Detection and Characterisation of Hepatitis E Virus in Animal Samples

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Outline

• Introduction to Hepatitis E virus (HEV)
• HEV surveillance in imported pigs
• Future perspectives
Introduction

• Virus family: Hepeviridae virus
• Icosahedral non-enveloped virus with a single stranded positive RNA (ssRNA)
• Genome: 7.2 kb, approximately 27 to 34 nm in size
• Stable in the environment
Introduction

• 8 main genotypes in mammals
• Genotypes 3 and 4
• Modes of transmission: Waterborne or Food borne.
• HEV infection in human is usually self limiting; severe case in pregnant woman; chronic infection in immunosuppressed patients
HEV in the Pig

- HEV infections in pigs are usually **asymptomatic**. Pigs become infected with HEV once maternal antibody wanes.
HEV in the Pig

• HEV is **ubiquitous**. HEV has been detected in nearly every pig production facility in the world since 1997.

• Prevalence: **80% – 100%** of commercial pigs farms in US show evidence of infection with HEV

• Current mitigation measures: Standard **biosecurity measures**, including regular cleaning and disinfection of swine facilities; there is no HEV vaccines for pigs

• Heating pork products to an internal temperature of **71°C for 20 min** is required to inactivate HEV
Heating pork products to an internal temperature of **71°C for 20 mins** is required to inactivate Hepatitis E virus.
Increase in Hepatitis E incidence

- Global trend of increasing Hepatitis E cases in human in recent years


* Data available for: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Estonia, Finland, France, Germany, Hungary, Italy, Latvia, the Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom.
What is responsible for the increase?

**Live animals**
- **Pigs**
  - Wild boar
  - Camels
  - Deers

**Food products**
- Pig liver
- Pig organs
- Seafood (Shellfish)
- Pork sausages

**Environment**
- Soil
- Contaminated water

**Humans**
- Pork-eaters
- Farmers
- Healthcare workers

**Human to human**
- Organ donors
- Blood donors

**Virus shedding**
**Faecal-oral route/Food**

**Biosecurity/Biosafety**
**Safe food practice/Health screening**
Sources of Pork for Singapore

• **Live pigs** (received at Buroh Lane abattoir)
  • ~1100 pigs six times a week from **Bulan Island**, Indonesia
  • ~300 pigs twice a week from Sarawak, Malaysia

• **Frozen pork meat**
  • Imported from Australia, Brazil, USA, the Netherlands, Spain, Sweden and Mexico

• **Chilled pork meat**
  • Imported from Australia, Canada, Netherlands, Brazil, Denmark and Poland
Surveillance of HEV in Bulan pigs:

Is there any change?

- Prevalence
- Viral evolution
- Strain diversity and transmission
## Surveillance of HEV in Bulan pigs: Prevalence

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>HEV antibodies detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (SeroPrevalence)</td>
<td>10/32 (31.25%) – 2017</td>
</tr>
<tr>
<td></td>
<td>6/20 (30.00%) - 2012</td>
</tr>
<tr>
<td></td>
<td>11/20 (55.00%) - 2010</td>
</tr>
<tr>
<td></td>
<td>7/20 (35.00%) – 2000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Viral prevalence</th>
<th>HEV RNA detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faecal swab</td>
<td>15/40 (37.5%) - 2017</td>
</tr>
<tr>
<td></td>
<td>6/268 (2.24%) - 2018</td>
</tr>
<tr>
<td>Liver</td>
<td>4/47 (8.5%) - 2018</td>
</tr>
</tbody>
</table>
Global HEV SeroPrevalence in pigs

(Data obtained during 2000-2014)
Global HEV Viral Prevalence in Pig Livers
(studies conducted between 2007-2017)

Global HEV Viral Prevalence in pig fecal samples

(Data obtained during 2002-2014)

Viral Prevalence

Country (No. of fecal samples tested)
Surveillance of HEV in Bulan pigs: Sequencing

1. Sample preparation and RNA extraction
   - 1:10 dilution of homogenized organ tissue/faeces in VTM;
   - Extract the RNA from the supernatant by using QIAamp Viral RNA Mini kit

2. Hep E full genome PCR (9 primer pairs) using One-step QIAGEN RT-PCR kit

3. NGS of PCR Products
   - Paired-end sequencing (2x150bp) by Illumina MiSeq
   - Contig generation using ref strain alignment (KT447528.1)
   - Phylogenetic analysis using RAxML (Model: GTR; 1000 bootstraps)
Surveillance of HEV in Bulan pigs: Sequencing

