

# Emerging viruses in irrigation waters

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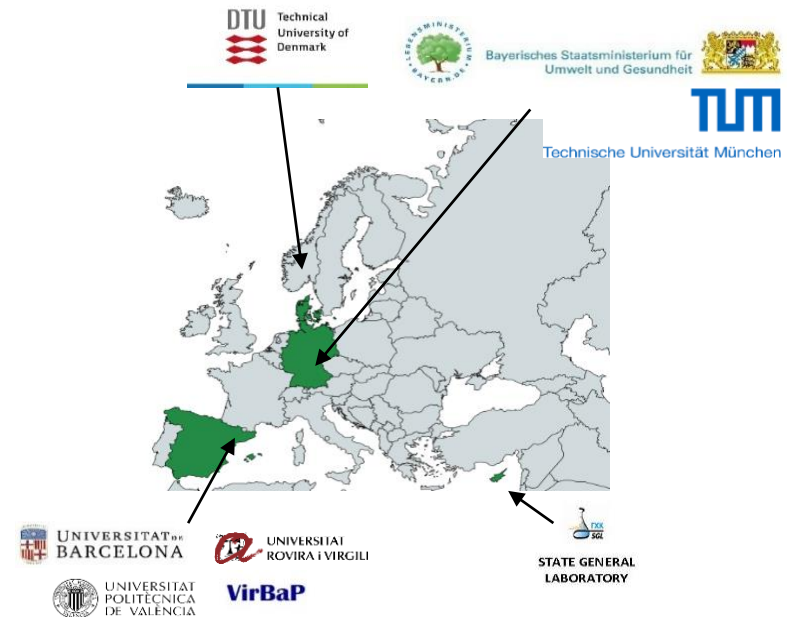
The 2<sup>nd</sup> Water JPI Conference  
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## Metawater

New Metagenomics and molecular based tools for European scale identification and control of emergent microbial contaminants in irrigation water



**Laboratory**  
of **Viruses** Contaminants  
of **Water** and **Food**

# Do we have methods for quantification of viruses?

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Characterization of the efficiency and uncertainty of skimmed milk flocculation for the simultaneous concentration and quantification of water-borne viruses, bacteria and protozoa



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- Evaluate the applicability of SMF for the simultaneous concentration of viruses, bacteria and protozoa in water.
- Determine the efficacy of the SMF recovery
- Compare q(RT)PCR and infectivity assays for viruses
- Define variability and uncertainty values of the method to use them in QMRA studies.

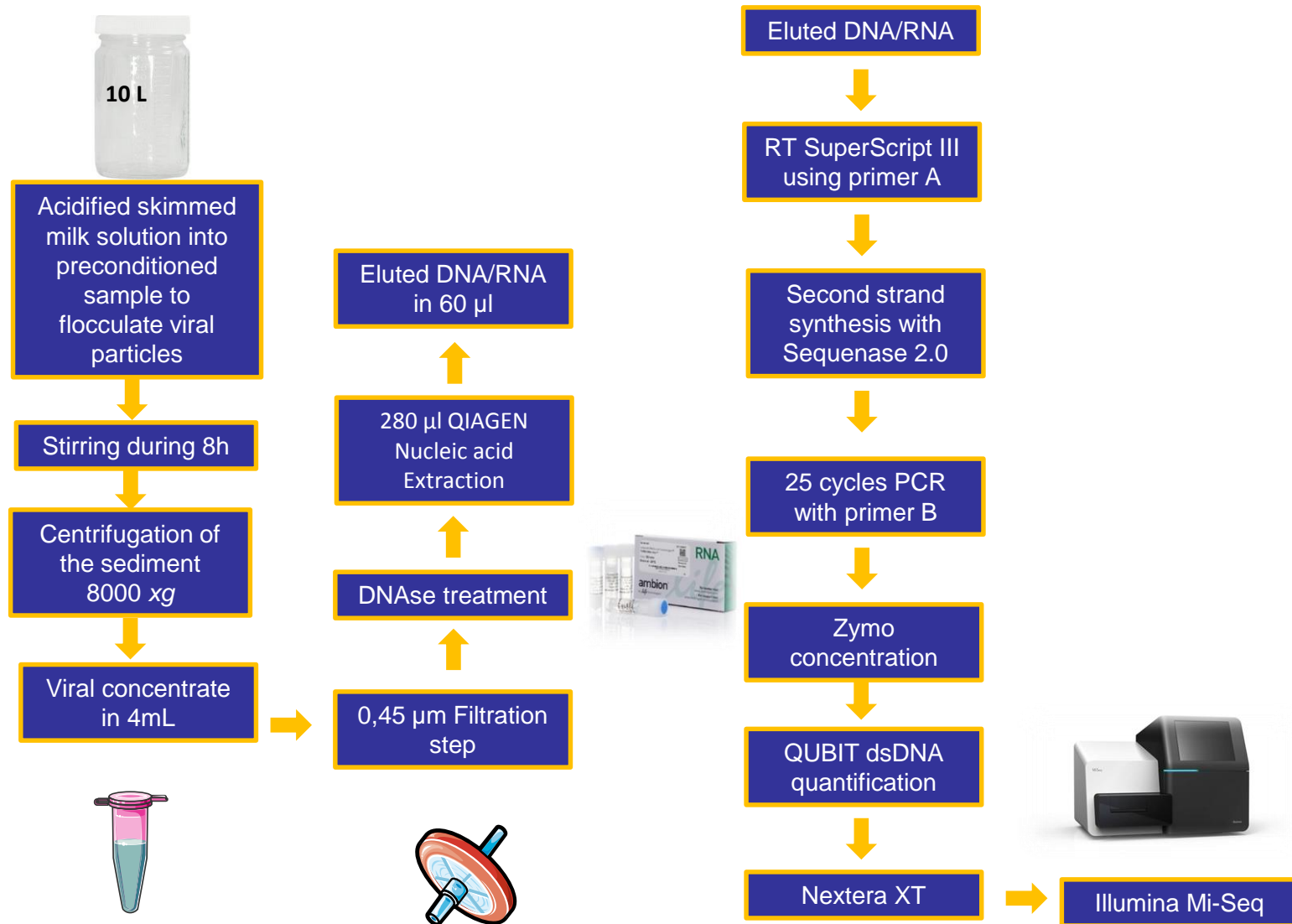
## Virus recovery by qPCR and Infectivity quantification

