UNIVERSITAT DE BARCELONA

Presence of HAV in the environment in Catalonia, Spain

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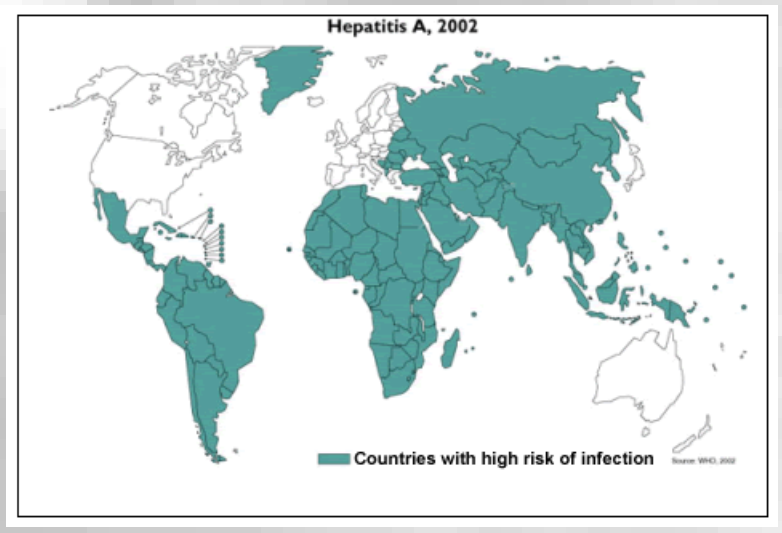
Epidemiology of hepatitis A infection

- 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.
- Since 1999 in Catalonia it has been established a pilot program (approved until the 2013-2014) for anti-hepatitis vaccination A+B for pre-adolescents



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

40 ml sewage water

Ultracentrifugation

Elution with glycine buffer pH 9.5

Neutralization

Centrifugation

Ultracentrifugation

Elution with 100µl PBS

Nucleic acid extraction

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

Clinical samples

Serum samples of acute hepatitis patients

Detection of IgM-VHA using ELISA

Positive samples

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

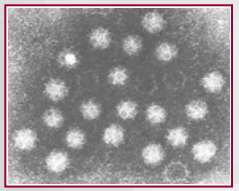
Presence of HAV in the environment (1994-2000)

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)

Nº patients	74
Aged	18-79 years old
Presence of IgM anti-HAV	26/74 (35.1%)
Presence of HAV-RNA	16/26 (61.5%)

Molecular Epidemiology of HEV and HAV in industrialized countries



Hepatitis E virus

Family *Hepeviridae,* Genus *Hepevirus*

- 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 estimated as 7.3%.

- Animal reservoirs: The detection of IgG anti-HEV by ELISA in pigs showed a seroprevalence of about 19% in Catalonia

Detection of HEV and HAV 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)

