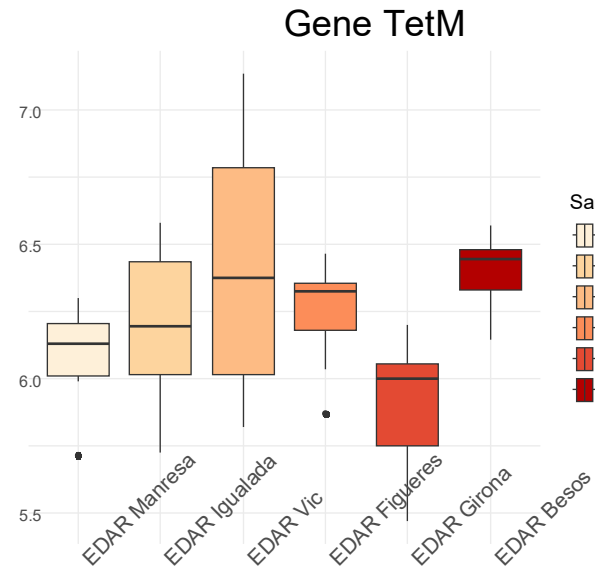
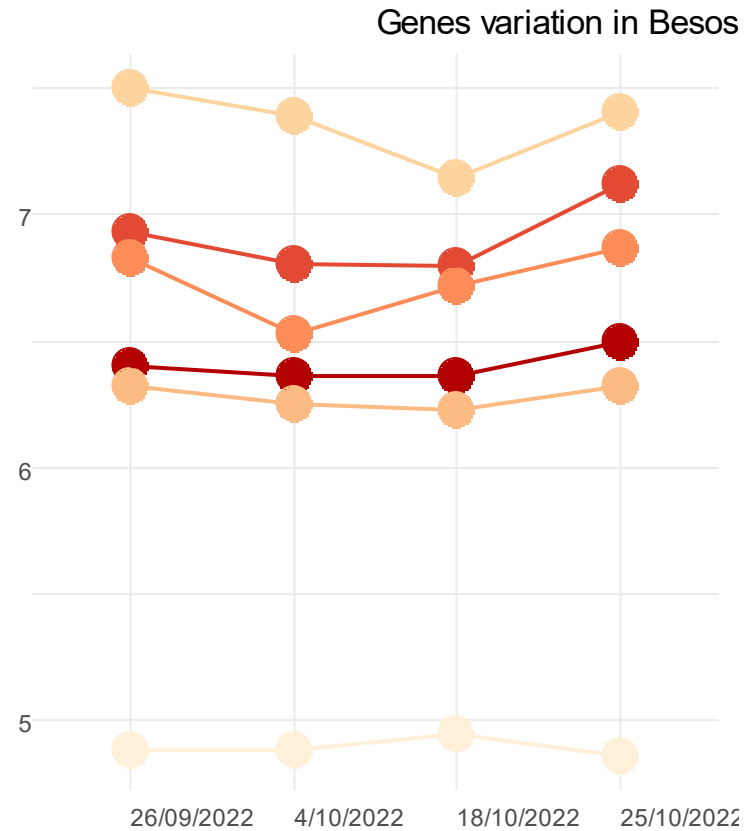
RESULTS (Characterize the presence of some ARGs)

The 6 genes were found in all the samples:

Low variations inside the same wastewater treatment plant



RESULTS (Characterize the presence of some ARGs)



RESULTS (Characterize the profile of proteins)



