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


Division of Viral Hepatitis, NCID, CDC and School of Public Health, University of North Carolina
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

59%
79%
25%
31%
41%
37%
30%
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

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<th>I</th>
<th>II</th>
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<th>IV</th>
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**Amino acid Identity (%)**

- ORF 1 region
- Nucleic Acid Similarity (%)
- Full genome

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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 primers capsid region, swine specific, Meng et al. 1998
- End-point titration of each concentrate by serial ten-fold dilution
  - $\sim 2 \times 10^6$ to $2 \times 10^7$ 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 bp
Phylogenetic Analysis of HEV Isolates
Summary

- 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

Spin 10,000 xg 30 min
Resuspend pellet in DPBS

Chloroform extract & PEG ppt ON

Manure 2 Supernatant

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

Chloroform extract & PEG ppt ON

Manure 3 Supernatant

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