



# METAWATER

## New Metagenomics And Molecular Based Tools For European Scale Identification And Control Of Emergent Microbial Contaminants In Irrigation Water

Water JPI Pilot Call  
Emerging water Contaminants –  
anthropogenic pathogens and pollutants  
Kick-off Meeting  
11th of March 2015, Brussels, Belgium



Laboratory of Virus Contaminants  
of Water and Food



## Partners in the project:



### **Spain:**

Rosina Girones, Universitat de Barcelona

Maria José Figueras, Universitat Rovira i Virgili

José Luis Alonso de la Universidad Politécnica de Valencia

### **Denmark:**

Charlotta Löfström, Anna Charlotte Schultz Technical University of Denmark

### **Cyprus:**

Georgios T. Papageorgiou, State General Laboratory

### **Germany:**

Christiane Höller, Bavarian Health and Food Safety Authority, Michael Seidel, Technische Universität München.

## University of Barcelona

Rosina Girones, (team leader, project coordinator, head of the Department of Microbiology)

Sílvia Bofill, (environmental virology and MST)

Josep Abril, (bioinformatics)

Rosa Araujo, (water-borne bacteria)

Marta Rusinyol, (environmental virology)

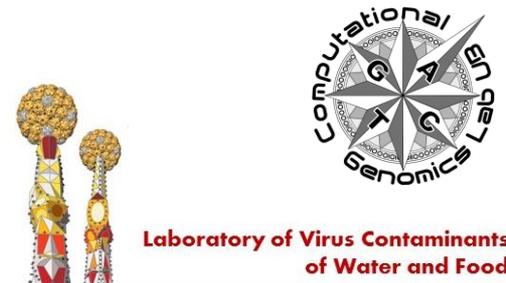
Humbert Salvado, (protozoa)

Miquel Calvo (statistics)

**Key areas of expertise:** Virology, viral contamination in water or food, bioinformatics, water protozoa and water-borne bacterial pathogens



**VirBaP**





UNIVERSITAT  
POLITÈCNICA  
DE VALÈNCIA

Technical University of Valencia  
METAWATER RESEARCH TEAM



## Research Institute of Water Engineering and Environment (IIAMA)

- José Luis Alonso Molina, PhD (UPV research team responsible)
- Yolanda Moreno Trigos, PhD
- Inmaculada Amorós Muñoz
- Laura Moreno Mesonero, PhD student

## Biotechnology Department

- M<sup>a</sup> Antonia Ferrús, Prof.

## Food Technology Department

- Eva M<sup>a</sup> Doménech Antich, Prof
- M<sup>a</sup> Isabel Escriche Roberto, Prof

**The key areas of expertise** include: waterborne bacteria and protozoa detection, molecular biology methods (NGS, qPCR, FISH), wastewater treatment microbiology, bacterial cell viability, risk assessment and food safety.



IIAMA

Instituto de Ingeniería del  
Agua y Medio Ambiente

## METAWATER RESEARCH TEAM

Prof. Dr. María José Figueras (principal investigator URV)

Dr. Carolina Silvera-Simón (postdoctoral research)

Fadua Latif (PhD student on the Epidemiology of *Aeromonas*)

Nuria Salas (PhD student on *Arcobacter* and shellfish)

Alba Pérez (PhD student, Molecular epidemiology)



### Key areas of expertise:

Faecal pollution and microbiological water quality, public health impact, molecular identification and genotyping of the emerging pathogens *Aeromonas* and *Arcobacter*, taxonomy and epidemiology. Water Safety Plans.

# Bavarian Health and Food Safety Authority (WP 6 & 8)

Prof. Dr. Christiane Höller (LGL research team responsible)  
Dr. Stefanie Huber (head of water department)  
Dipl.-Ing. Diana Calomfirescu (scientist for project)  
Dr. Giuseppe Valenza (specialist medical microbiology)  
Dr. Christian Tuschak (specialist molecular biology)  
Silke Nickel (technical assistant)

## Associated partner:

Dr. Margit Schade (Bavarian Environmental Authority)

## Key areas of expertise:

Water hygiene incl. water microbiology, hospital hygiene, public health issues, antibiotic resistant bacteria and molecular typing, detection of pathogens