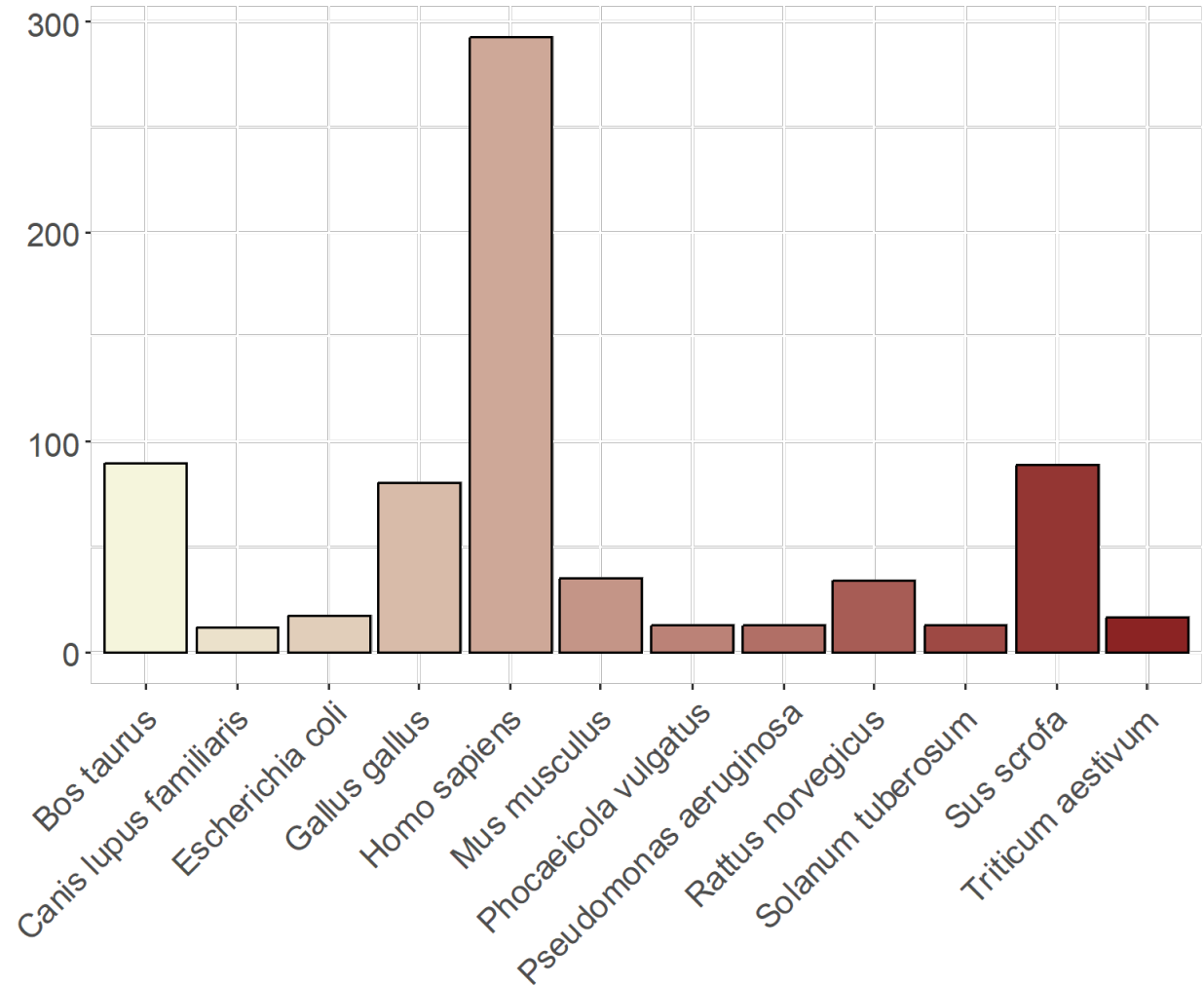
Proteome Discoverer:

