



UNIVERSITAT DE BARCELONA



# Distribution of HEV in the environment

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# Epidemiology of hepatitis E virus

- ❑ Traditionally, North America and Europe have been considered as non-endemic regions with a seroprevalence of anti-HEV antibodies of 1 to 5%.
- ❑ The amount of IgG anti-HEV detected in the population of Spain and Catalonia are 5.5% and 7.3%, respectively.
- ❑ Animal Reservoirs: it is known that HEV was present in Spanish pig farms with 97% farms positive for anti-HEV IgG antibodies.

# Comparison of HAV and HEV

	HAV	HEV
Incubation period	~30 days	~40 days
Mortality	0.1-2 %	1-4 %
Mortality in pregnancy	No difference	>20 %
Age	Older children, younger adults	Older children, younger adults

Characteristic	HAV	HEV
Family	<i>Picornaviridae</i>	<i>Hepeviridae</i>
Genus	<i>Hepatovirus</i>	<i>Hepevirus</i>
Size / RNA genome	27-32 nm / 7.5 Kb	27-34 nm / 7.5 Kb
Genotypes	Six 1,2,3: human; 4,5,6: simian	Five 1,2: human; 3,4: human, swine; 5: avian
Serotypes	One	One (1-4)

# Objectives

1. To evaluate the evolution of the molecular epidemiology and the dissemination of HEV in the environment and in comparison with the outcomes of previous studies.
2. To characterize the strains of E virus present on the studied populations.
3. To evaluate the effect of sanitation in the reduction of the dissemination of HEV in the environment.
4. To evaluate whether HEV is a significant emergent pathogen in Spain, as a model of developed country.



# Environmental Samples

## WATER

Ter River (~ 100 l)

Llobregat River (1-250 l)

## BIOSOLIDS

Biosolids from WWTP (100ml)

## SLUDGE

Slaughterhouse sludge (100ml)

## WASTE WATER

Urban sewage (50ml)



# Methodology



**Samples**

**Concentration of viral particles into small volumes**

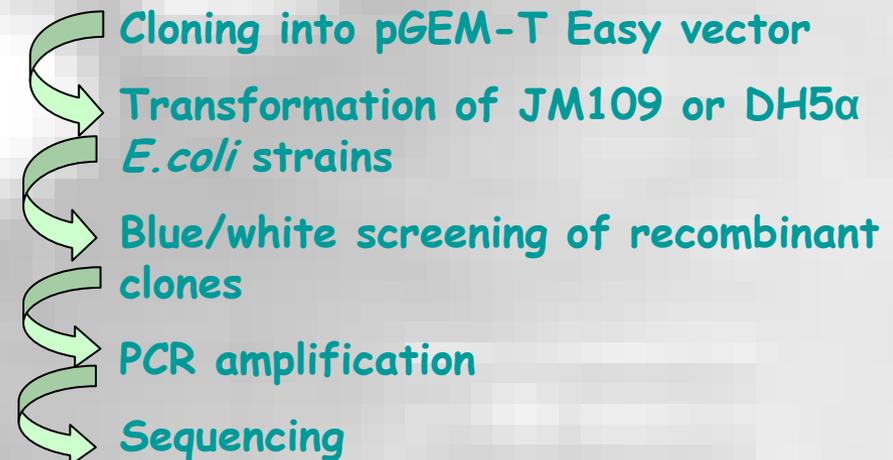
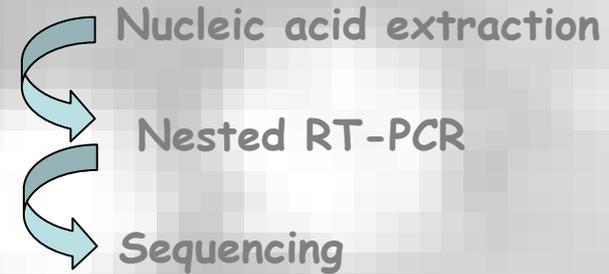
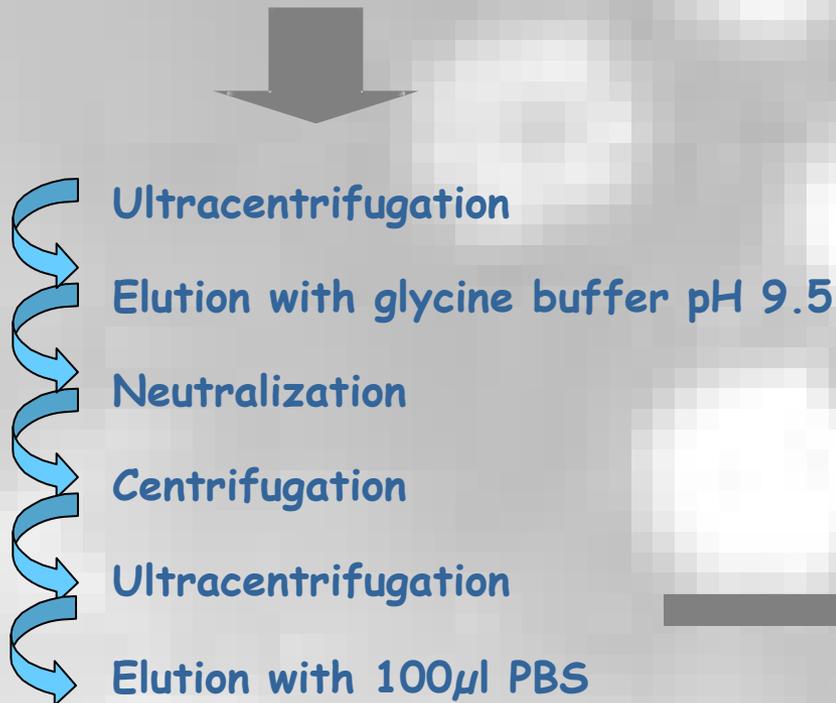
Nucleic acid extraction  QPCR  
Nested- PCR  
Sequencing