Detection of HAV and 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

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*

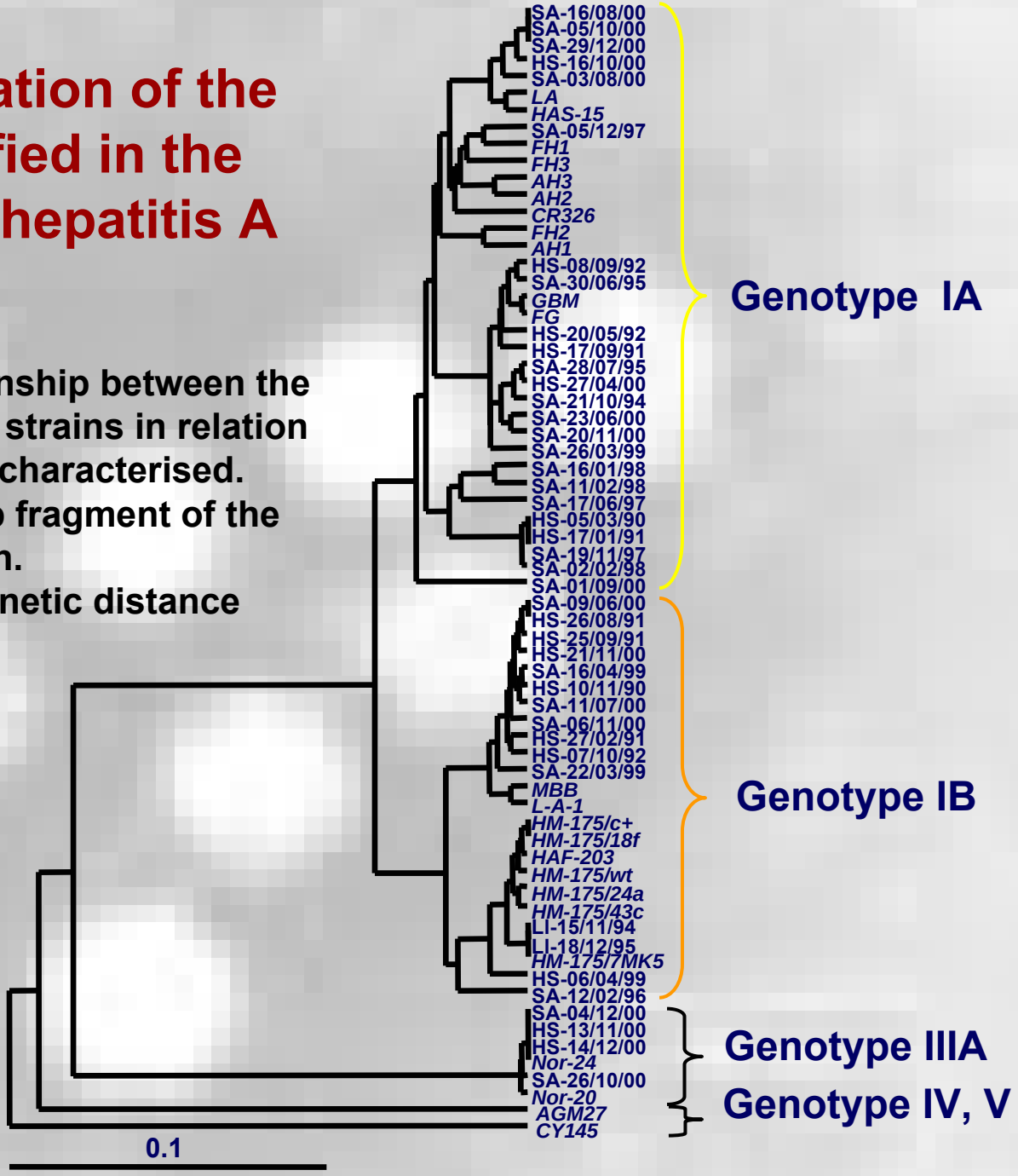
- The sequence of nucleotides in the hypervariable region is used for *typification*

Identification of HAV strains

Samples	Differences with the control (398 nt)	Identifyed 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%
Clínical (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 for the period 1990-2000

- Genotype I strains were 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 until 2004 produces a situation of sanitary risk, especially considering the low prevalence of antibodies in the young population.
- HAV was the most abundant enteric hepatitis virus detected in the environment until 2000, with estimated percentage of positive samples in urban sewage of 57.4% and values of 43.5% for HEV (1996-2002)

Molecular epidemiology of HAV

Present situation 2006-2007

- Evaluation of the HAV strains circulating in the area of Barcelona in last years
  - 18 sewage samples collected between 2000-2004
  - 24 sewage samples collected over 2006-2007
- Comparative analysis of the presence of HEV
- Evaluation of HAV in environmental samples of other geographical areas

Conclusions

- The study of environmental samples provides a framework for the global analysis of the HAV and HEV strains that circulate among the population

- The epidemiological pattern of excretion of HAV has changed in the studied area in the lasts few years. HAV was highly disseminated in the environment with about 57.4% of urban sewage positive samples from 1990-2000. However, in the years 2006-2007 HAV has not been detected in the urban sewage studied.

- The vaccination program established in the region could be directly related to the absence of HAV strains in the sewage of this area.

- At the present HEV is the most abundant enteric hepatitis virus detected in the urban sewage, with a percentage of positive HEV sewage samples of 43.5-33.3%.

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