1462 peptides from 456 species

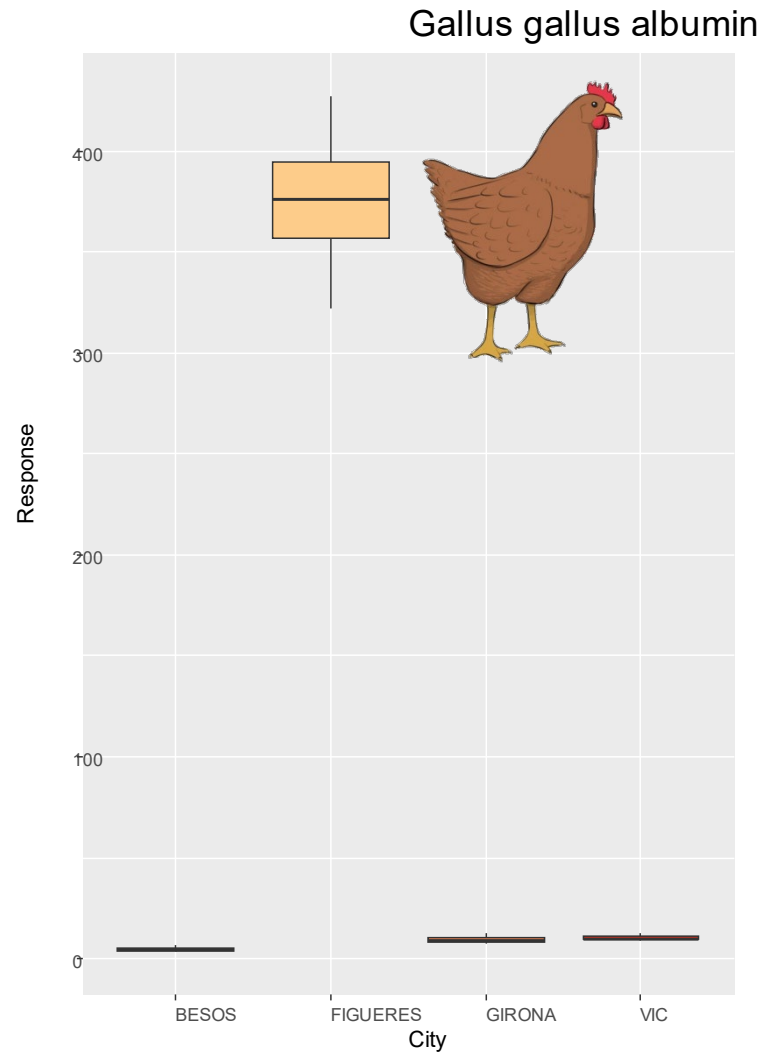
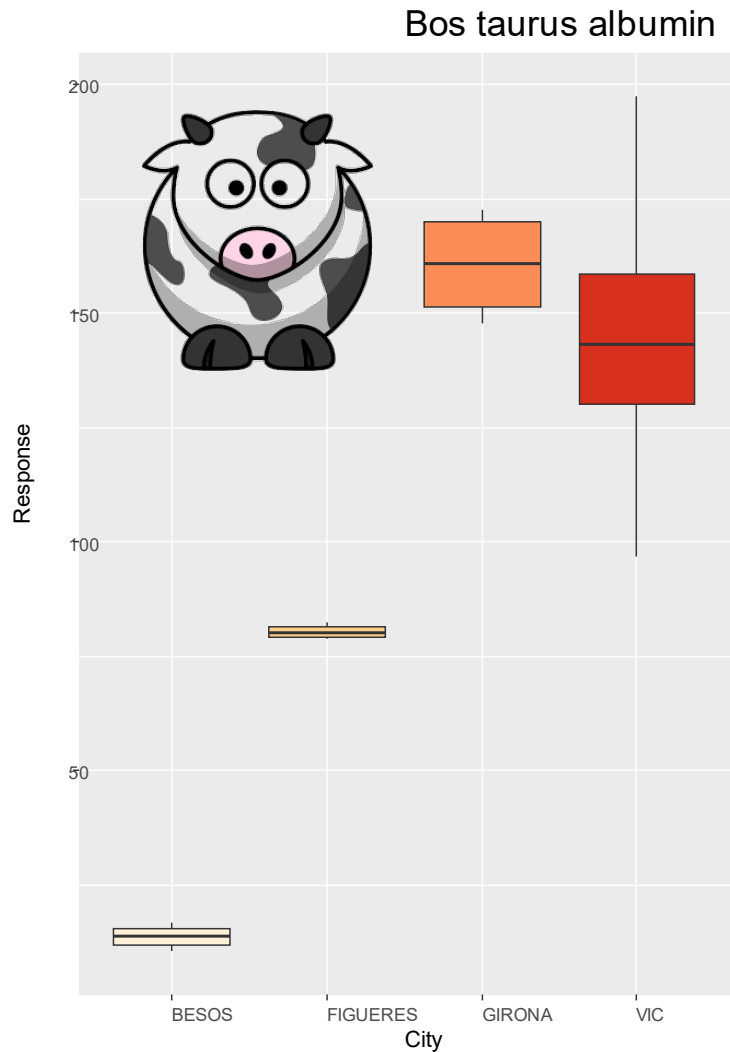


26 species has more than 5 peptides

- Humans (293)
- Animals: Cow(90), Dog(12), Chicken (81), Rat & Mice (158)
- Food: Potato (13) & Wheat (17)
- Bacteria: E. Coli (18), P. vulgatus (13), P. Aeruginosa (13)