Virus	Method	% Recovery			
		Mean %	CI 95% of mean	min	max
HAdV	qPCR	66	53.5 - 78.5	32.2	86.7
	IFA	58.7	4.5 - 100	8.1	49.8
MS2	q(RT)PCR	23.9	19.6 - 28.1	13.8	36.8
	PFU	11.9	9 - 14.7	9.5	13.9
RoV	q(RT)PCR	28.2	25.6 - 30.7	16	37.1
	TCID50	26.1	17.1 - 35.1	43.5	83.7
BVDV	q(RT)PCR	14.7	10.8 - 18.7	12.9	15.8
	TCID50	0.7	0.4 -1.1	0.67	0.89

## Bacteria and protozoa recovery with qPCR and IFA quantification

	Method	% Recovery			
		Mean %	CI 95% of mean	min	max
<i>E. coli</i>	qPCR	59.6	40.3 - 79	15.6	98.7
<i>H. pylori</i>	qPCR	30.2	24.4 - 36.1	20.8	41.5
<i>A. castellanii</i>	qPCR	20.5	14.9 - 26.1	13	32.1
<i>G. lamblia</i>	IFA	17.8	15 - 20.7	12.8	21.5
<i>C. parvum</i>	IFA	12.8	12.5 - 15.2	9.6	17.4

# Protocol for the treatment of water samples for metagenomics



# NGS and molecular techniques for the analysis of emerging bacteria, protozoa and viruses in irrigation water

One year sampling, 72 irrigation water samples + 12 sewage + 12 secondary treated effluents

## Conventional irrigation water sources

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Drinking water tank (DW) and Sediment of the DW tank

Reservoir water

Reservoir water for orchard irrigation

Groundwater

River water

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## Wastewater and recycled water

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Raw sewage

Secondary treated effluent (CAS)

