Challenges in diagnosis of Hepatitis E virus infections

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Hepatitis E Virus

- Hepeviridae family
- Non-enveloped virus
- Positive sense, single stranded RNA of ±7200 bp
- 27-34 nm in diameter
Clinical presentation of hepatitis E virus

- Fever
- Fatigue
- Loss of appetite
- Nausea
- Vomiting
- Abdominal pain
- Jaundice
- Dark urine
- Clay-colored stool
- The ratio of symptomatic to asymptomatic infection is reported to range from 1:2 to 1:13.
- Mortality: overall 1-4%, pregnant women 15-25%
Number of reported HEV-cases in Germany

Source: Robert Koch Institute, Germany
Global distribution of HEV genotypes

Kamar, Lancet 2012
HEV transmission in developed countries - zoonose

~55% of fecal tanks in Dutch pig farms HEV RNA positive
EID 2007

~ 6% of Dutch porcine livers HEV RNA positive.
J Food Prot. 2007

Dalton et al Lancet 2008
Course of HEV infection in the immunocompetent

Dalton et al, Lancet 2008
Chronic HEV in solid organ recipients

- Chronic HEV infection reported in the transplant setting
  - Persistent viraemia
  - Persistently raised transaminase activity
  - Histological features associated with chronic hepatitis
  - Evidence of rapid development of cirrhosis

- Association with a more profound immunosuppression

Haagsma, Liver Transplantation 2009;15(10):1225-8
Chronic HEV infection misdiagnosed as Graft v Host

- IgM -
- IgM +
- IgG -
- IgG +

ALAT (u/l)

HEV-RNA (Ct-value)

Apr-01 Sep-02 Jan-04 Mei-05 Okt-06 Feb-08 Jul-09 Nov-10 Apr-12
HEV in living adult SOT transplant recipients

HEV infections among SOT patients

<table>
<thead>
<tr>
<th></th>
<th>HTX</th>
<th>LTX</th>
<th>LungTX</th>
<th>NTX</th>
<th>Multiple SOT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>confirmed HEV infection</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>no HEV infection</td>
<td>254</td>
<td>297</td>
<td>52</td>
<td>573</td>
<td>12</td>
</tr>
</tbody>
</table>

Mixed Tx group:
confirmed HEV case: 1 NTX-LTX, 1 NTX-HTX
no HEV infection: 8 NTX-LTX, 3 NTX-HTX, 1 NTX-LuTX

Pas et al, EID 2012
Current status of HEV diagnostics

* Pathology
  - not specific
  - Invasive
Current status of HEV diagnostics - Histopathology

Liver biopsy overview
Current status of HEV diagnostics - Histopathology
Current status of HEV diagnostics

* Pathology  not specific
  Invasive

* Virus culture  -  inefficient
Virus culture - HEV

Efficient cell culture systems for hepatitis E virus strains in feces and circulating blood
Hiroaki Okamoto
Division of Virology, Department of Infection and Immunity, Jichi Medical University School of Medicine, Shimotsuke-Shi, Tochigi, Japan

PLC/PRF/5 (hepatocellular carcinoma) and A549 (lung cancer) cells

long incubation period, low sensitivity
Current status of HEV diagnostics

* Pathology  not specific
  Invasive

* Virus culture - inefficient

* HEV serology
  - validation of commercial assay
  - conformational testing using blot
HEV serology - literature

A lot of in house assays described

Use of Serological Assays for Diagnosis of Hepatitis E Virus Genotype 1 and 3 Infections in a Setting of Low Endemicity

M. Herremans, J. Bakker, E. Duizer, H. Vennema, and M. P. G. Koopmans


A Comparison of Two Commercially Available Anti-HEV IgG Kits and a Re-Evaluation of Anti-HEV IgG Seroprevalence Data in Developed Countries

Richard Bendall, Vic Ellis, Samreen Ijaz, Rachel Ali, and Harry Dalton

IgM and IgG Genelabs, Mikrogen Recomblot

Genelabs, Wantai, 4.5x higher seroprevalence with Wantai
HEV serology - literature

Serologic Assays Specific to Immunoglobulin M Antibodies against Hepatitis E Virus: Pangentotypic Evaluation of Performances

Jan Drobeniuc,¹ Jihong Meng,¹² Gábor Reuter,³ Tracy Greene-Montfort,¹ Natasha Khudyakova,¹ Zoya Dimitrova,¹ Saleem Kamili,¹ and Chong-Gee Teo¹

