



UNIVERSITAT DE BARCELONA



Molecular Epidemiology of Hepatitis A and E viruses

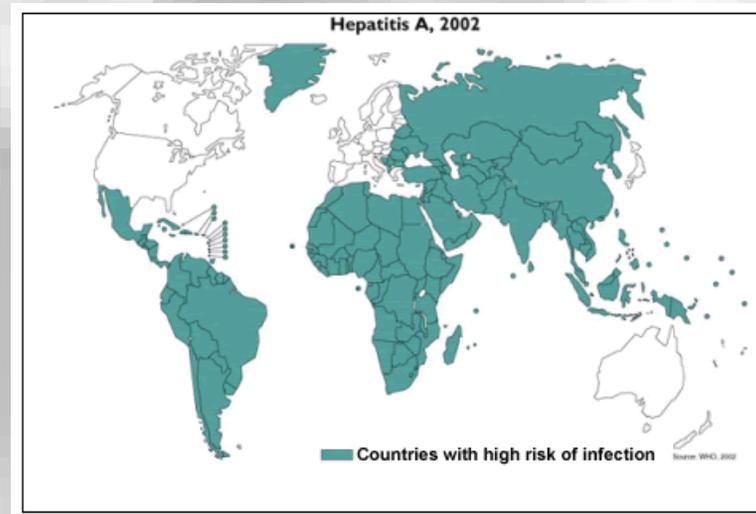
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The hepatitis A virus

- Hepatitis A virus (HAV) has been classified as the only member of the genus *Hepatovirus* in the family *Picornaviridae*
- HAV is transmitted by the faecal-oral route, and causes acute hepatitis in humans and some other primates
- HAV has a single antigenic serotype and a single infection is considered to confer lifelong immunity. To define the genetic relatedness of different strains we analyze the virus detected in the environment or in clinical samples using nested-PCR and comparing nucleotide sequences.
- Previous comparative studies of the nucleotide sequences of different HAV strains have suggested that sequence relatedness can be correlated with the geographical origin of viruses.

Epidemiology of hepatitis A infection

- Spain is considered an area of low endemicity for the HAV infection and is characterised by diminishing HAV seroprevalence in the population.
- Within Catalonia (Spain), a seroprevalence of 67.8% in the general population has been documented, less than 5% being seropositive in the group from 5 to 14 years old. Therefore, a large percentage of the Spanish population under 30 years of age is not protected against hepatitis.



Viral hepatitis cases reported in the “Boletín Epidemiológico Nacional” and in the “Butlletí Epidemiològic de Catalunya”

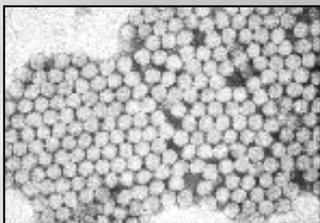
	2003		2004		2005
	España	Cataluña	España	Cataluña	España
Virus Hepatitis A	796	225	838	214	1062
Virus Hepatitis B	736	123	751	134	636
Otras Hepatitis Víricas	688	---	870	---	662

Molecular Epidemiology of HEV in a non-endemic area, comparative study with HAV infections

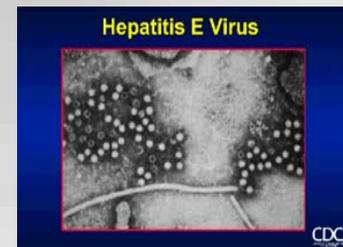
Previous studies: Development of PCR-based techniques for the study of the molecular epidemiology of water- and food-borne viruses

Analysis of clinical and environmental samples for the identification of the viral strains infecting the population.

HEV is considered an emergent pathogens according to the generic definition: Emergent water or food borne diseases can be defined as those that have newly appeared, have existed but are rapidly increasing in incidence and/or geographic range, or those for which water or food borne transmission routes have only recently been recognized.

