Detection and Genetic Analysis of Swine Hepatitis E Virus in Farm Waste

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Hepatitis E

- First described in a large outbreak in India that was not caused by hepatitis A virus
- Outbreaks frequently associated with contaminated water, few secondary cases occur
- Infects mainly young adults, higher mortality in pregnant women-up to 25%
Hepatitis E and Seroprevalence of anti-HEV in Humans Worldwide

- **Endemic areas:** India, Pakistan, Nepal, China, Middle Asia
  - Epidemics reported
  - High seroprevalence of anti-HEV

- **Non-endemic areas:** US, Western Europe
  - No epidemics, low seroprevalence
  - Usually reported in travelers
  - Rare domestically-acquired cases identified in US, United Kingdom, Greece, Austria, New Zealand
Serologic Evidence of Non-human HEV Infection Worldwide

- Non-domestic animals
  - Rats
  - Rhesus monkey

- Domestic animals
  - Chickens
  - Dogs
  - Sheep and goats
  - Cattle
  - Swine
Worldwide Seroprevalence of HEV Antibodies in Swine
Indication of Potential Cross Species Transmission of HEV

- Cross-sectional studies: increased prevalence of anti-HEV in persons who work with swine in Moldova and the US*
- Anti-HEV among 2-20% U.S. blood donors
- Non-human primates infected with US-1 swine HEV
- Specific-pathogen-free pigs were infected with US-2 (a swine-like human isolate)

# HEV Genotypes Identified Worldwide

<table>
<thead>
<tr>
<th></th>
<th>Genotype I Burma</th>
<th>Genotype II Mexico</th>
<th>Genotype III US 1 Swine</th>
<th>Genotype IV China-new</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid Identity (%) ORF 1 region</td>
<td>83</td>
<td>83</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Nucleic Acid Similarity (%) Full genome</td>
<td>76</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
</tbody>
</table>

### Table Details:
- **Amino acid Identity (%) ORF 1 region**
- **Nucleic Acid Similarity (%) Full genome**

The table shows the percentage similarity of nucleic acid and amino acid identities between different HEV genotypes. The matrix is symmetric, indicating the similarity between each pair of genotypes.
Objective

Isolate and characterize swine HEV from combined waste found in pits/lagoons on swine farms
Summary of Waste Samples Processed and Analyzed by RT-PCR

- Obtained from Midwestern farm where animals arrive (~50 lbs) and grow to ~200 lb
- Sample of liquid manure taken from pit waste at time of field application
- Five one liter samples:
  - Fall: 2 liters, pre- and post-application
  - Spring: 3 liters, one pre-application and two post-application
- Fall and spring samples represent collection from two different groups of animals
Concentration of Virus from One Liter of Manure

Centrifugation

Liquid ~800 ml
Concentrate, extract
~10 ml

Solids
Resuspend, centrifuge
supernatant
Concentrate, extract
~10 ml

solids
Extract, concentrate
~10 ml
Evaluation of Concentrates

- Tested 140 ul sample of each 10 ml concentrate by RT-PCR for presence of swHEV ſprimers capsid region, swine specific, Meng et al. 1998
- End-point titration of each concentrate by serial ten-fold dilution
  - ~2x10⁶ to 2x10⁷ RT-PCR units (genome equivalents) per liter
- Direct sequence analysis of amplicons
Genome Organization of HEV

- ORF 1: 5079 bp
- ORF 2: 1980 bp
- ORF 3: 369 bp

- RNA polymerase
- Helicase
- Capsid: 5572-5860
Phylogenetic Analysis of HEV Isolates

Isolates

Mexico 14

US2

US_Sw1

US1

sMW-US_Nov

sMW-US_May

Egypt

India

Nepal

Burma

Japan

China/Taiwan

Pakistan

II

III

IV

I

China 4

Japan-new

Mexico 14

Phylogenetic Analysis of HEV Isolates
Swine HEV was isolated from samples of pooled liquid manure

Sequence analysis of one region (220 bp) of the genome yielded two unique, but related, sequences obtained at different time points

Different time points represent different groups of animals

These sequences are most similar to swUS-1 and two human US isolates US-1 and US-2 (Genotype III)

Further epidemiological and environmental studies are needed
Manure 1 Pellet

Resuspend in .5 M Threonine
Stir 1 hr RT

Chloroform extract & PEG ppt ON

Spin 10,000 xg 30min
Resuspend pellet in DPBS

Manure 2 Supernatant

PEG ppt ON
Resuspend pellet in .5M Threonine
Stir 1 hr RT

Chloroform extract & PEG ppt ON

Spin 10,000 x g 30min
Resuspend pellet in DPBS

Manure 3 Supernatant

PEG ppt ON
Resuspend pellet in .5M Threonine
Stir 1 hr RT

Chloroform extract & PEG ppt ON

Spin 10,000 xg 30 min
Resuspend pellet in DPBS