# Institute of Hydrochemistry, TU Munich (IWC-TUM)



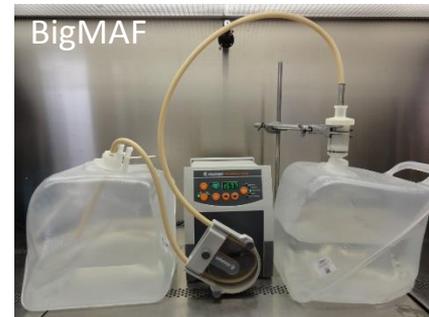
## 25 years expertise in (bio)analytical measurement methods for environmental analysis

- design and construction of multiplex microarray readout system MCR3
- multiplexed microarray analysis techniques on antibody and DNA microarrays for microorganisms and pathogens
- rapid concentration methods of pathogens in water by crossflow microfiltration, immunomagnetic separation or monolithic filtration (MAF)
- automated microorganism concentrator combining crossflow ultrafiltration and MAF

**PD Dr. M. Seidel** (project leader)

**Dennis Elsaesser** (Dipl.-Ing.)

**Sandra Schaefer** (M.Sc. Bio.Chem.)



# Technical University of Denmark (DTU)



- National Food Institute, DTU
  - Charlotta Löfström, PhD, Ass. Prof. (Danish project lead)
  - Anna Charlotte Schultz, PhD, Senior Researcher (WP4 lead)
  - Frank M Aarestrup, PhD, Prof.
  - Maria Hellmér, PhD student
- Associate partners
  - Center for Biological Sequence Analysis, Dept of Systems Biology, DTU
    - Ole Lund, PhD, Prof.
  - Lynettefællesskabet I/S
    - Kim Rindel (Sewage treatment)
  - HOFOR A/S
    - Ann-Katrin Pedersen (Drinking water producer and distributor)
  - The Danish Nature Agency, Danish Ministry of Environment
    - Anne-Marie Madsen (government policies on nature and environment)

## Combined key areas of expertise

Bacteria and virus detection and characterization by microbiological, molecular and metagenomics methods (culture, PCR, NGS including bioinformatics tools and databases) in food, sewage and drinking water systems for the purpose of use in e.g. studies of monitoring and support to governmental policies.

# State General Laboratory of Cyprus

## Water Microbiology Laboratory



### Metawater project, WP 7

Georgios Papageorgiou PhD

Nicolas Pissarides MSc

Katerina Antoniou MSc

Elli Karavi MSc

Panagiota Pieri MSc

Antonis Sotiriou MSc

### Key areas of expertise

Water hygiene, water microbiology, molecular biology methods (RT-PCR), waste water treatment microbiology, concentration of viruses from small and large volumes of water samples, ecotoxicity in recycled waste water surface water and potable water.



**The final objective is to prevent epidemics and to produce the scientific bases to support the development of European/national regulation for water use for irrigation.**

Specific objectives are:

1. Developing new bioinformatics tools and novel methods for water-borne emergent pathogens, NGS systems for nucleic acid detection and novel sample preparation protocols suitable for irrigation water and implementation in end-user laboratories. **Preparation of internationally harmonized Standard Operation Procedures for the control of pathogens in irrigation water.**
2. **Characterization of viral/bacterial/protozoa communities/pathogens** in critical points of **source water and distribution networks**, including antibiotic-resistant bacteria and cyanobacterial toxins.
3. **Characterization of microbial communities and pathogens** in **wastewater treatment plants using diverse technologies**, and **reclaimed water at the point of use.**



## Specific objectives (continue):

5. Development of the list of microorganisms identified representing a risk in **irrigation water in Europe: virome and bacteriome.**
6. Identifying transmission routes, and defining prevalence and behavior of **antibiotic resistant bacteria in irrigation water.**
7. Evaluation of **microbial removal efficiency in wastewater treatment plants** and **risk assessment studies** to build new evidence-based analysis of the suitability of **bacterial and viral indicators and current water regulations for controlling irrigation water quality.**
8. **To integrate sequence and annotation databases into a web-based dynamic interface** for implementation of the developed resources at the European level.



