The Development of Chronic Hepatitis in Rabbits Experimentally Infected with HEV Isolate from Rabbit

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Hepatitis E Virus (HEV)

**Characteristics**

- **Faecal-oral transmission:** primarily through contaminated water and undercooked pork.

- **Self-limited disease:** Fulminant form of Hepatitis E in few patients.

- **High mortality (~20%) in pregnant woman:** 1~3%

- **Chronic hepatitis and cirrhosis in immunocompromised patients (SOT and HIV patients)**

- **Poor prognosis in patients with chronic liver diseases and HEV superinfection:** Most cases of severe HEV represent “acute on chronic”

**Milestones**

- **1983, Balayan MS:** Demonstration of enterically transmitted non-A non-B hepatitis (ET-NANBH).

- **1990, Reyes GR:** Cloning and sequencing of HEV genome.

- **1998, Meng XJ:** Identify the first HEV strain of animal origin (Swine HEV); Zoonosis.

- **2004, ICTV:** HEV was classified in the new genus Hepevirus in the family Hepeviridae.

- **2010, China:** The world’s first recombinant hepatitis E vaccine.

- **2012, Emerson SU:** Adaptation of a Genotype 3 Hepatitis E Virus to Efficient Growth in Cell Culture.
HEV: Virology

Positive sense, single-stranded RNA non-enveloped virus
HEV genome ~7.2kb, 3 ORFs
Virion: 32~34nm icosahedral,
Genotype and distribution

Kamar N et al. Lancet 2012
Temporal distribution of genotypes of human HEV isolates in Mainland China (1986-2011)

- **Northeast China**
  - Human: 1a*, 4a, 4b, 4c, 4g*
  - Swine: 4a*, 4b, 4d, 4g*, 4n

- **North China**
  - Human: 1a, 4a*, 4b, 4d*, 4h
  - Swine: 4a*, 4d

- **East China**
  - Human: 1a, 3b, 4a*, 4b, 4c, 4d, 4e, 4h*, 4n
  - Swine: 3b, 4a, 4b, 4n*

- **Central China**
  - Human: 1a*, 4b*
  - Swine: 3b, 4a, 4b, 4n*

- **Southwest China**
  - Human: 1a

- **South China**
  - Human: 1a, 4a, 4b*
  - Swine: 4a, 4b*

Incidence of hepatitis E in China


Data from China CDC
HEV: Zoonosis and animal reservoirs

Human HEV (G1, G2, G3, G4)

G3, G4

G3, G4

First isolated from China in 2009;

Rabbit HEV

Out-group

G3
## The epidemiology of rabbit HEV around the world

<table>
<thead>
<tr>
<th>Nation</th>
<th>Area</th>
<th>Year</th>
<th>Rabbit Species</th>
<th>Sample for detection</th>
<th>Anti-HEV antibody (%)</th>
<th>HEV RNA (%)</th>
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<td>Serum</td>
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<td>2011</td>
<td>Japanese White, Chinchilla</td>
<td>Serum</td>
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<td>Rex</td>
<td>Serum</td>
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<td>0.00</td>
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<tr>
<td>Inner Mongolia</td>
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<td>2012</td>
<td>Rex</td>
<td>Serum</td>
<td>57.30</td>
<td>71.60</td>
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<tr>
<td>USA</td>
<td>Virginia</td>
<td>2011</td>
<td>Californian, Flemish, Lop, Mini Rex, New Zealand, Salitan, etc</td>
<td>Serum/feces</td>
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<td>22.00</td>
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<tr>
<td>France</td>
<td>Western France</td>
<td>2012</td>
<td>Farmed, Wild</td>
<td>Bile (farmed), Liver tissue(Wild)</td>
<td>Un-detected</td>
<td>7.00(farmed) 23.0(wild)</td>
</tr>
</tbody>
</table>

*Source: Liu L et al. Chin J Viral Dis, 2014*
Study on prevalence and genotype of hepatitis E virus isolated from Rex Rabbits in Beijing, China

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Department of Microbiology, Peking University Health Science Center, Beijing, China