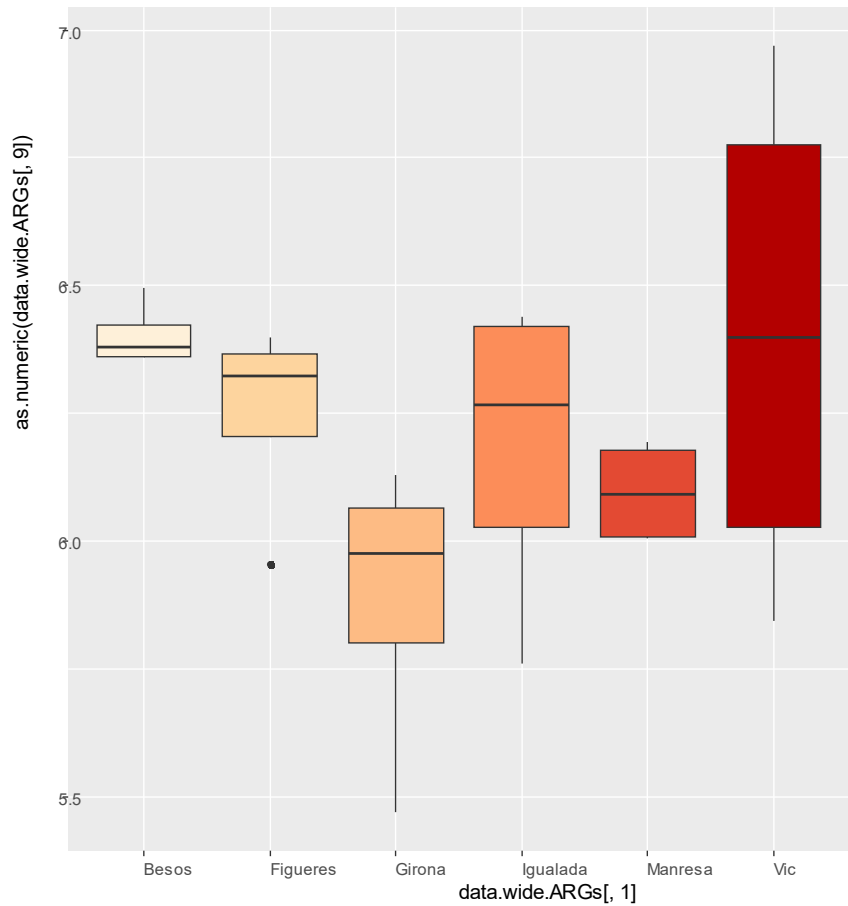


RESULTS (Characterize the profile of proteins)



RESULTS (Study relationships)

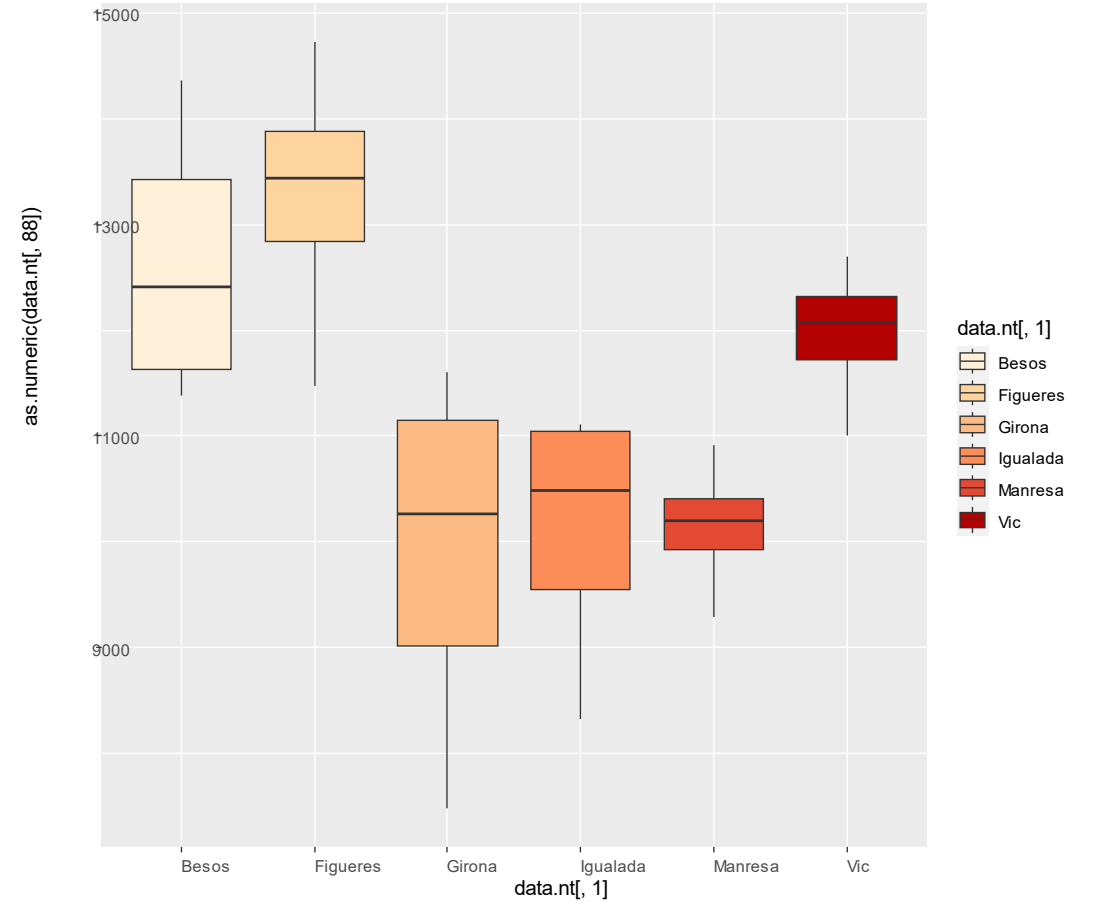
Gene tetM



```
> p.value[88,1]  
[1] 0.04649818
```