WORK PACKAGE		Year 1 (M 1-12)				Year 2 (M 13-24)				Year 3 (M 25-36)			
No.	Title	3	6	9	12	15	18	21	24	27	30	33	36
1	Bioinformatics - Development of new tools for the study of waterborne emergent pathogens	x	x	x	x	x	x	x	x	x	x	x	
2	Development of novel sample preparation protocols suitable for implementation in end-user laboratories (large scale sewage treatment plants and water distribution systems).	x	x	x	x	x	x	x	x	x	x	x	x
3	Development of pre-NGS-processing methods for detection of emergent pathogens and high-throughput sequencing	x	x	x	x	x	x						
4	Using NGS and molecular techniques for the analysis of emerging bacteria, protozoa and viruses in irrigation water	x	x	x	x	x	x	x	x	x	x	x	x
5	Traceability of the fecal contamination	x	x	x	x	x	x	x	x	x	x	x	
6	Prevalence and behaviour of multiresistant gram-negative bacteria	x	x	x	x	x	x	x	x	x	x		
7	Prevalence and behaviour of biotoxin producing cyanobacteria	x	x	x	x	x	x	x	x	x	x	x	
8	Implementation at the European level, communication to European society and dissemination to stakeholders			x	x	x	x	x	x	x	x	x	x



## Movility Actions:

Each partner will participate in mobility activities according to the following plan:

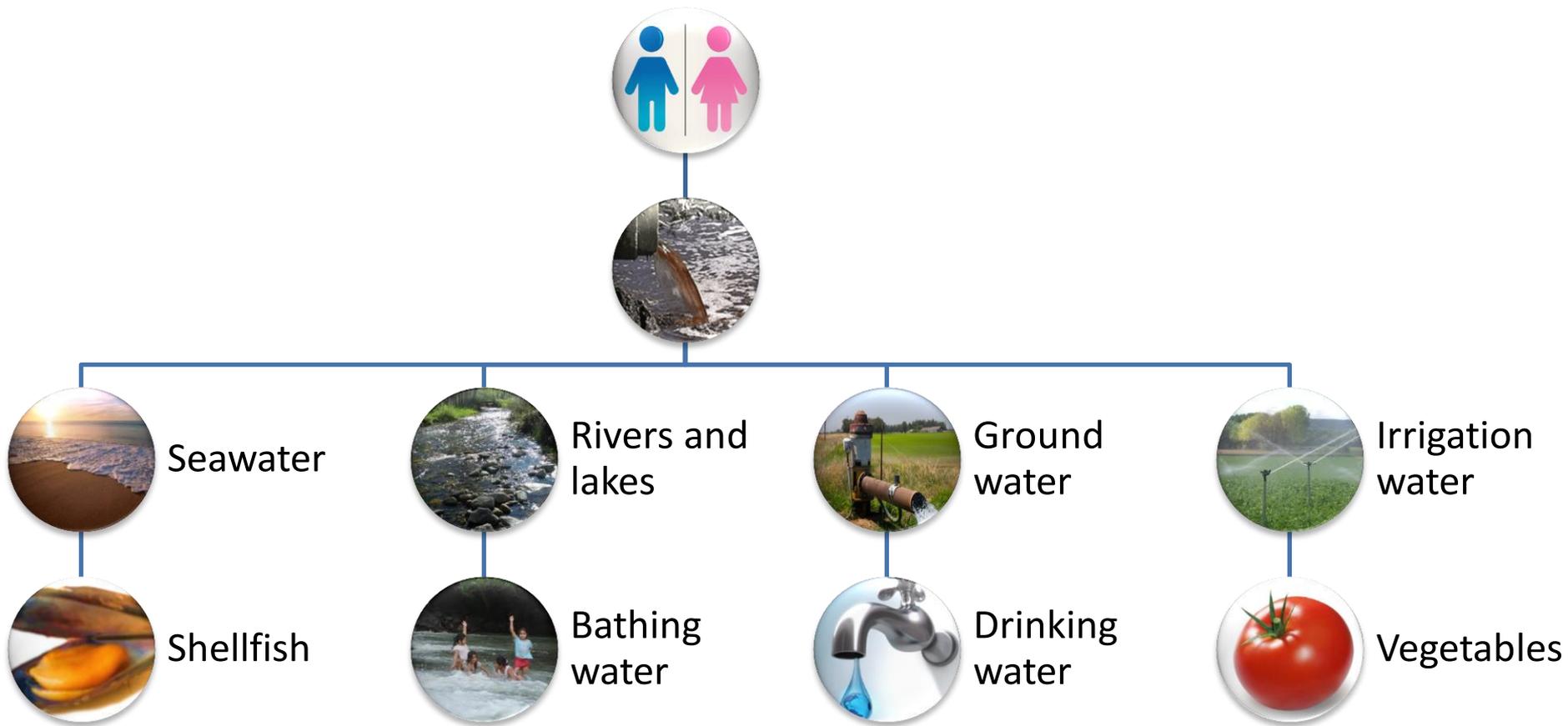
- **DTU and UB** will exchange a staff research member each during 1 month for sharing and training of NGS related procedures.
- **UB will host a SGL** research staff member for 0.5 months for training on molecular viral methods.
- **Two members of LGL will move to TUM and LfU** for 0.5 and 0.2 months to learn DNA-marker method, micro array method and sampling large volumes of water.
- **One member of UPV will move to WIG** (Water Institute of Granada) for 0.5 month training in NGS related procedures.