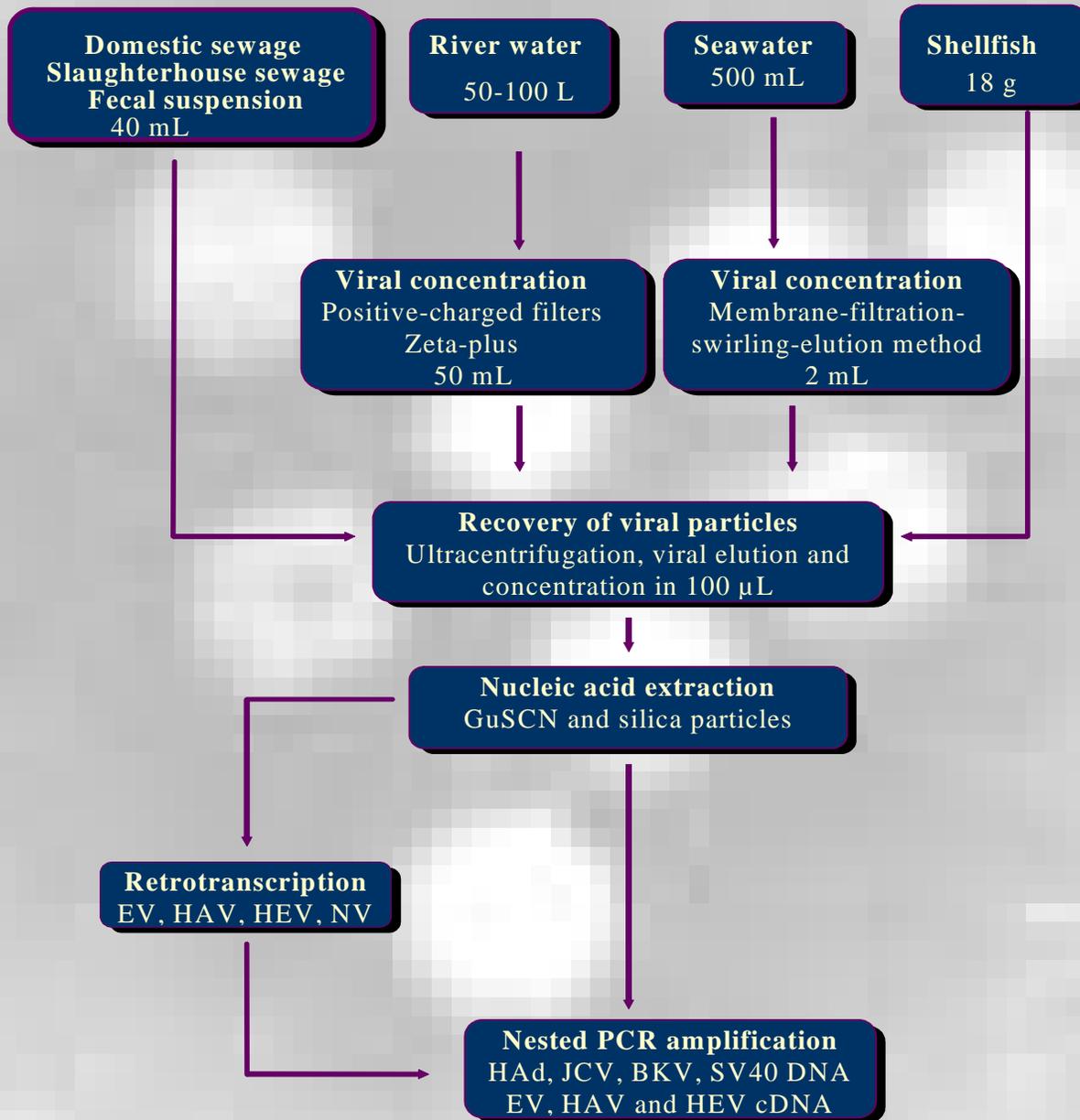


Hepatitis A Virus



Hepatitis E virus

Methodology



Methodology for urban sewage samples



40 ml sewage water

- 
- Ultracentrifugation
 - Elution with glycine buffer pH 9.5
 - Neutralization
 - Centrifugation
 - Ultracentrifugation
 - Elution with 100µl PBS

- 
- Nucleic acid extraction
 - (Semi)Nested RT-PCR
 - Sequencing

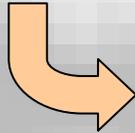
- 
- Cloning into pGEM-T Easy vector
 - Transformation of JM109 or DH5α *E.coli* strains
 - Blue/white screening of recombinant clones
 - PCR amplification
 - Sequencing