<table>
<thead>
<tr>
<th></th>
<th>Sens. (%)</th>
<th>Spec. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int. Immuno-Diagnostics</td>
<td>82</td>
<td>91,2</td>
</tr>
<tr>
<td>MP biomedicals (former Genelabs)</td>
<td>72</td>
<td>93</td>
</tr>
<tr>
<td><strong>RPC Diagnostic Systems</strong></td>
<td><strong>98</strong></td>
<td><strong>95</strong></td>
</tr>
<tr>
<td>Mikrogen</td>
<td>92</td>
<td>95,6</td>
</tr>
</tbody>
</table>

2 in-house assay, 4 commercial available assays

Sens. panel 50 samples, 4 genotypes
Spec. panel 229 samples
# HEV Serology validation - included ELISAs

<table>
<thead>
<tr>
<th>IgM/IgG</th>
<th>Name</th>
<th>Company</th>
<th>Country</th>
<th>Genotype inclusivity</th>
<th>Antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEV IgM/IgG</td>
<td>HEV Elisa v3.0</td>
<td>MP diagnostics</td>
<td>Singapore</td>
<td>gt 1 en 2</td>
<td>mix of peptides from ORF2 and complete ORF3</td>
</tr>
<tr>
<td>HEV IgM/IgG</td>
<td>recomWell HEV IgM/IgG</td>
<td>Mikrogen Diagnostik</td>
<td>Germany</td>
<td>gt 1</td>
<td>synthetic, ORF2 en ORF3 (e.coli)</td>
</tr>
<tr>
<td>HEV IgM/IgG</td>
<td>recomWell HEV IgM/IgG new</td>
<td>Mikrogen Diagnostik</td>
<td>Germany</td>
<td>gt 1 and 3</td>
<td>synthetic, ORF2 en ORF3 (e.coli)</td>
</tr>
<tr>
<td>HEV IgM/IgG</td>
<td>HEV IgM/IgG</td>
<td>DRG</td>
<td>Germany</td>
<td>gt 1 en 2</td>
<td>synthetic, ORF2 en ORF3</td>
</tr>
<tr>
<td>HEV IgM/IgG</td>
<td>HEV IgM/IgG</td>
<td>Dia.Pro</td>
<td>Italy</td>
<td>gt 1 en 2</td>
<td>synthetic, ORF2 en ORF3</td>
</tr>
<tr>
<td>HEV IgM/IgG</td>
<td>HEV IgM/IgG</td>
<td>RPC Diagnostic systems / DSI</td>
<td>Italy</td>
<td>gt 1 en 2</td>
<td>artificial ag. Composed of 12 antigenic regions derived from ORF2 and ORF 3</td>
</tr>
<tr>
<td>HEV IgM/IgG</td>
<td>HEV IgM/IgG</td>
<td>DiaCheck</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HEV IgM/IgG</td>
<td>HEV IgM/IgG PE2</td>
<td>Wantai HEV IgG PE2</td>
<td>Singapore</td>
<td></td>
<td>PE2 peptide from structural region of ORF2</td>
</tr>
</tbody>
</table>

Pas et al, in preparation
### Sensitivity panel: HEV PCR confirmed patients

<table>
<thead>
<tr>
<th>Time of Drawal</th>
<th># samples</th>
<th># patients</th>
<th># samples immune status</th>
<th>genotypes included</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ID</td>
<td>non-ID</td>
</tr>
<tr>
<td>Prior to infection</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>&lt; 6 wks</td>
<td>34</td>
<td>31</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>6 wks &lt;t&lt; 6 months</td>
<td>22</td>
<td>19</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>&gt;6 months</td>
<td>20</td>
<td>16</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>total</td>
<td><strong>88</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Division was made on basis of clinical symptoms in combination with retrospective HEV RT-PCR analysis
Specificity panel HEV- IgM

N= 89 samples
Specificity panel  HEV- IgM
HEV serology – validation of commercial assays