.

# Pathogens in wastewater disseminate in ground water, river catchments and impacted irrigation water and seawater



- ✓ Environmental waters are susceptible to fecal contamination from point and nonpoint sources. Excreted viruses may contaminate water and food.

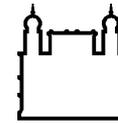


# Concentration of viruses from river, seawater, groundwater, using direct flocculation with skimmed milk

Direct Flocculation Procedure:  
A: Detail of the flocculated sample.  
B: Image of the sedimented flocs.



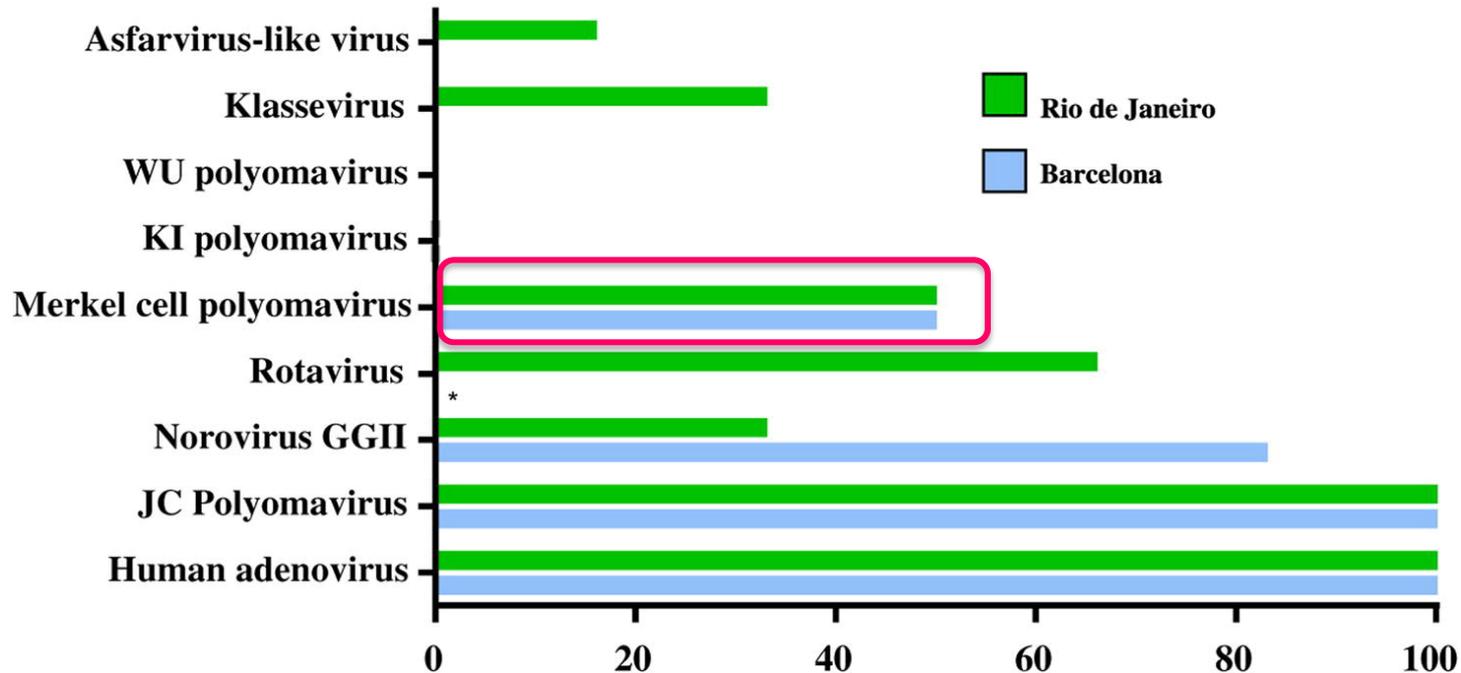