Identification of HAV strains

Urban sewage and superficial water samples



Recovery and concentration of viral particles



Detection of HAV by nested RT-PCR

-  conserved region 5'-NTR
-  hypervariable region VP1/2A



Analysis of the amplified sequences



Annealing of the sequences



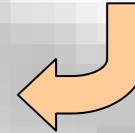
Phylogenetic analysis

Clinical samples

Serum samples of acute hepatitis patients



Detection of IgM-VHA using ELISA



Positive samples



Presence of HAV in the environment (1994-2000)

Type of sample	Positives by nested PCR	Estimated concentration
Sewage water	31/54 (57.4%)	$10-10^2$ GE/ml
Llobregat River	22/56 (39.2%)	$10-10^2$ GE/l
Ter River	2/10 (20%)	10 GE/l
Shellfish	4/104 (3.8%)	10^2-10^3 GE/g

Presence of HAV in sera from patients with acute hepatitis (1990-2000)

N° patients	74
Aged	18-79 years old

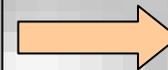
Presence of IgM anti-HAV	26/74 (35.1%)
Presence of HAV-RNA	16/26 (61.5%)

Detection and identification of strains of HAV

- The sequence of nucleotides of the region 5'-NTR shows at least two differences in relation to the control strain

G-551 G-591 in the control strain (related to adaptation to cell culture)

A-551 A-591 in the environmental and clinical strains



Field strains of HAV

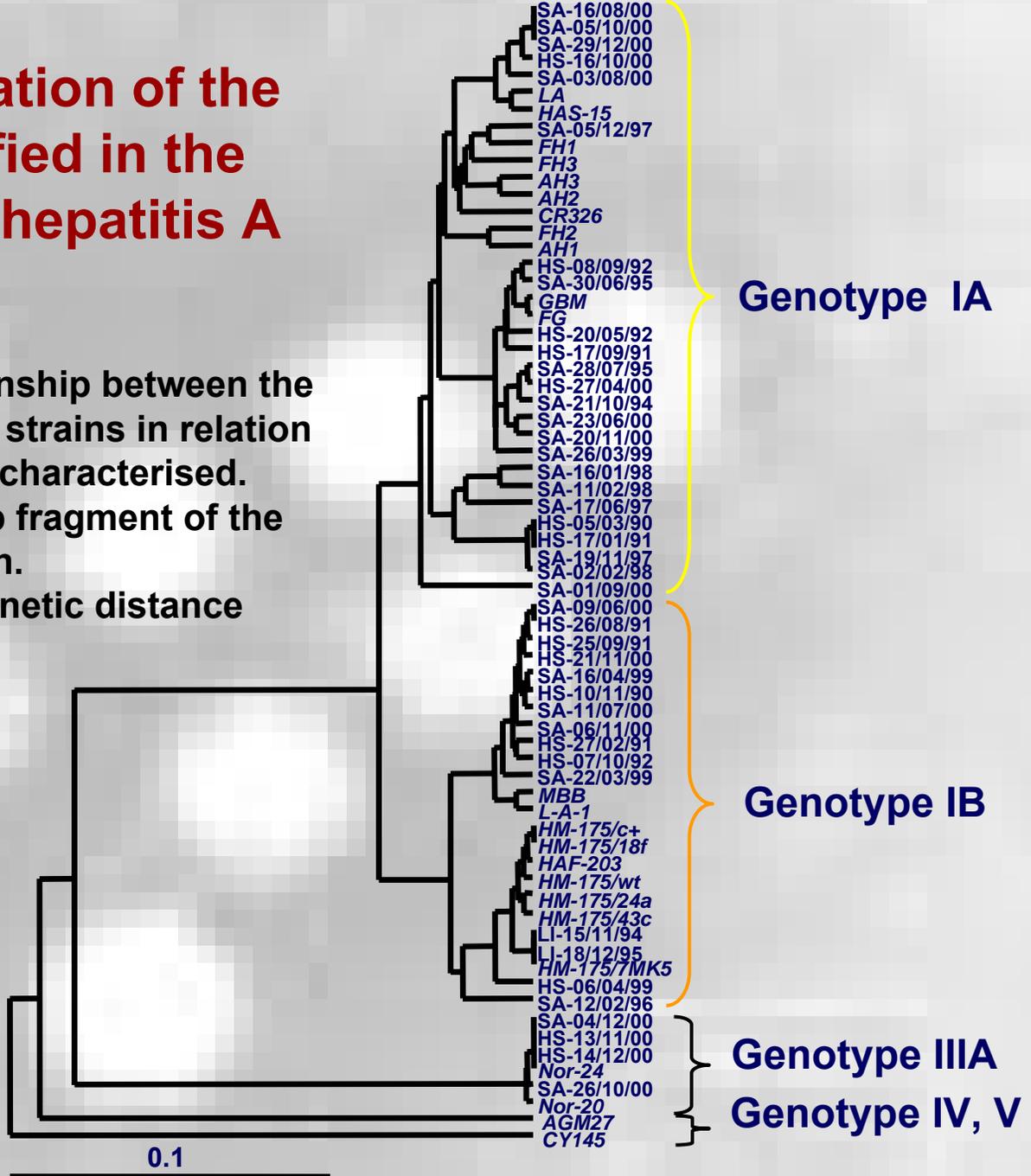
- The sequence of nucleotides in the hypervariable region is used for *typification*