- Obtained 6569 nt long sequence
- Sequencing of missing region in progress

9 primer pairs designed

**PCR**

HEV genome:
- S28-Bulan pig (PCR) 131-903, 1341-2150, 2853-4600, 5305-7000 (~7.2kb)
- S28-Bulan pig (PCR + NGS) 120-2457, 2802-7032 (~5.1/7.2kb, ~6.5/7.2kb)

**Sanger sequencing**

**NGS**
### Surveillance of HEV in Bulan pigs: Sequence analysis – comparison with historical strains

<table>
<thead>
<tr>
<th>Region</th>
<th>Sequence</th>
<th>1998 Swine HEV (USA)</th>
<th>2001 Swine HEV (Japan)</th>
<th>2007 Swine HEV (South Korea)</th>
<th>2016 Swine HEV (China)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleotide</td>
<td></td>
<td>91.7%</td>
<td>85.5%</td>
<td>88.9%</td>
<td>87.8%</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>ORF1</td>
<td>96.6%</td>
<td>95.2%</td>
<td>96%</td>
<td>95.7%</td>
</tr>
<tr>
<td>ORF2</td>
<td></td>
<td>98.0%</td>
<td>98%</td>
<td>97.7%</td>
<td>97.7%</td>
</tr>
<tr>
<td>ORF3</td>
<td></td>
<td>93.4%</td>
<td>92.6%</td>
<td>95.4%</td>
<td>93.5%</td>
</tr>
</tbody>
</table>
## Surveillance of HEV in Bulan pigs: Sequence analysis – critical mutations

<table>
<thead>
<tr>
<th>Amino acid change or Nucleotide Substitution</th>
<th>Domain/Region</th>
<th>Functional significance</th>
<th>Mutation in imported Pig HEV strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y1320H</td>
<td>RdRp/ORF1</td>
<td>Increased efficiency of viral replication and infectivity</td>
<td>Y1320</td>
</tr>
<tr>
<td>V1213A</td>
<td>ORF1</td>
<td>Associated with increased virulence</td>
<td>V1213</td>
</tr>
<tr>
<td>L477T and L613T</td>
<td>ORF2</td>
<td>Involved in formation of neutralization-sensitive epitopes</td>
<td>L477 and L613</td>
</tr>
<tr>
<td>T735I</td>
<td>ORF1</td>
<td>Frequent among the major human pathogenic HEV genotypes 1, 3, and 4 and correlated with liver failure</td>
<td>T735</td>
</tr>
<tr>
<td>Nucleotide Substitution at C5907, U3148</td>
<td>ORF2</td>
<td>Silent substitutions; may associate with fulminant hepatitis</td>
<td>5907T, 3148G</td>
</tr>
</tbody>
</table>

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*Emerging Infectious Diseases, Vol. 15, No. 5, May 2009*
HEV Strain Diversity and Transmission

• One Health Partners

• Sharing of materials, methods, and sequence information for comparative analysis.
Phylogenetic analysis using RAxML

- Model: GTR
- Converged after 250 replicates
- ‘Unique Singapore cluster’
- HEV Sequences of local patients are from “Zhu et al 2018, Journal of Viral Hepatitis.” (NUH and GIS)

Legend:
- Orange: Local
- Red: Asia
- Blue: Europe
- Green: North America
Phylogenetic analysis using RAxML

- Model: GTR
- Converged after 250 replicates
- ‘Unique Singapore cluster’
Surveillance of HEV in Bulan pigs:

Summary

• **Prevalence** – Consistent across the years; comparable to other countries

• **Viral evolution** – Nucleotide substitution at C5907 and U3148 observed

• **Transmission and strain diversity** – Local HEV cluster; ongoing
What is responsible for the increase?

• HEV surveillance data did not find significant changes of HEV in the pig population. The annual incidence of HEV fluctuates but remained stable over the past years.

• More studies under One-Health platform are required to evaluate if there are additional risk factors, which in turn would inform intervention measures.
AVA’s messages

Cook Your Food Well

- Ensure the centre of meat and poultry are well-cooked.
- Cook food at high temperatures (above 75°C).
- Bring food like stews, soups and curries to boiling temperatures when cooking.
- Take special note when cooking minced meat or meat patties as mince meat becomes more exposed to bacteria during the mincing process.

Separate Raw Food From Cooked Food

- Disinfect tea towels or dishcloths regularly, once a week, with either chlorine or hot water.
- Wash your hands before and after handling food, especially raw meat and seafood.

Food Safety Is a Shared Responsibility!
Future Perspectives

• Detailed transmission pathway in the swine population, in the community, and between swine and human

• Impact of reverse zoonosis in disease introduction to pig population

• Swine HEV vaccine development

• Host-pathogen interaction (pig and human)

• Molecular epidemiology using new technologies

• Surveillance and impact measurement
Acknowledgements
Thank you

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