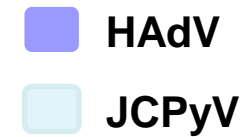
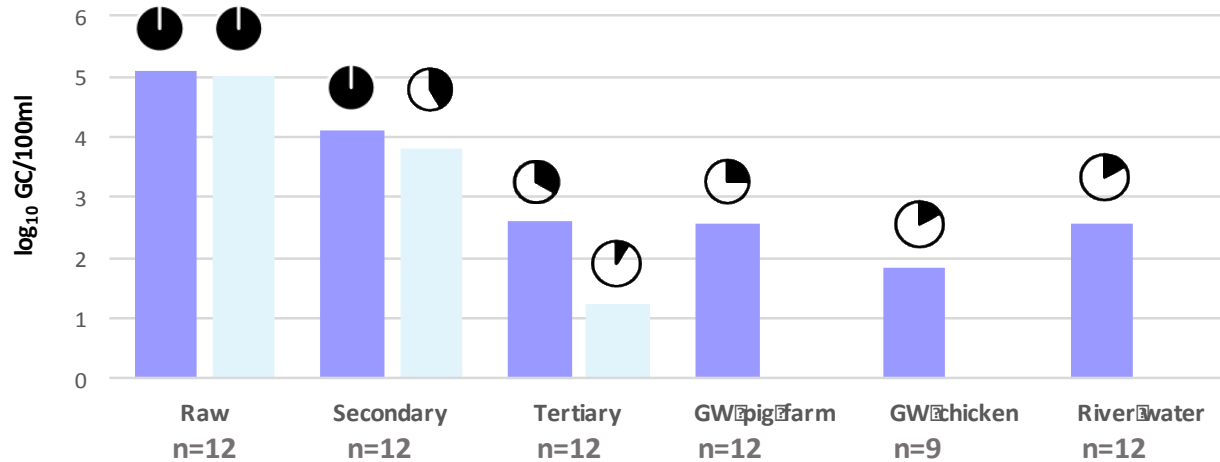
Wetland effluent (tertiary treatment)

Wetland treated effluent (Filtration+Cl<sub>2</sub>)

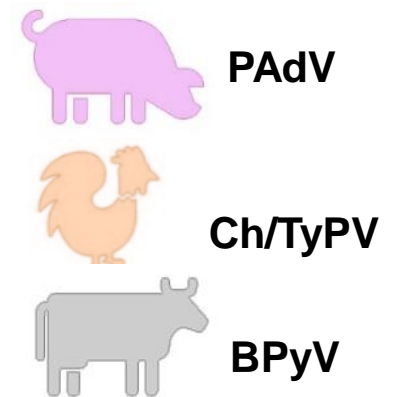
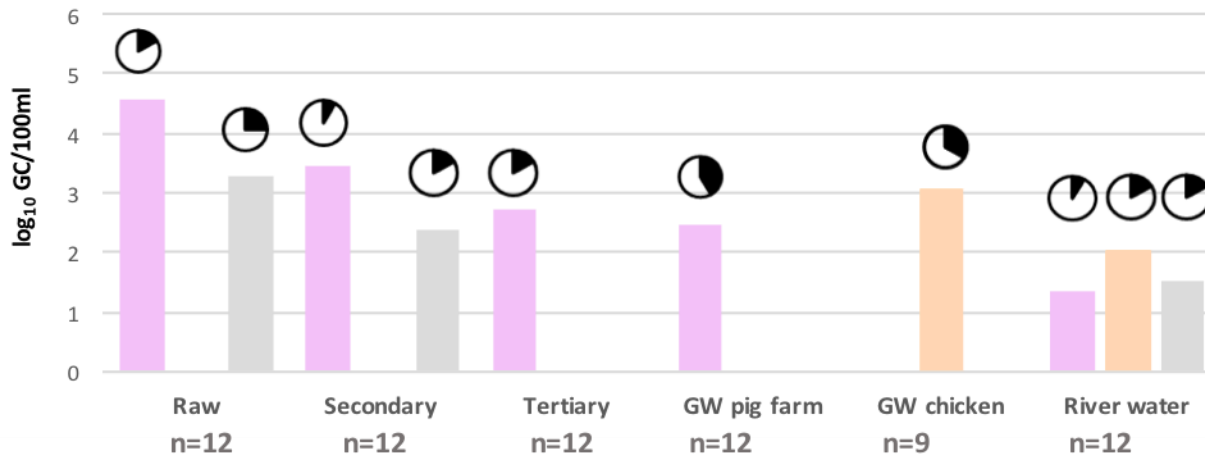