 Cloning into pGEM-T Easy vector  
 Transformation of JM109 or DH5α *E.coli* stains  
 PCR amplification  
 Sequencing

# Methodology for urban sewage samples



50 ml sewage water



# Detection of viruses in river water

## Glass wool filtration

**Pre-acidification** pH  
3,5



**Filtration** through glass  
wool columns 1l/min



Elution by gravity flow with 200ml of **Glycine  
buffer** 0.05M pH 9.5 + 3% beef extract



**Organic flocculation** at pH 3.5



**Centrifugation** at  
7,000xg, 30 min, 4°C

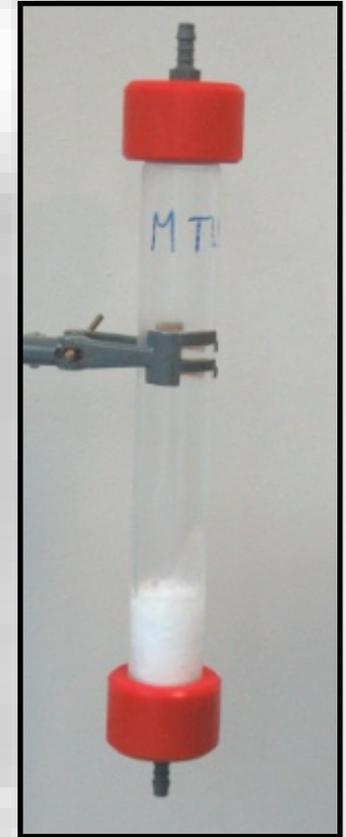
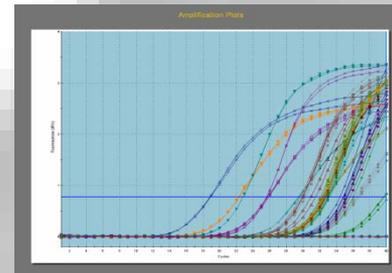