<table>
<thead>
<tr>
<th></th>
<th>IgM geno 1</th>
<th>IgM geno 3</th>
<th>IgG WHO</th>
<th>IgG geno 1</th>
<th>IgG geno 3</th>
<th>Sens. IgM</th>
<th>Spec. IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mikrogen old</td>
<td>4000</td>
<td>250</td>
<td>1600</td>
<td>6400</td>
<td>800</td>
<td>52%</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Mikrogen new</td>
<td>32000</td>
<td>16000</td>
<td><strong>3200</strong></td>
<td><strong>&gt;12800</strong></td>
<td><strong>3200</strong></td>
<td>79%</td>
<td>90%</td>
</tr>
<tr>
<td>MP diagnostics</td>
<td><strong>&gt;64000</strong></td>
<td>4000</td>
<td>800</td>
<td>3200</td>
<td>100</td>
<td>74%</td>
<td>84%</td>
</tr>
<tr>
<td>DSI</td>
<td>8000</td>
<td>4000</td>
<td>800</td>
<td>3200</td>
<td>800</td>
<td>71%</td>
<td>90%</td>
</tr>
<tr>
<td>DiaPro</td>
<td>32000</td>
<td>32000</td>
<td>800</td>
<td>6400</td>
<td>100</td>
<td><strong>81%</strong></td>
<td><strong>98%</strong></td>
</tr>
<tr>
<td>Wantai</td>
<td><strong>&gt;64000</strong></td>
<td><strong>&gt;64000</strong></td>
<td>1600</td>
<td><strong>&gt;12800</strong></td>
<td>800</td>
<td>75%</td>
<td><strong>&gt;99%</strong></td>
</tr>
<tr>
<td>DRG</td>
<td>32000</td>
<td>32000</td>
<td>800</td>
<td>6400</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diacheck</td>
<td>4000</td>
<td>250</td>
<td>400</td>
<td>3200</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Diacheck* was excluded.
IgM ratio’s sensitivity panel

per immune status group

- Immunocompromised
- Immunocompetent
- Unknown

per genotype

Geno 1
Geno 3
IgG ratio’s sensitivity panel

*per immune status group*

- Immunocompromised
- Immunocompetent
- Unknown

*per genotype*

- Geno 1
- Geno 3

Pas et al, in preparation
Course of chronic HEV infection
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Median</th>
<th>Range</th>
<th>ULN (F/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak ALT (U/L)</td>
<td>301</td>
<td>81 - 909</td>
<td>30/40</td>
</tr>
<tr>
<td>Peak AST (U/L)</td>
<td>172</td>
<td>66 - 1016</td>
<td>30/36</td>
</tr>
<tr>
<td>Peak γ-GT (U/L)</td>
<td>299</td>
<td>72 - 1740</td>
<td>34/49</td>
</tr>
<tr>
<td>Peak Billirubine (µmol/l)</td>
<td>16</td>
<td>5 - 100</td>
<td>16/16</td>
</tr>
<tr>
<td>Peak HEV-RNA (Ct values)</td>
<td>20.0</td>
<td>16.7 - 26.6</td>
<td>NA</td>
</tr>
<tr>
<td>Period of HEV-RNA positivity (months)</td>
<td>16</td>
<td>6 - 55</td>
<td>NA</td>
</tr>
<tr>
<td>Time between the SOT and first HEV-RNA positive</td>
<td>2.0</td>
<td>-0.3 - 20.1</td>
<td>NA</td>
</tr>
<tr>
<td>Time of HEV-RNA positivity prior to HEV IgM (days)</td>
<td>32</td>
<td>0 - 826</td>
<td>NA</td>
</tr>
<tr>
<td>Time of HEV-RNA positivity prior to HEV IgG (days)</td>
<td>124</td>
<td>0 - 826</td>
<td>NA</td>
</tr>
</tbody>
</table>

*HEV IgM and IgG (Wantai Biochemicals)*

Pas et al, EID 2012
Current status of HEV diagnostics

* Pathology  not specific
  Invasive

* Virus culture  inefficient

* HEV serology
  - validation of commercial assay
  - conformational testing using blot

* Molecular diagnostics
  - real time vs conventional RT-PCR
  - standardisation
  - genotyping
HEV real time RT-PCR
# HEV real time RT-PCR