# MST markers as irrigation water management tools

## Human MST

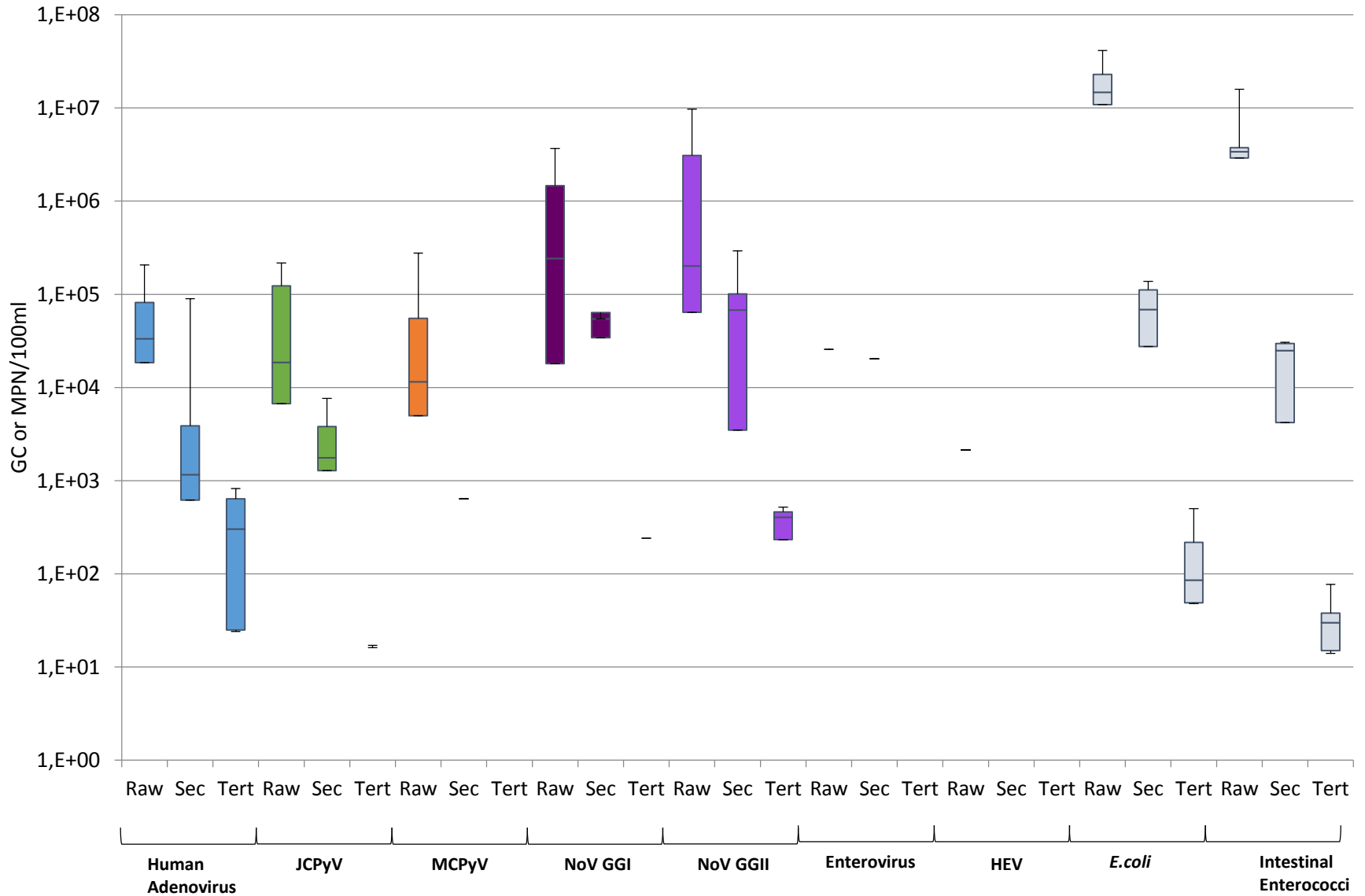


## Animal MST





# qPCR results in raw sewage, secondary and tertiary treated effluents (wetland)



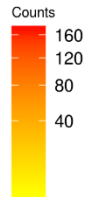
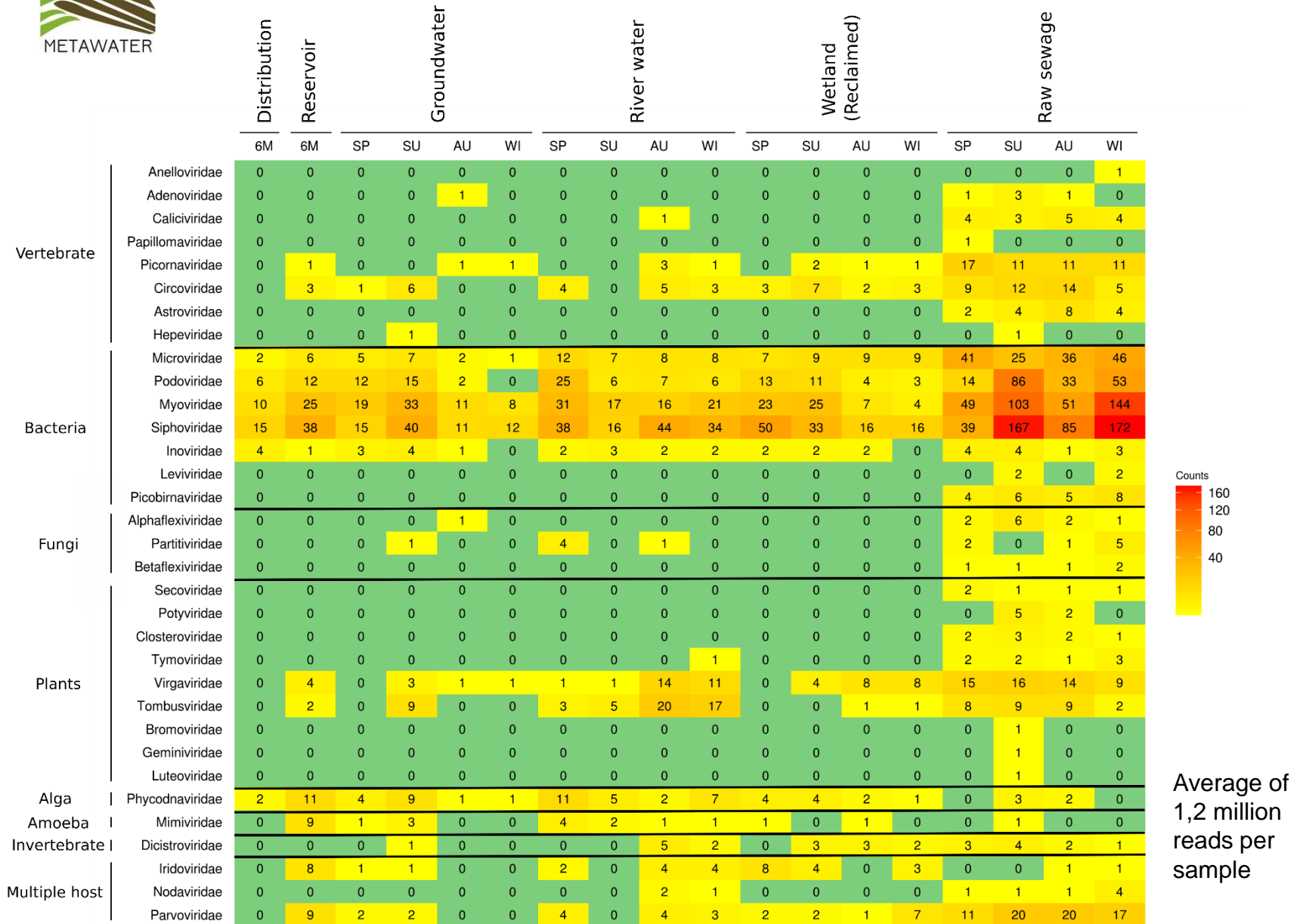
## Microbial removal efficiency in wastewater treatment plants and risk assessment studies

Quantitative Microbial Risk Assessment to estimate the health risk associated with the ingestion of lettuce irrigated with tertiary effluents from 2 WWTPs

WWTP	Virus	Observed	To reach $10^{-6}$ DALYs
1 UV, chlorin. Actiflo®	HAdV	2.5	5.6
	NoV GII	1.9	7
2 Wetlands	HAdV	2.8	5.1
	NoV GII	3.9	6.7



Mean of the best fit distributions of reductions in tertiary effluent by each virus in actual scenario and required reductions to reach suggestions of WHO ( $10^{-6}$  DALYs).



Average of 1,2 million reads per sample

## Main conclusions and recommendations

1. Concentration protocols for the efficient (low-cost) quantification of pathogens in water are useful for improving QMRA studies to facilitate evidence based decisions for water safety management.

**Recommendation:** There is a need of implementing highly advanced sewage treatments (such as MBR) or additional disinfection treatments to achieve acceptable microbial quality for water reuse in irrigation of fresh vegetables

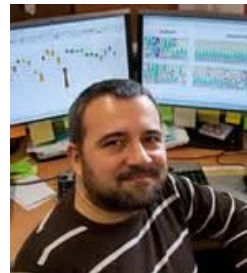
2. Analysis of the virome using metagenomics in raw sewage has shown the presence important human pathogens including emerging strains of both RNA and DNA viruses

**Recommendation:** Implementation of metagenomic public health surveillance systems using NGS techniques in urban sewage as a valuable data base with information of the circulating viruses in the population.

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[http://www.ub.edu/microbiologia\\_virology/en](http://www.ub.edu/microbiologia_virology/en)



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