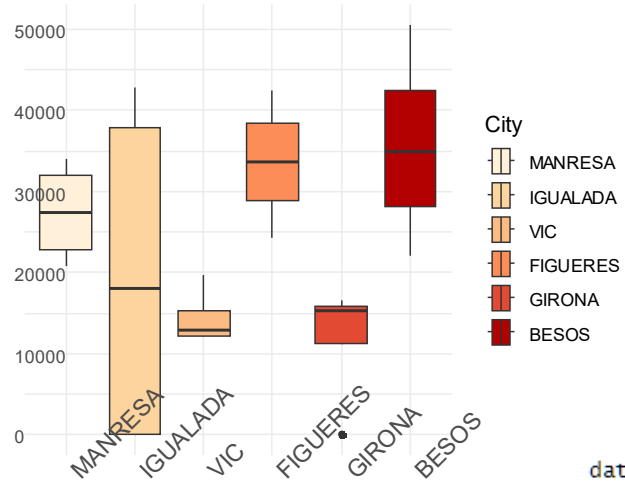


2,3-diaminopropionic acid



RESULTS (Study relationships)

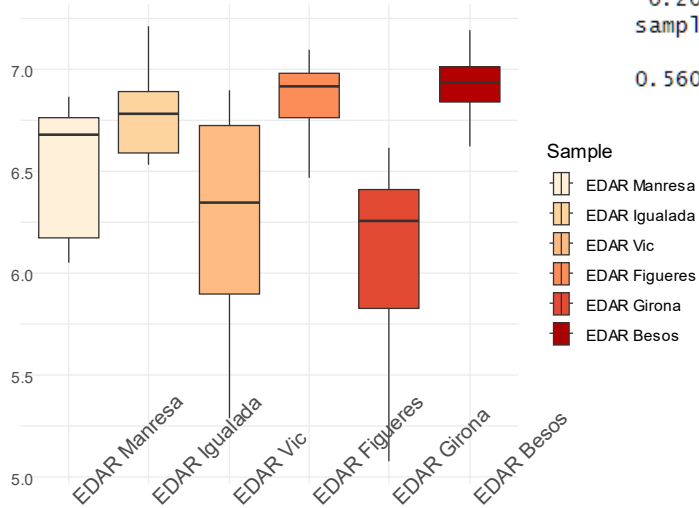
Sulfamethoxazole levels



Pearson's product-moment correlation

```
data: sul1[, 2] and sul1[, 3]
t = 3.1721, df = 22, p-value = 0.004412
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.2025974 0.7859844
sample estimates:
cor
0.5602098
```