**Resuspension** of the pellet in 5 ml of  
PBS and storage at -80°C



Nucleic acid extraction

nPCR HEV  
and/or  
qPCR



# Primers and probes used

## Hepatitis A virus

Reaction	Region	Publication
Nested PCR	5'NTR	Pina et al., 2001
Nested PCR	VP1/2A	Pina et al., 2001
QPCR	5'NTR	Jothikumar et al., 2005

## Hepatitis E virus

Reaction	Region	Publication
Nested PCR	ORF2	Ercker et al., 1999
QPCR	ORF3	Jothikumar et al., 2006

## Human adenoviruses

Reaction	Region	Publication
QPCR	Ad hexon	Hernroth et al., 2002

# Evaluation of the diversity of HEV strains simultaneously circulating

□ Selected representative samples were analyzed by cloning amplicons and sequencing analysis (10-12 clones):

- √ 6 samples from two different sewage treatment plants in the area of Barcelona
- √ 2 samples of biosolids from a urban sewage treatment plant
- √ 2 samples from sludge generated in a slaughterhouse treating pigs



# Diversity of HEV strains

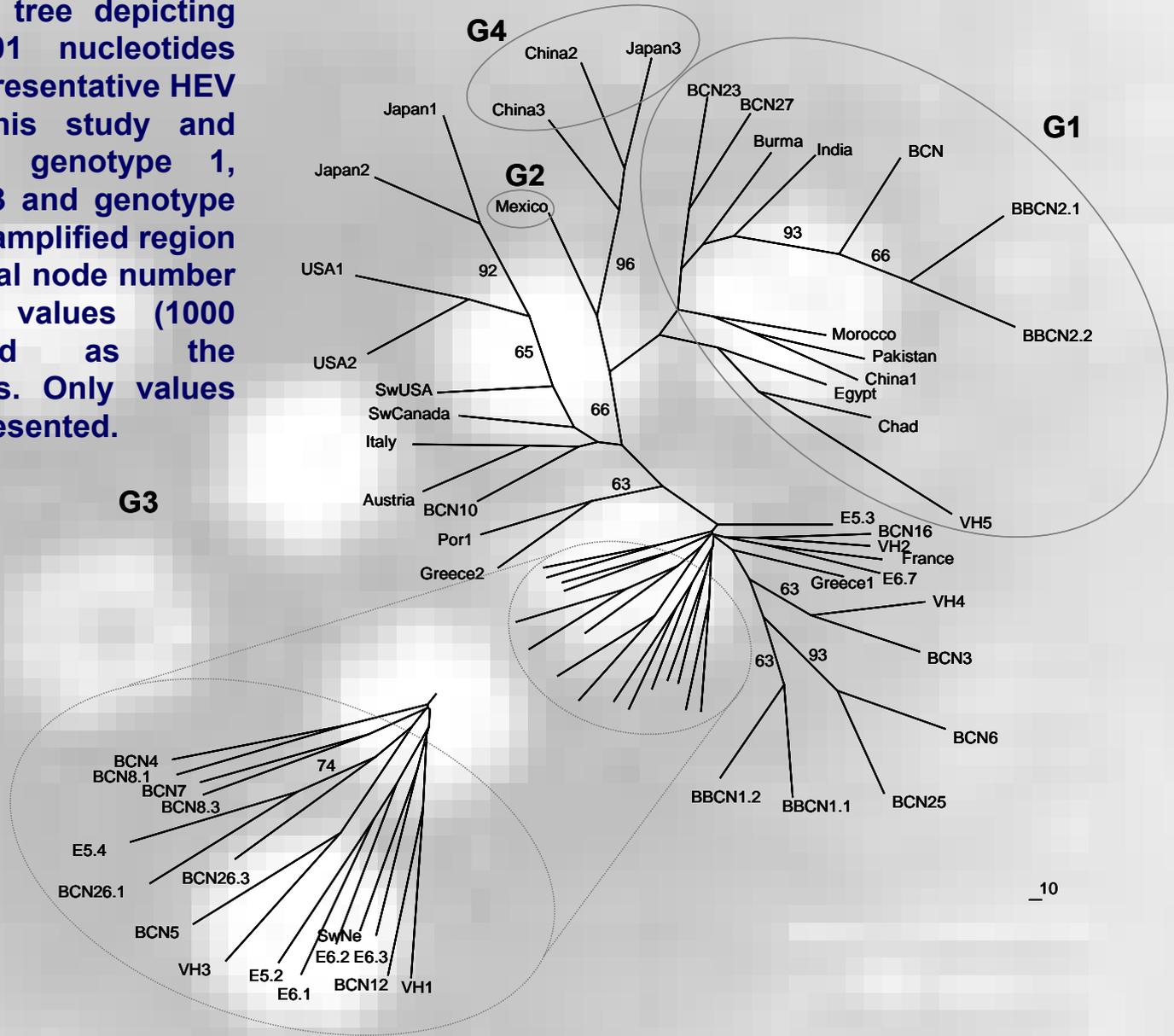
Typification and diversity of the HEV strains identified by cloning the amplicons obtained from the analyzed samples