Identification of HAV strains

Samples	Differences with the control (398 nt)	Identified strains	Genotype	Prevalence
Environmental (16 samples)	2-44	3 HM-175 1 MBB 1 L-A-1	IB	55%
		7 GBM 3 FG 1 FH1	IA	44%
Clinical (11 samples)	16-43	1 HM-175 5 MBB	IB	54%
		5 GBM	IA	45%

Genetic characterization of the HAV strains identified in the environment and in hepatitis A patients

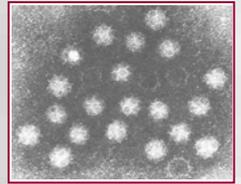
Phylogram depicting the relationship between the environmental and clinical HAV strains in relation to other isolates previously characterised. The region analyzed is 296-bp fragment of the VP1/2A junction. The scale represents the genetic distance



Conclusions

-  **Genotype I strains are the most frequently detected (genotype IA and IB, 50% each) in the environment and in hepatitis patients. Genotype III strains were detected with lower prevalence in the environment and in hepatitis patients during the same period of time (two environmental and two clinical samples were positive).**
-  **It was not possible to identify strains belonging to a common endemic genotype.**
-  **The abundance of HAV in the environment produces a situation of sanitary risk, especially considering the low prevalence of antibodies in the young population.**

Hepatitis E virus



Family *Hepeviridae*, Genus *Hepevirus*

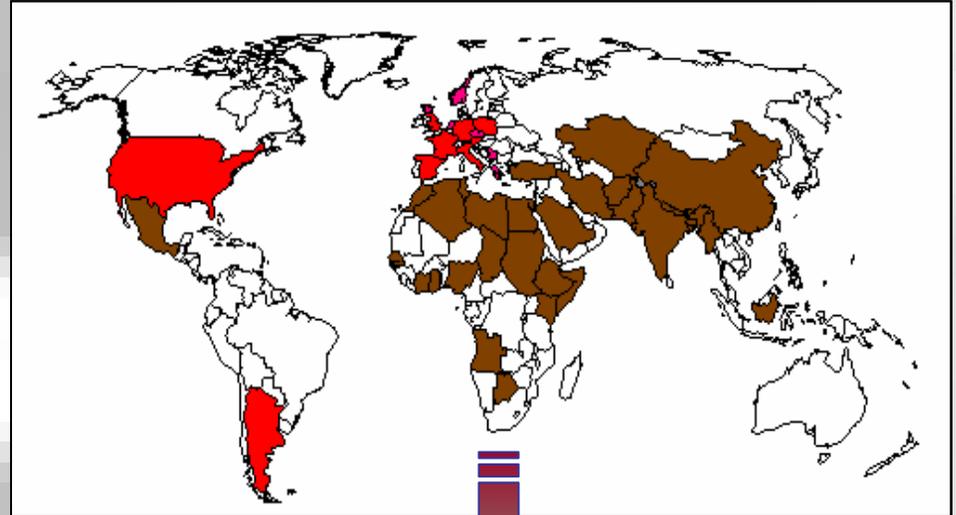
- ❑ Important pathogen in Asia, Africa, Middle East and Central America
- ❑ HEV presents a mortality rate of about 1% but it could be up to 20% in pregnant women
- ❑ The prevalence of HEV antibody in non-endemic areas is considered to be from 3 to 20%. In Catalonia, Northeast of Spain the seroprevalence has been evaluated as 7.3%.