- Viruses in river water



Ministério da Saúde

FIOCRUZ  
Fundação Oswaldo Cruz  
Instituto Oswaldo Cruz

Occurrence of viruses analyzed in river water

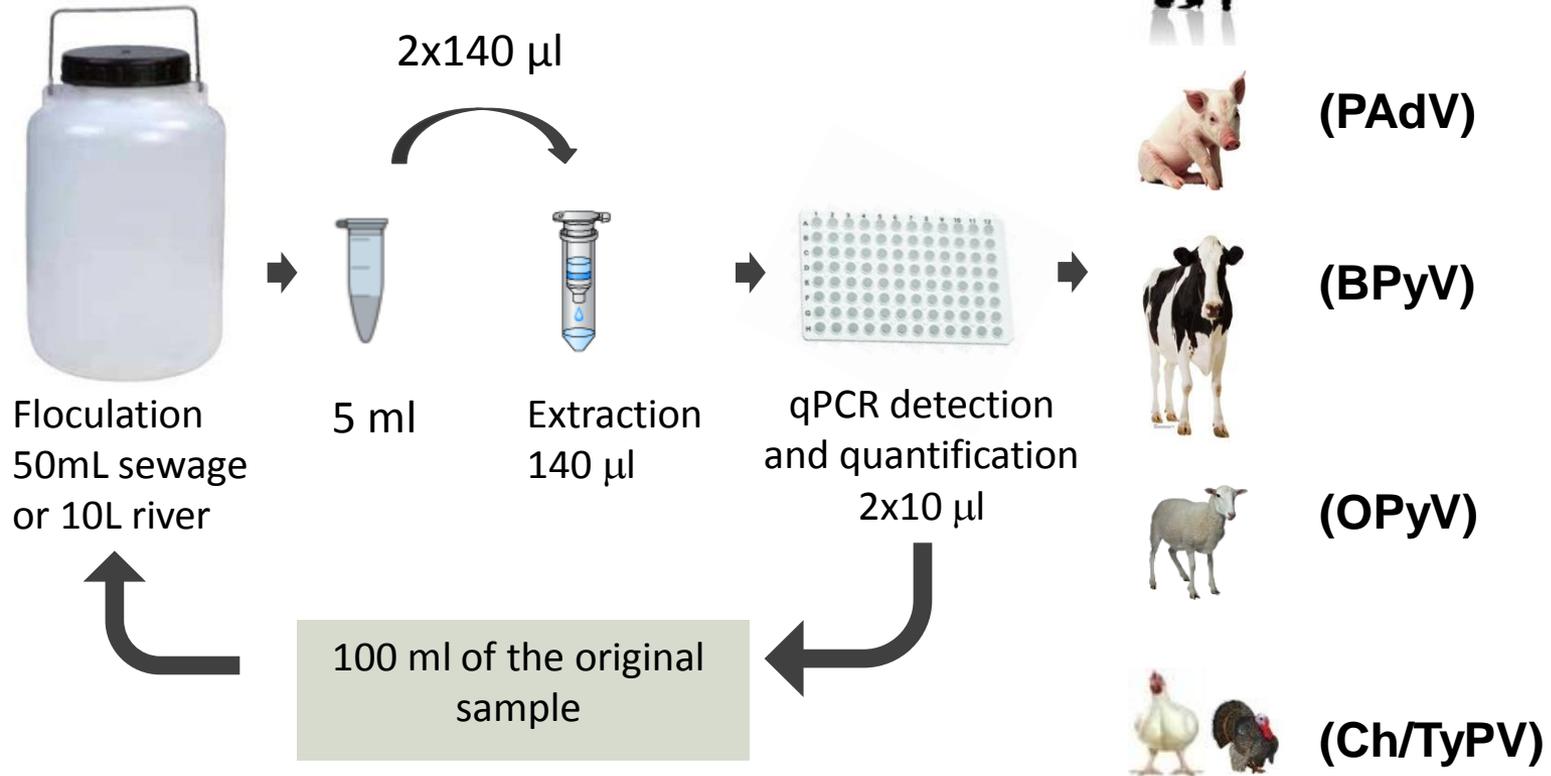


% of positive samples (n=6 percity)

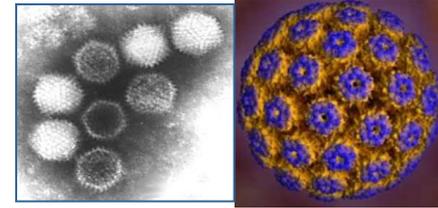
\*Rotavirus not analyzed in Barcelona

Detection and quantification of classic and emerging viruses by skimmed-milk flocculation and PCR in river water from two geographical areas. Calgua B, Fumian T, Rusiñol M, Rodriguez-Manzano J, Mbayed VA, Bofill-Mas S, Miagostovich M, Girones R. 2013 May 15;47(8):2797-810.