Sample	Type of sample	Sampling (year/month)	Sequences found	% intra-sample similarity	Genotype
BCN8	urban sewage	2001/April	3 (BCN8.1 to 3)	90-96%	G3
BCN10	urban sewage	2001/May	1 (BCN10)	-	G3
BCN25	urban sewage	2003/March	1 (BCN25)	-	G3
BCN26	urban sewage	2003/May	5 (BCN26.1 to 5)	94-99%	G3
BCN23	urban sewage	2005/February	1 (BCN23)	-	G1
BCN27	urban sewage	2007/December	1 (BCN27)	-	G1
BBCN1	biosolid <sup>a</sup>	2005/January	2 (BBCN1.1 & 2)	99%	G3
BBCN2	biosolid <sup>a</sup>	2005/February	3 (BBCN2.1 to 3)	98-99%	G1
E5	sludge <sup>b</sup>	2004/May	4 (E5, E5.2 to 4)	92-99%	G3
E6	sludge <sup>b</sup>	2006/February	8 (E6.1 to 8)	89-99%	G3

<sup>a</sup> from an urban sewage treatment plant

<sup>b</sup> from a slaughterhouse (more than 80% of the processed animals in this slaughterhouse were pigs)

Unrooted phylogenetic tree depicting relationships over 101 nucleotides within ORF2 among representative HEV strains reported in this study and others isolates from genotype 1, genotype 2, genotype 3 and genotype 4, when comparing the amplified region within ORF2. The internal node number represents bootstrap values (1000 replicates) expressed as the percentage of all trees. Only values greater than 60 are represented.



# Data on sanitation and clinical cases

- From 1992 to 2008, the region of Catalonia experienced a significant increase in the number of sewage treatment plants (from 91 to 343) and in the total volume of waste water depurated (from 991,892 to 2,786,871 m<sup>3</sup>/day).
- During the period from 2004 to 2007, HEV was also analyzed in 19 serum samples from patients with acute hepatitis symptoms attending the Hospital General Vall Hebron (Barcelona, Spain). Three positive cases were identified presenting HEV genotype 3 and one presenting HEV genotype 1, probably imported in a recent trip to India.

# Conclusions

- **The results obtained in this study strongly suggest that HEV has replaced HAV as the most frequently detected hepatitis virus potentially transmitted through local faecal contaminated water or food in SW Europe.**
- **The dramatic reduction in the presence of HAV in sewage observed in SW Europe would be attributed to considerable improvements in sanitation. However, these improvements have not had an equivalent effect on the circulation of HEV genotype 3 in the area. The continued circulation of this genotype would be maintained considering other animal hosts as HEV infections in swine representing an external source of HEV in the population.**
- **The results proved the presence of HEV strains belonging to genotype 3 and also sporadically to genotype 1 in urban sewage and biosolids. Contamination of food and water through their contact with sewage not properly treated and biosolids presenting HEV may represent a significant risk for human populations in relation to HEV even in industrialized areas.**

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