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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HAV</th>
<th>HEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation period</td>
<td>~30 days</td>
<td>~40 days</td>
</tr>
<tr>
<td>Mortality</td>
<td>0.1-2 %</td>
<td>1-4 %</td>
</tr>
<tr>
<td>Mortality in pregnancy</td>
<td>No difference</td>
<td>&gt;20 %</td>
</tr>
<tr>
<td>Age</td>
<td>Older children, younger adults</td>
<td>Older children, younger adults</td>
</tr>
</tbody>
</table>

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

Nested-PCR

Sequencing

Cloning into pGEM-T Easy vector

Transformation of JM109 or DH5α

E.coli stains

PCR amplification

Sequencing

QPCR
Methodology for urban sewage samples

50 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
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

nPCR HEV and/or qPCR

Nucleic acid extraction
### Primers and probes used

#### Hepatitis A virus

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Region</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nested PCR</td>
<td>5’NTR</td>
<td>Pina et al., 2001</td>
</tr>
<tr>
<td>Nested PCR</td>
<td>VP1/2A</td>
<td>Pina et al., 2001</td>
</tr>
<tr>
<td>QPCR</td>
<td>5’NTR</td>
<td>Jothikumar et al., 2005</td>
</tr>
</tbody>
</table>

#### Hepatitis E virus

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Region</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nested PCR</td>
<td>ORF2</td>
<td>Ercker et al., 1999</td>
</tr>
<tr>
<td>QPCR</td>
<td>ORF3</td>
<td>Jothikumar et al., 2006</td>
</tr>
</tbody>
</table>

#### Human adenoviruses

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Region</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>QPCR</td>
<td>Ad hexon</td>
<td>Hernroth et al., 2002</td>
</tr>
</tbody>
</table>
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 an 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

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

a from an urban sewage treatment plant
b 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 m³/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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