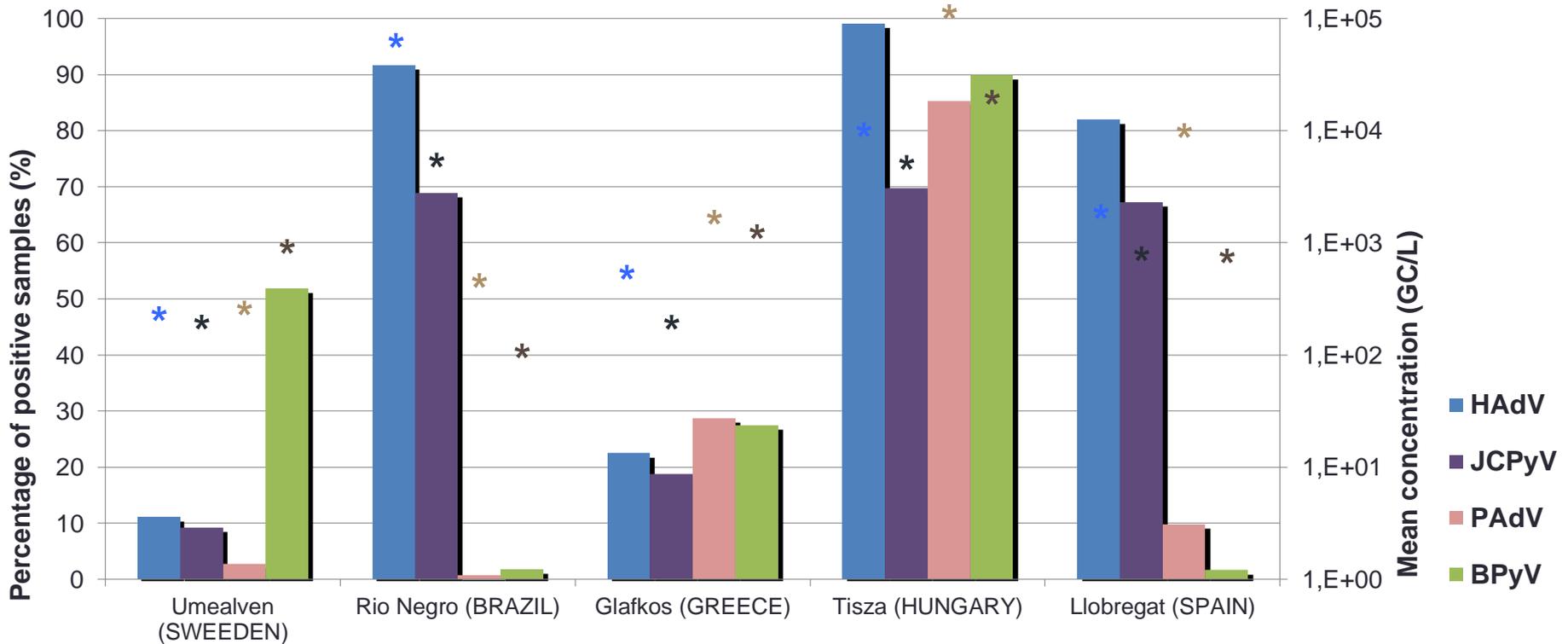
## Analysis of viruses

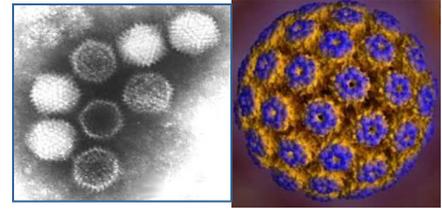


Process control: HAdV 35

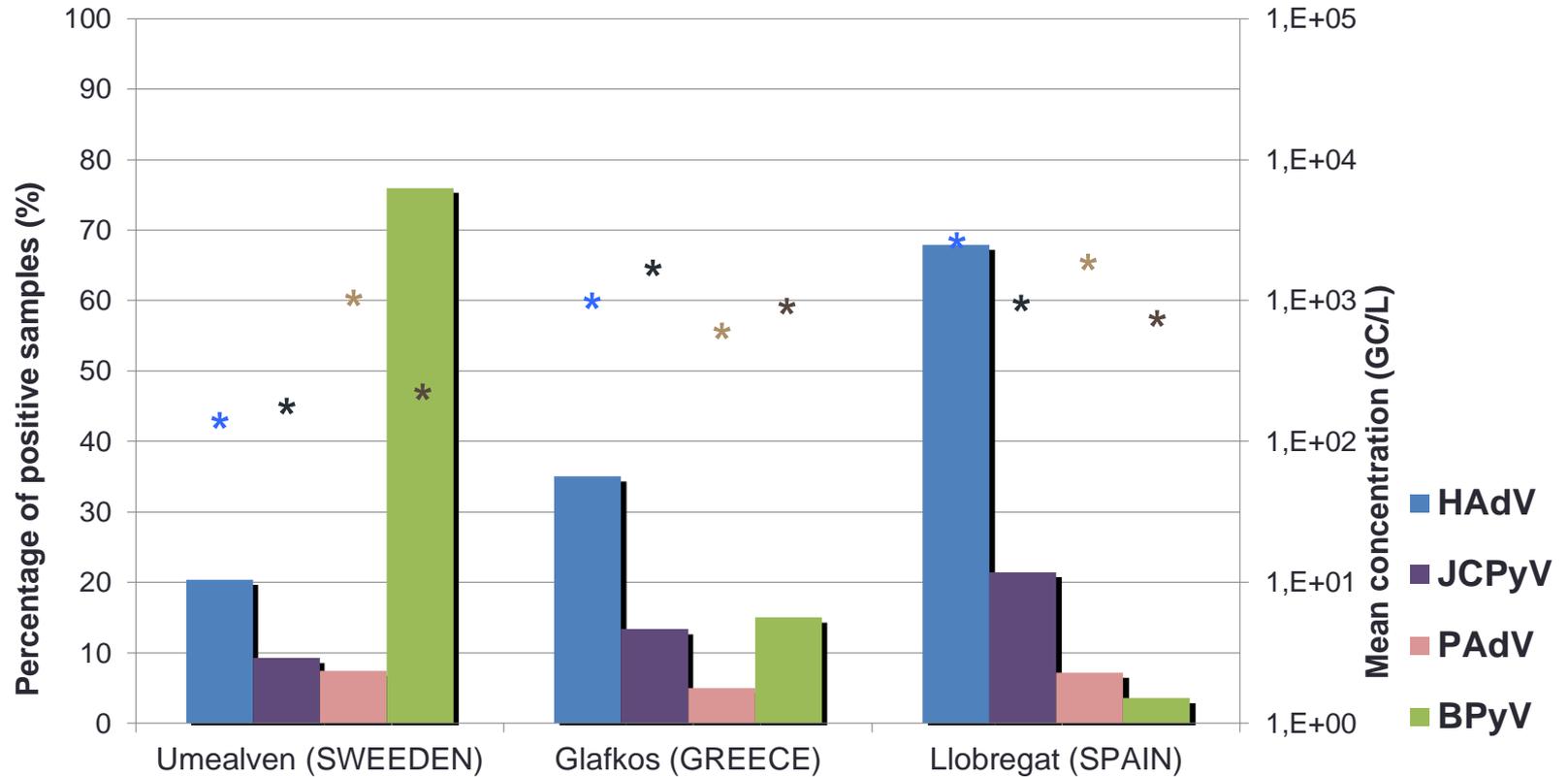


### Human and animal viruses in diferent river cathments





### Human and animal viruses in seawater



## Water Reuse: Using Reclaimed Water for Irrigation

Sampling point	Number of samples (n)	HAdV GC/L	JCPyV GC/L	NoVGI GC/L	NoVGII GC/L	<i>E.coli</i> (NMP/100 ml)	Intestinal enterococci (NMP/100 ml)
Secondary treatment	8	1,51E+04 (8/8)	2,27E+03 (7/8)	1,31E+04 (6/8)	6,43E+04 (8/8)	8,47E+04 (8/8)	9,72E+03 (8/8)
Tertiary treatment	8	2,75E+04 (8/8)	3,94E+03 (8/8)	3,59E+04 (7/8)	1,53E+04 (8/8)	1,29E+02 (2/8)	3,80E+01 (1/8)
Irrigation water	15	2,26E+04 (12/15)	3,59E+04 (12/15)	1,32E+05 (8/15)	1,06E+04 (14/15)	5,66E+01 (7/15)	5,65E+01 (7/15)



**Food samples.** Food vegetables (mostly lettuce) irrigated with reclaimed water were analyzed following the CEN concentration method TS 15216-1 and using MS2 bacteriophage as a process control.