Gene sul1

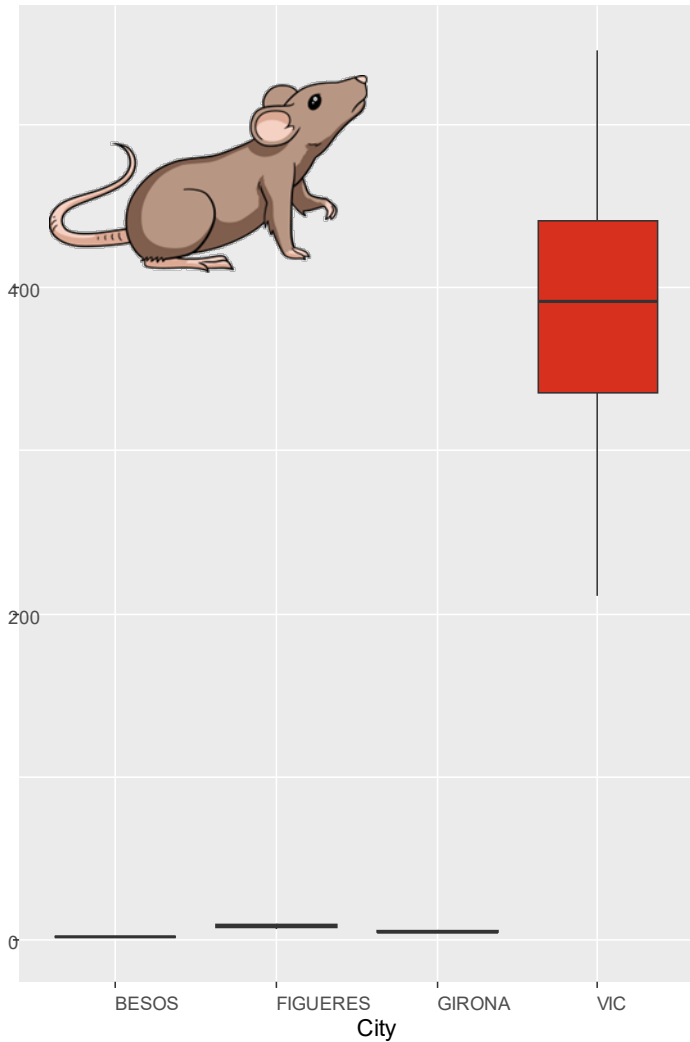


Correlation between sulfamethoxazole levels and sul1 gene expression

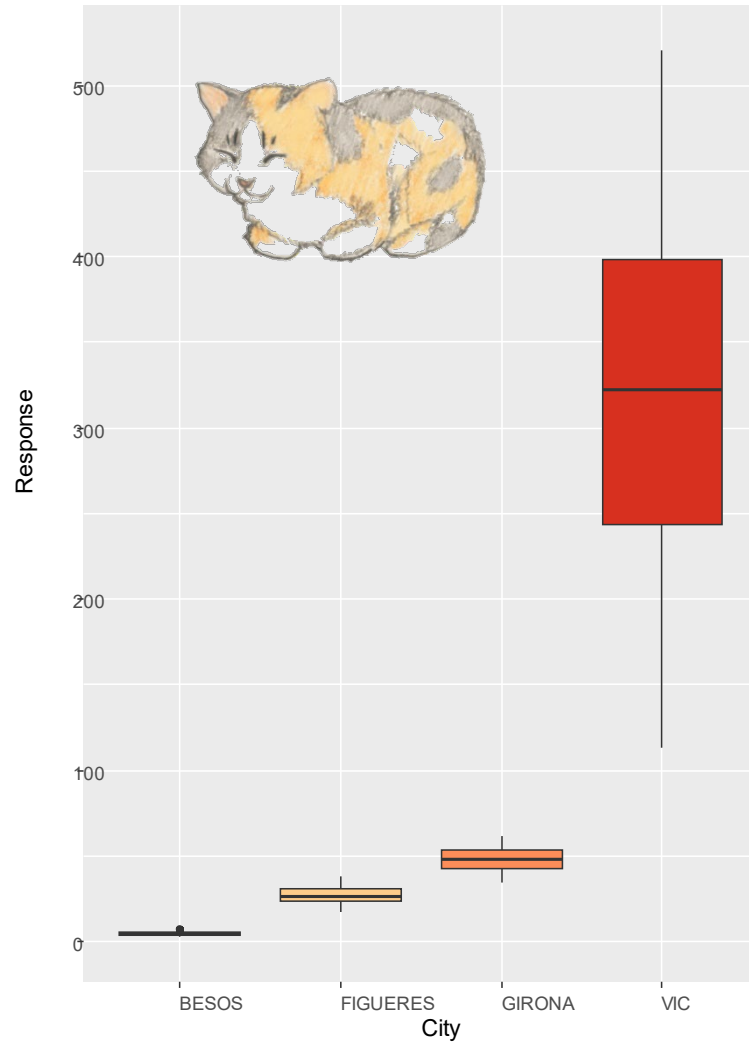


RESULTS (Study relationships)