Outbreaks

Place	Year	No. cases
New Delhi (India)	1955	29000
Kyrgyz Republic	1955-56	10800
Katmandu Valley (Nepal)	1973-74	10000
Ahmedabad (India)	1975-76	30000
Mandalay (Burma)	1976-77	20000
Kashmir (India)	1978-82	52000
Algeria	1980-81	780
Tortiya (Côte d'Ivoire)	1983-84	800
Ethiopia	1985-86	2000
Xinjiang (China)	1986-88	120000
Somalia	1988	11400
Kanpur (India)	1990-91	79000
Islamabad (Pakistan)	1993-1994	3827
Sadr City (Iraq)	2004	100
Chad	2004	1442
Sudan	2004	6861

Distribution in the world of the HEV clinical cases

▶ ***“ENDEMIC”*** areas serologically confirmed, with documented epidemic outbreaks or frequent sporadic cases.



▶ ***“NON ENDEMIC”*** areas with documented sporadic cases, most of them considered as imported.



Molecular epidemiology of Hepatitis E in Spain

- ❑ **Detection of 6 HEV strains isolated from serum samples from patients with acute hepatitis (3 positive samples between 1989-99, and 3 in 2003)**
 - 4 autochthonous strains (genotype 3)
 - 1 imported strain (genotype 1)

- ❑ **Detection of 11 acute hepatitis patients with IgG anti-HEV in the acute phase of the hepatitis (disappearance of IgG after few months, could be an indication of HEV?)**
 - 3 with IgM anti-HEV and HEV-RNA

Detection of HEV in urban sewage (I)



□ **Analysis of HEV-RNA presence in 46 raw urban sewage samples from the entry of a wastewater treatment plant in Barcelona (Spain)**

✓ 20/46 (43.5%) positive samples:

1 sample from 1996 (BCN15)

1 sample from 1999 (BCN16)

18/34 samples from 2000-2002 (BCN2 to BCN14)

□ **Analysis of HAV-RNA presence in 27 of those 46 raw sewage samples**

✓ 21/27 (77.8%) positive samples

Detection of HEV in urban sewage (II)

□ Analysis of sewage samples from other industrialized countries:

◆ 5 sewage samples from Washington (USA)

✓ 1 positive sample for HEV → W1 strain

✓ 5 positive samples for HAV → genotype IA

◆ 4 sewage samples from Nancy (France)

✓ 1 positive samples for HEV → N1 strain

✓ 3 positive samples for HAV → genotype IA

◆ 4 sewage samples from Umeå (Sweden)

✓ 0 positive samples for HEV

✓ 1 positive sample for HAV → genotype IB

◆ 5 sewage samples from Patras (Greece)

✓ 0 positive samples for HEV

✓ 1 positive sample for HAV → genotype IA

Diversity of VHE strains infecting simultaneously the population



□ Isolation of HEV RNA in samples from a urban sewage treatment plant

✓ Afluent (raw urban sewage):

4/5 positive samples → genotypes 3 and 1

✓ Sludge and biosolids generated:

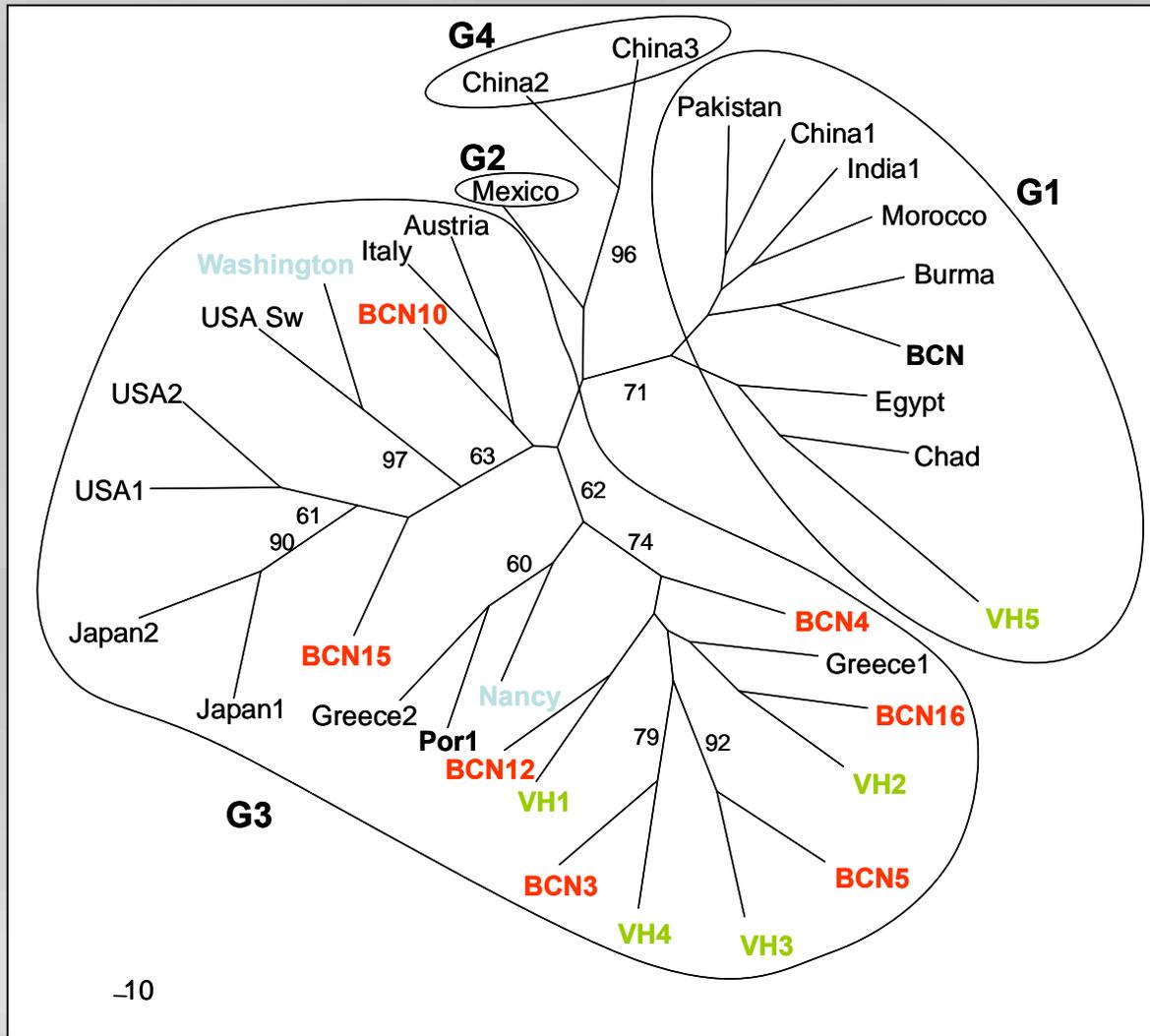
2/5 positive samples → genotypes 3 and 1

Natural infection in pigs with HEV-related strains



- ❑ The detection of IgG anti-HEV by ELISA showed a seroprevalence of about 19% in Catalonia
- ❑ HEV-RNA was detected in 6/36 pools (1/9 farms) of fecal samples (Por1 strain)
- ❑ One HEV strain (probable porcine origin) was isolated from 1 slaughterhouse sewage sample (E11 strain)
- ❑ Thirteen HEV strains (probable porcine origin) were isolated from 2 (2/5 samples) slaughterhouse biosolid sample
- ❑ All HEV strains identified from porcine origin were typified as genotype III