Received February 2010, accepted for publication March 2010

SUMMARY. A novel genotype of hepatitis E virus (HEV) isolated from rabbits is reported. This is the first study to confirm and further investigate the prevalence of the novel HEV genotype in rabbits in China. Sera and fecal samples were collected from licensed pet rabbits in Beijing, China. All serum samples were tested for anti-HEV antibodies by ELISA. Both the sera and the fecal samples were evaluated for detection of HEV RNA using a nested RT-PCR assay. The nucleotide sequences of rabbit HEV were then analyzed and sequence homology of rabbit HEV compared against human HEV genotypes 1–4, and avian HEV. Results: The prevalence of positive serum anti-HEV from rex rabbits was 54.62% (65/119). The detection rate of HEV RNA using RT-PCR primers was 6.96% (8/115) amongst rabbit fecal samples. All eight amplicons shared 98.3–100% nucleotide homology with each other and had identities of 75.8–78.6%, 73.2–75.0%, 77.4–81.0%, 74.2–78.6%, and 54.8–57.6% with the corresponding regions of genotypes 1–4 and avian HEV, respectively. Phylogenetic analysis showed that the eight sequences formed one individual branch and were on the same branch with G6C9 and G6C16, both of which were reported to be a novel genotype of HEV isolated from rabbits. The conclusion is that this study provides further information about HEV infecting rabbits, which may be a new animal host of HEV, as well as genetic evidence of a new mammalian genotype of HEV.

Keywords: antibody, genotype, hepatitis E virus, rabbit.

INTRODUCTION

Hepatitis E virus (HEV) is a small, non enveloped, single-strand, positive-sense RNA virus that is transmitted via the fecal-oral route, primarily through contaminated water supplies. Cases in which HEV infection has been identified after ingestion of meat from HEV-infected deer or swine have been reported, and the HEV sequence isolated from the meat identical to cases that were isolated from the patients, definitively demonstrating an zoonotic transmission of HEV [1-2]. HEV, which was previously referred to as an enterically transmitted non-A, non-B hepatitis virus, is the causative agent of acute self-limited or fulminating hepatitis E and is considered to be endemic in many developing countries where sanitation conditions are suboptimal [3,4]. A study showed that hepatitis E accounts for a significant proportion of enterically transmitted forms of viral hepatitis in humans [5, 6] and poses an important public health problem in both developing countries and some industrialized countries, including the United States and several European countries [7]. In epidemics, disease mortality usually affects young adults, with a relatively high mortality of up to 25% in infected pregnant women [8].

HEV is classified under the family Hepatoviridae consisting of four recognized major genotypes that infect humans and other animals [9]. Genotypes 1 and 2 HEV are restricted to humans and are often associated with large outbreaks and pandemics in developing countries with poor sanitation conditions [10]. Genotypes 1 and 4 HEV infect humans, pigs and other animal species and are responsible for sporadic cases of hepatitis E in both developing and industrialized countries [11-14]. The avian HEV associated with hepatitis-splenomegaly syndrome in chickens is genetically and antigenically related to mammalian HEV and likely represents a new genus in the family [14, 15]. Various species of animals, such as non-human primates, rodents, swine, cows, and horses, have been found with a high prevalence of anti-HEV antibodies since Wang [16] isolated HEV from swine in 1997.

Geng JB et al. JVH. 2010

The rabbit HEV isolate from fecal samples with the detection rate of HEV RNA was 6.96%.
Phylogenetic analysis of the full genome of rabbit hepatitis E virus (rbHEV) & molecular biologic study on the possibility of cross species transmission of rbHEV

Table 3. Specific amino acid substitutions in the ORF2 region among different HEV genotypes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Sample number</th>
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</thead>
<tbody>
<tr>
<td>Nonzoonotic</td>
<td>HEV-1</td>
<td>105, 131</td>
</tr>
<tr>
<td></td>
<td>HEV-2</td>
<td>497, 506, 511</td>
</tr>
<tr>
<td>Zoonotic</td>
<td>HEV-3</td>
<td>613</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Novel type</td>
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Conserved amino acids and their sites:

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<th>40</th>
<th>62</th>
<th>83</th>
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<th>1061</th>
<th>1151</th>
<th>1256</th>
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<tr>
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<td>F</td>
<td>S</td>
<td>N</td>
<td>V</td>
<td>D</td>
<td>A</td>
<td>S</td>
<td>F</td>
<td>L</td>
<td>V</td>
<td>A</td>
<td>Q</td>
<td>D</td>
<td>E</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>HEV-2</td>
<td>H</td>
<td>F</td>
<td>S</td>
<td>N</td>
<td>T</td>
<td>D</td>
<td>P</td>
<td>S</td>
<td>A</td>
<td>Y</td>
<td>M</td>
<td>L</td>
<td>E</td>
<td>P</td>
<td>E</td>
<td>D</td>
</tr>
<tr>
<td>HEV-3</td>
<td>R</td>
<td>L</td>
<td>A</td>
<td>V</td>
<td>S</td>
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<td>M</td>
<td>L</td>
<td>E</td>
<td>P</td>
<td>E</td>
<td>D</td>
<td>R</td>
</tr>
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<td>HEV-4</td>
<td>R</td>
<td>L</td>
<td>A</td>
<td>V</td>
<td>S</td>
<td>F</td>
<td>S</td>
<td>A</td>
<td>Y</td>
<td>M</td>
<td>L</td>
<td>E</td>
<td>P</td>
<td>E</td>
<td>D</td>
<td>R</td>
</tr>
<tr>
<td>Novel type</td>
<td>R</td>
<td>F</td>
<td>A</td>
<td>V</td>
<td>S</td>
<td>F</td>
<td>S</td>
<td>A</td>
<td>Y/F</td>
<td>M</td>
<td>L</td>
<td>Q/P</td>
<td>P</td>
<td>E/G</td>
<td>D</td>
<td>R</td>
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</table>

* The amino acid positions are in accordance with CHN-XJ-SW13 (GU119961).

Table 4. Specific amino acid substitutions in the ORF1 region among 94 HEV isolates form different genotypes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype</th>
<th>Sample number</th>
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</thead>
<tbody>
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<td>Nonzoonotic</td>
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<tr>
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<td>HEV-4</td>
<td>39</td>
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<tr>
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<td>Novel genotype</td>
<td>3</td>
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</table>

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<th></th>
<th>19</th>
<th>105</th>
<th>131</th>
<th>497</th>
<th>506</th>
<th>511</th>
<th>613</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEV-1</td>
<td>P</td>
<td>A</td>
<td>H</td>
<td>S</td>
<td>V</td>
<td>S</td>
<td>A</td>
</tr>
<tr>
<td>HEV-2</td>
<td>P</td>
<td>A</td>
<td>H</td>
<td>S</td>
<td>V</td>
<td>S</td>
<td>A</td>
</tr>
<tr>
<td>HEV-3</td>
<td>A</td>
<td>S</td>
<td>P</td>
<td>T</td>
<td>M</td>
<td>T</td>
<td>G</td>
</tr>
<tr>
<td>HEV-4</td>
<td>A</td>
<td>S</td>
<td>P</td>
<td>T</td>
<td>M</td>
<td>T</td>
<td>G</td>
</tr>
<tr>
<td>Novel genotype</td>
<td>A/V</td>
<td>S</td>
<td>P</td>
<td>T</td>
<td>M</td>
<td>T</td>
<td>G</td>
</tr>
</tbody>
</table>

* The amino acid positions are in accordance with CHN-XJ-SW13 (GU119961).

Geng JB et al. Infect Genet Evol. 2011
Pathogenecity of Hepatitis E Virus from Rabbits

Hepatitis E virus and neurologic disorders

Kamar N, et al.
Emerg Infect Dis. 2011
Extrahepatic Replication of HEV in Experimentally Infected Rabbits

Liver, stomach, small intestine, kidney, spleen, heart, brain, bladder and lung

(+ and (-) HEV RNA

Experimental Infection of Monkeys with Rabbit HEV

Prior to inoculation: Anti-HEV antibodies and HEV RNA were negative, ALT were normal (0-40 U/L);

Sample Collection: Blood and Feces (twice a week), monitor for 16 weeks;

ALT in serum: Hepatitis (≥twice the pre-challenge ALT);
Cross-species transmission of HEV to cynomolgus macaques

Comparing the complete genome sequence of HEV passed in the monks with that of the inoculum showed 99.8% nucleotide identity.