<table>
<thead>
<tr>
<th>1st Author</th>
<th>YoP</th>
<th>Validated</th>
<th>Target</th>
<th>Principle</th>
<th>Length</th>
<th>Limit of Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mansuy et al</td>
<td>2004</td>
<td>Gt1 and 3</td>
<td></td>
<td>Two-step Taqman on Light cycler</td>
<td></td>
<td>1E3 copies/ml</td>
</tr>
<tr>
<td>Orru et al</td>
<td>2004</td>
<td></td>
<td></td>
<td>SyBRgreen</td>
<td></td>
<td>10GEC</td>
</tr>
<tr>
<td>Jothukumar et al</td>
<td>2004</td>
<td>Gt1-4</td>
<td>ORF2/ORF3</td>
<td>taqman</td>
<td>70 bp</td>
<td>4GEC, others claim no quant.</td>
</tr>
<tr>
<td>Ahn et al</td>
<td>2006</td>
<td>gt3</td>
<td>ORF2/ORF3</td>
<td>taqman</td>
<td>103bp</td>
<td>16 copies/ml</td>
</tr>
<tr>
<td>Enouf et al</td>
<td>2006</td>
<td>Gt1-4</td>
<td>ORF2/ORF3</td>
<td>Taqman on Light cycler</td>
<td>86bp</td>
<td>10 copies/rx</td>
</tr>
<tr>
<td>Li et al</td>
<td>2006</td>
<td>GT 1-2, macaques</td>
<td>ORF2</td>
<td>SyBRgreen</td>
<td>207bp</td>
<td>4.5x103 copies/rx</td>
</tr>
<tr>
<td>Gyarmati et al</td>
<td>2007</td>
<td>Gt1-4, 2 human samples</td>
<td>ORF2</td>
<td>Taqman on Light cycler</td>
<td>113bp</td>
<td>1-20 geq/rx</td>
</tr>
<tr>
<td>Zhao et al</td>
<td>2007</td>
<td>Gt1-4, theoretical</td>
<td>ORF2/ORF3</td>
<td>taqman</td>
<td>103bp</td>
<td>5.6E3 copies/ml pseudovirus</td>
</tr>
<tr>
<td>Ward et al</td>
<td>2009</td>
<td>swine isolates only</td>
<td></td>
<td>comparison of four of the above; Jothukumar et al most sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasichova et al</td>
<td>2012</td>
<td>swine isolates only</td>
<td>dual target</td>
<td>taqman in LC480</td>
<td>70/113bp</td>
<td>50 copies/ml</td>
</tr>
</tbody>
</table>
HEV NAT quality assessment 2011 – Sanguin/ R’dam

• 6 participating labs of Dutch HEV workgroup

• Samples were randomized and included:
  • 10 log dilution series of genotype 3 sample
  • calibrated against candidate WHO HEV standard*
  • four (diluted) patient samples, 3 x gt3 and 1x gt1
  • four negative controls (EDTA-(mini) pools)

HEV NAT quality assessment – Results

Result of analysis of HEV-RNA panel by 6 participants

- Dilution series of R'dam patient sample
- Diluted patient samples (Sanquin / AMC)
- Negative controls (pooled donor plasma)
Quality of HEV nucleic acid amplification assays

24 laboratories, 22 HEV-positive plasma, 10-fold serial dilutions of HEV genotypes 3a, 3b, 3f, and 4c.

*International standardisation needed*

→ 1st HEV RNA WHO standard available (Gt3, 250,000 IU/ml)!
Distribution of available sequences along the genome

Courtesy: Harry Vennema, RIVM, The Netherlands
genotype 3

genotype 4

genotype 2

2 M74506.1

1b AY204877.1

1b AY230202.1

NL2007 S08000076

NL2010 E10011341

NL2010 S10026102

FJ457024.1

NL2008 S08014746

1a AF076239.3

1a AF459438.1

1a D10330

M73218.1

1a AF185822

1a AF051830.1

1a X99441

DQ459342.1

NL2009 S09027398

NL2010 S10015037

X98292.1

1a D11092.1

M94177.1

NC 001434.1

L08816.1

1a AF444002.1

1a L25595

1a D11093.1

1a M80581

0.05

ORF1d
Conclusies

- Bewustzijn van HEV infecties in transplantatie settings is stijgende, daarom is accurate en snelle diagnostiek nodig voor de juiste interventie strategie

- Om een HEV infectie te diagnosticeren is zowel serologie als moleculaire diagnostiek (real time RT-PCR) van belang

- De nauwkeurigheid van de huidige HEV serologische assays varieert enorm. In onze validatie waren voor IgM DiaPro en Wantai de beste testen, en voor IgG de nieuwe Mikrogen en Wantai assay de beste.

- Kwaliteit van moleculaire assays varieert tussen de verschillende labs, daarom zijn kwaliteitsrondzendingen en standardisatie van belang
# Acknowledgements

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- Prof Dr. Ab Osterhaus
- Dr. A. A. van der Eijk
- Dr. R.A. de Man
- Dr. P. Th.W van Hal
- Prof. Dr. W. Weimar
- Dr. A.H.M.M. Balk
- RIVM
- Dr. H. Vennema
- Dr. J. Reimerink
- Sanquin
- Dr. B. Hogema