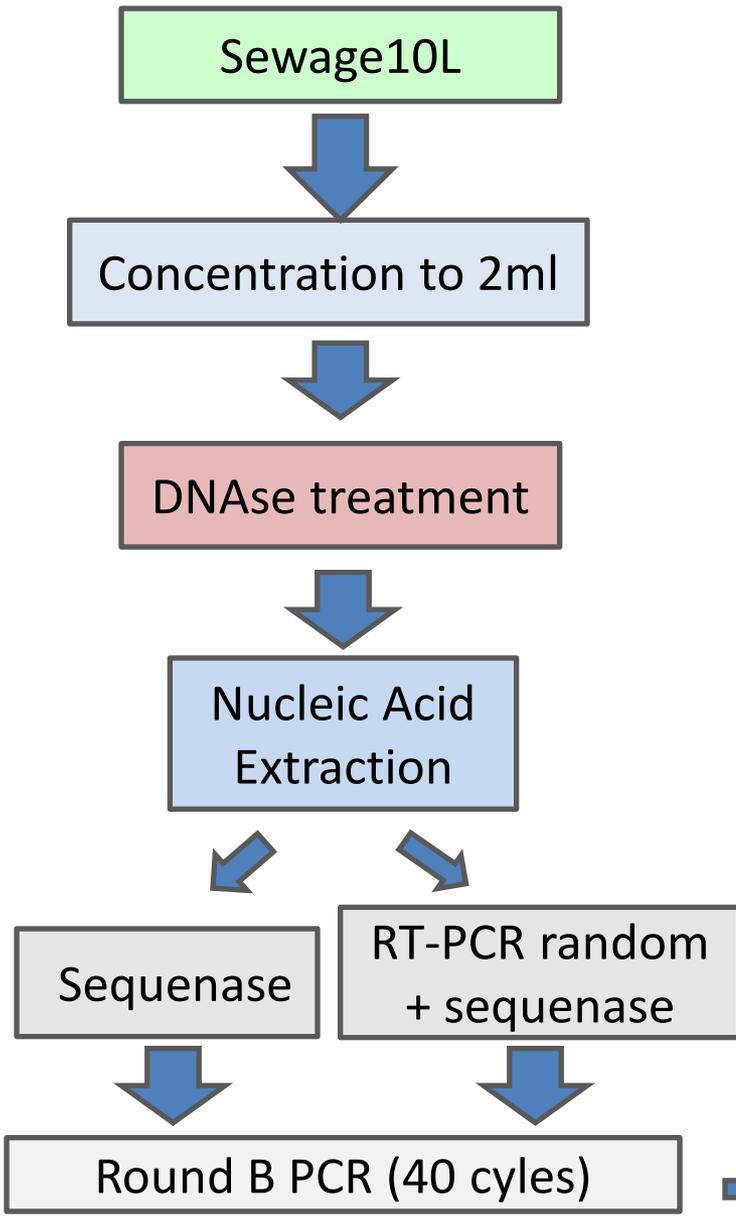
Food matrices	Number of samples	HAdV	NoVGI	NoVGII
Vegetables (Lettuce, mint, chard and basil)	20 (8 month sampling)	1/20 (Lettuce)	(0/20)	1/20 (chard)

## Metagenomics contribute to analyze microbial populations

- ❑ Advances in high-throughput, deep sequencing technology make it possible to characterize virome richness, gene functions, and association with disease. The human virome is the collection of all viruses that are found in or on humans
- ❑ Viral infections of humans include acute, self-limited infections; fulminant, uncontrolled acute infections; and chronic infections that may be asymptomatic or that may be associated with serious, even fatal diseases such as AIDS.
- ❑ Furthermore, many diseases of unknown etiology are thought to be of viral origin



# Metagenomics of excreted viruses in sewage samples from Barcelona using Illumina MiSeq



Sample	Reads	Mean length
Winter	2828219	231
Autum	796157	243
Spring	1177145	227