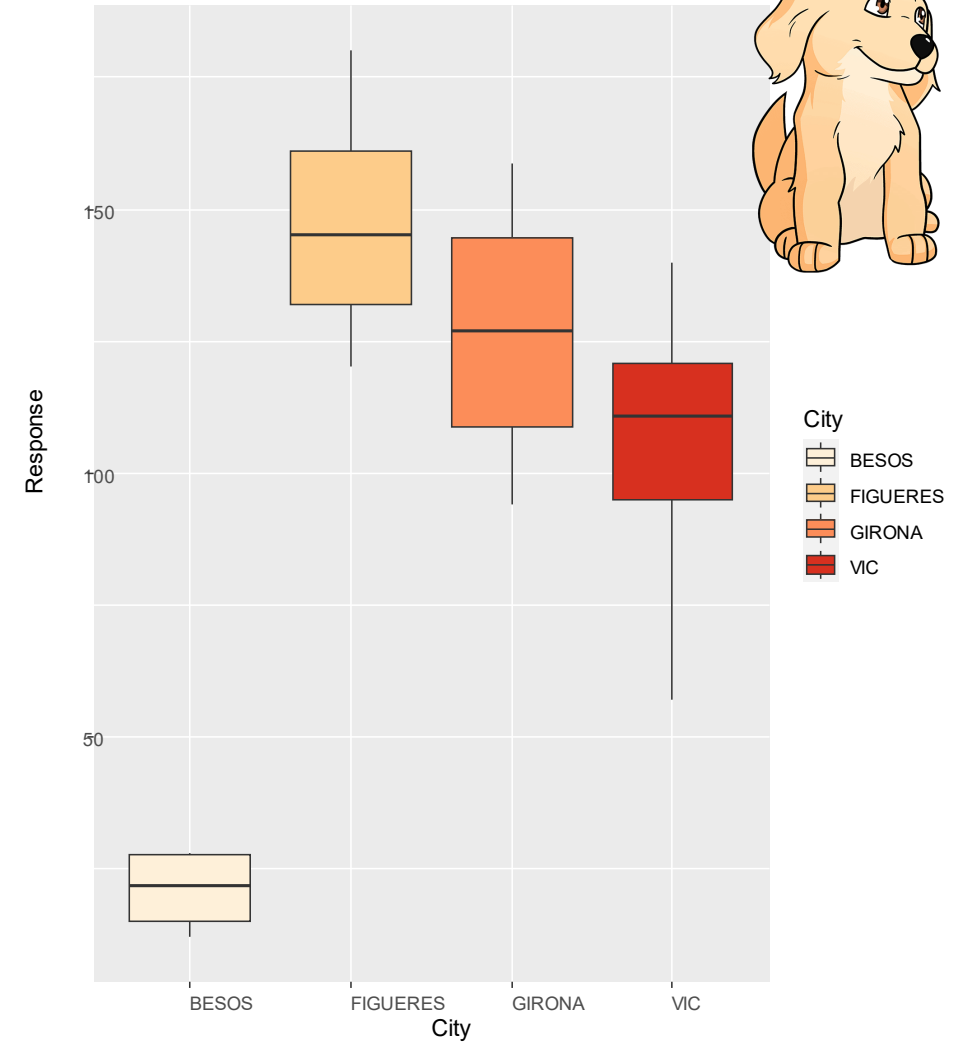
Sus scrofa albumin



Felis catus albumin



Canis lupus familiaris albumin



CONCLUSIONS

- Wastewater in a huge information storage, in terms of small and big chemicals
- Using different sampling sites may help to clarify the correlation between ARGs and Antibiotics presence
- Combining multidisciplinary strategies, we can disentangle important correlations between chemicals present in WWBE

TAKE-HOME MESSAGE: We have the analytical capabilities ready for obtaining a holistic understanding of population health status using WWBE