Liu P et al, Emerg Infect Dis 2013
Pathogenesis study of rHEV: Chronicity

SPF Rabbits Infected with Rabbit Hepatitis E Virus Isolate Experimentally Showing the Chronicity of Hepatitis

**Group 1** was control including 2 rabbits inoculating with PBS, C1&C2.

**Group 2** including 4 rabbits (R1—R4) inoculating a homologous rabbit HEV isolate.

**Group 3** including 4 rabbits (S1—S4) inoculating a heterologous swine HEV isolate.
Pathogenesis study of rHEV: Chronicity

Figure 1. Dynamic seroconversion of anti-HEV, HEV RNA, ALT and AST observed in rabbits.

Rabbit C1 in group 1 inoculated with sterile PBS.
Pathogenesis study of rHEV: Chronicity

Figure 2. Dynamic seroconversion of anti-HEV, HEV RNA, ALT and AST observed in rabbits.

Rabbit R3 in group 2 inoculated with rabbit HEV strain.
Pathogenesis study of rHEV: Chronicity

Figure 3. Liver histology.
A–D (H & E stain, original magnification, 610),
E–G (Masson's trichrome stain, 610),
H–I (Immunohistochemistry, 640).

(A) Liver section from a control rabbit with no visible pathological signs of HEV infection.
(B)-(C) Lymphocytes distributed focal or scattered in hepatic lobule, the inflammatory cells gathered along blood vessel walls.
(D) Chronic inflammatory cells infiltrate the portal area, blood vessel walls thickening associated with fibrosis, local hyaline degeneration.
(E) No histopathological changes with minimal staining limited to areas immediately adjacent to portal structures.
(F) Artery wall thickening associated with moderate to severe fibrosis.
(G) More advanced portal and periportal fibrosis with short fibrous septa.
(H) Negative immunohistochemistry result for HEV antigen in liver sections from the control rabbits.
(I) Positive results for HEV antigen in liver sections of experimental groups.
Figure 4. Dynamic seroconversion of anti-HEV, HEV RNA, ALT and AST observed in rabbits.

Rabbit S4 in group 3 inoculated with a genotype 4 swine HEV at 0wpi, and rabbit HEV at 25wpi.

Note: ↑ indicates group 3 rabbits were inoculated with rabbit HEV strain at 25wpi (when recovered from initial infection).
Table 1. Detection of HEV RNA in fecal/serum samples collected weekly from rabbits.

<table>
<thead>
<tr>
<th>Group</th>
<th>Rabbit ID</th>
<th>Positive(+) or Negative(-) in Fecal/Serum Samples at Indicated Wpi</th>
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<td></td>
<td></td>
<td>0  1  2  3  4  5  6  7  8  9  10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36</td>
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</table>
| 1a C1 | -/-       | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -/- | -|-
HEV Replication in Extrahepatic Tissue of Infected Rabbits

Figure 5. Extrahepatic tissue histology.

A–E (Immunohistochemistry, 640), F–I (H & E stain 610).

(A) Negative immunohistochemistry result for HEV antigen in extrahepatic tissue sections from the control rabbits.

(B)–(E) Positive results for HEV antigen in brain, stomach, duodenum and kidney.

(F) Duodenum section from a control rabbit with no visible pathological signs of inflammation.

(G) A large number of lymphocytes infiltrate mucosal interstitial, focal lymph follicles formed in duodenum sections.

(H) Kidney section from a control rabbit with no visible pathological signs of HEV infection.

(I) Multifocal lymphocytes and mononuclear cells infiltrate in renal interstitial.
Summary

In summary, on rabbit HEV study, our group has provided the first experimental evidences on the following aspects:

- 6.96% detection rate of HEV RNA amongst rabbit fecal samples meaning another important reservoir of HEV
- Phylogenetic analysis of the whole genome showing the potential possibility of rabbit HEV cross-species transmission.
- Rabbit HEV transmissible to cynomolgus macaques suggesting that rabbits may be a new source of human HEV infection
- SPF rabbits experimentally infected with a homologous rabbit HEV isolate developed signs of chronic hepatitis providing the laboratory evidence of chronic hepatitis E
- Both positive and negative-stranded HEV RNA and HEV antigen expression detected in extrahepatic tissues indicating the existence of HEV replication in many tissues in addition to the liver
- Based on our studies, Rabbit HEV possesses strong pathogenecity and possibility to infect human, and rabbit can be used as animal model to further investigate HEV.
Acknowledgements

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Thanks!