Phylogenetic studies with human HEV strains

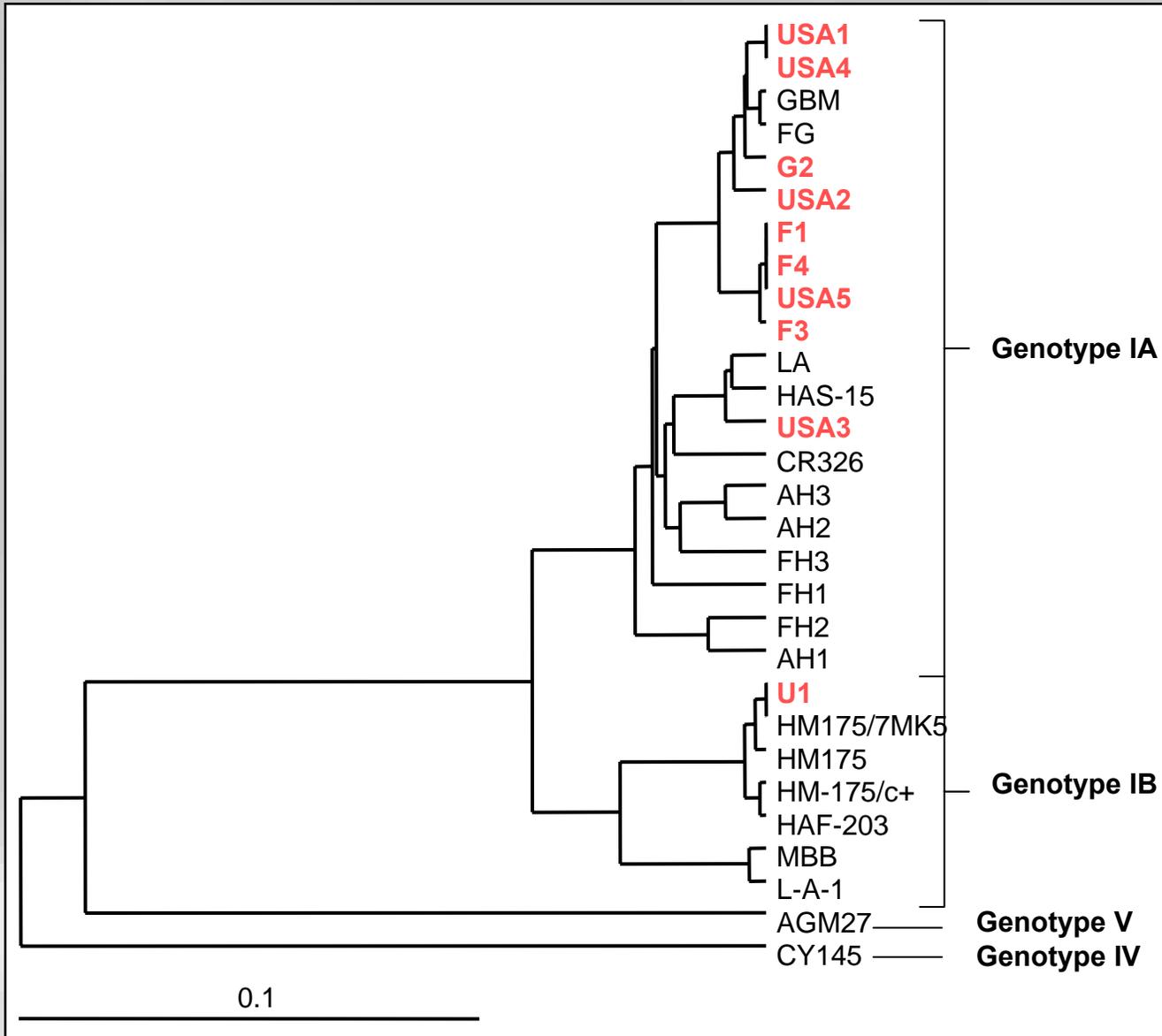


HEV

Neighbor joining tree constructed for representing phylogenetic relationships among the representative sequences found in Barcelona (in bold) and other isolates from "endemic areas" and "non-endemic areas". The bootstrap confidence levels obtained for 1000 replicates are shown (only values greater than 60 are indicated).

Phylogenetic studies

HAV



Conclusions (I)

- **The study presented here provides a framework for the global analysis of the HAV and HEV strains that circulate among the population**
- **A significant prevalence of HEV infections in industrialized countries has been proven by testing urban sewage samples from wide diverse geographical areas. These locations also presented a high percentage of samples positive for HAV.**
- **It has been showed that for HEV and HAV diverse viral strains are circulating in the same location, being the HAV strains more globally disseminated than HEV. Different common HEV genotypes are detected in the diverse geographical areas and in infected animal hosts.**
- **HEV virus are widespread also among swine populations (genotype III) in Spain. It is important to point out the great similarity between strains from human and swine origin and the fact that pigs represent a host for the infection. These swine HEV strains are more similar to the human HEV strains from the same area than to swine HEV strains from other areas**

Conclusions (II)

- **HEV strains isolated from sewage samples are identical or very similar to the strains causing acute hepatitis and to the swine HEV strains identified in the same area.**
- **HEV should be considered as an environmental contaminant even in highly industrialized countries and the transmission routes of the infection must be investigated.**
- **Further monitoring will be needed in order to evaluate:**
 - **A potential reduction of the presence of HAV in the environment in several years after the implementation of the vaccination program**
 - **The dissemination of HEV genotypes that can cause outbreaks (i.e. GI) over the regions previously considered non-endemic, where GIII is the common genotype identified**

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