Hepatitis E – virus and vaccination

(virology, epidemiology, diagnostics and vaccines)

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Melbourne, Australia
Hepatitis E virus

- Similarities and contrasts with hepatitis A virus
- The (unmet) need for reliable diagnostics
- Diagnostics - Discovery and development
  - Antigens and antibodies
- Vaccines – Current status
Hepatitis E virus and infection

- Acute, generally self-limiting
  - 25% mortality in 3rd trimester of pregnancy
- Most common in developing nations
- Strain differences
Physical characteristics

HAV
◆ Naked, 27-30 nm particles
◆ (+) RNA, 7.5 kb
◆ 1 polyprotein
◆ Three capsid proteins (VP1, VP2, VP3)
◆ Enteric transmission

HEV
◆ Naked, 32-34 nm particles
◆ (+) RNA, 7.2 kb
◆ 3 primary proteins
◆ One capsid protein (PORF2)
◆ Enteric transmission
Geographic distribution of human HEV

(CDC data)
Swine HEV - Meng et al, 1997, NIH

- ≈100% of USA swine anti-HEV positive
- Unique HEV sequence
- Similar strain isolated from patients in USA (Schlauder et al, 1998)
Many other HEV-related viruses....

Disease burden

**HAV**
- Very common in developing world, but low morbidity
- Common in developed countries with wide socioeconomic gaps
- Foodborne outbreaks
  - International trade in fresh foods, eg strawberries, lettuces from Mexico-USA

**HEV**
- Common in much of developing world; high morbidity
- Rare in developed countries*
  - Travellers, zoonotic
- Foodborne and vectors in developed countries
  - Consumption of raw liver and meat in Japan, swine HEV
Control of HEV - the role of diagnostics

- Effective vaccine should be available soon for developing countries (Hecolin; Xiamen Innovax Biotech Co Ltd)
  - GSK/Novavax vaccine passed Phase 3 in 2007, but not moving?

- Price and supply, duration of protection uncertain?

- Need to identify outbreaks/high incidence areas so that vaccine can be used, and water purification can be addressed

- Less concern about contact transmission (vs HAV)
Ideal diagnostics for HEV

- Inexpensive and robust (field use and developing countries)
- Sensitive and specific, in areas where there is high prevalence
- High positive predictive value in all countries, including low prevalence
- Rapid, Point of care (RPOC) ideal for developing countries
Serological responses to HAV and HEV

- **HAV IgG**
- **HAV IgM**
- **HEV IgG**
- **HEV IgM**

**Graph:**
- X-axis: Months post infection
- Y-axis: Serological response levels
- Curves for HAV IgG, HAV IgM, HEV IgG, and HEV IgM over time.
Diagnostic challenges

**HAV**
- Good antigen (virus), but expensive and difficult to make
- Good ELISAs, but expensive
- No rapid (RPOC) tests

**HEV**
- Variable antigens, easy to make
- Poor ELISAs (low sensitivity and/or specificity)
- No rapid (RPOC) tests
The unmet need for HEV diagnostics

- Genelabs IgG, IgM and Abbott IgG
- Highly variable sensitivity (≈60%) and sensitivity (≈80%) – not related to virus strain differences
- All based on ORF2 and ORF3 proteins expressed in *E. coli*
HEV Genome structure

7.2 kb (+) RNA

5’ ——— NON-STRUCTURAL ——— STRUCTURAL ——— AAAan 3’

ORF1
- AUG
- UGA
- 185kDa

ORF2
- AUG
- UAG
- 71kDa

ORF3
- AUG
- UAA
- 13kDa
HEV proteins - PORF2

- PORF2: major capsid protein
- Protective antibody response (Tsarev et al, 1994)
Antigenic structure of HEV

- Which antigens are appropriate for diagnosis and vaccine development?
- Which are the immunodominant epitopes?
- Are these epitopes associated with the protective immune response?
## Recombinant HEV Antigens (1)

<table>
<thead>
<tr>
<th>Protein</th>
<th>aa</th>
<th>Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF3</td>
<td>124</td>
<td>++/-</td>
</tr>
<tr>
<td>ORF2</td>
<td>660</td>
<td>+/-</td>
</tr>
<tr>
<td>C-2</td>
<td>435</td>
<td>++/-</td>
</tr>
<tr>
<td>SG3</td>
<td>327</td>
<td>++/-</td>
</tr>
</tbody>
</table>

**Legend:**
- **ORF3:** 124 aa, Reactivity: ++/-
- **ORF2:** 660 aa, Reactivity: +/-
- **C-2:** 435 aa, Reactivity: ++/-
- **SG3:** 327 aa, Reactivity: ++/-
Genelabs HEV IgM ELISA

Blood donors
HEV epidemic
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<th>Protein</th>
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<tr>
<td></td>
<td></td>
<td>Acute</td>
</tr>
<tr>
<td>ORF3</td>
<td>124</td>
<td>++/-</td>
</tr>
<tr>
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</tr>
<tr>
<td>SG3</td>
<td>327</td>
<td>++/-</td>
</tr>
<tr>
<td>ORF2 (baculovirus) VLPs</td>
<td>548</td>
<td>++++</td>
</tr>
<tr>
<td>Population</td>
<td>anti-HAV</td>
<td>anti-HEV</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Intravenous drug users</td>
<td>66.4</td>
<td>23.0</td>
</tr>
<tr>
<td>Homosexual men</td>
<td>32.3</td>
<td>15.9</td>
</tr>
<tr>
<td>Blood donors</td>
<td>16.0</td>
<td>22.9</td>
</tr>
<tr>
<td>(Baltimore, Sacramento, New York)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mast et al, 1997
## Recombinant HEV antigens (3)

<table>
<thead>
<tr>
<th>Protein</th>
<th>aa</th>
<th>Reactivity</th>
</tr>
</thead>
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<tr>
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<td>ORF2 (VLPs)</td>
<td>548</td>
<td>+++</td>
</tr>
<tr>
<td>GST-ORF2.1</td>
<td>273</td>
<td>+++</td>
</tr>
</tbody>
</table>

*VLPs*
Western blot of patient sera, ORF2.1 deletion series

Acute patient

Convalescent patient
Western blot of patient sera, ORF2.1 deletion series

Acute patient

Convalescent patient
Western blot of MAbs against the deletion series
<table>
<thead>
<tr>
<th>MAb</th>
<th>Isotype</th>
<th>Epitope</th>
<th>PORF2 IF</th>
<th>Blocking, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>4B5</td>
<td>IgG1</td>
<td>394-414, conformational</td>
<td>++++</td>
<td>0-4</td>
</tr>
<tr>
<td>3B2</td>
<td>IgG1</td>
<td>414-434</td>
<td>++++</td>
<td>0</td>
</tr>
<tr>
<td>1E6</td>
<td>IgG2b</td>
<td>434-457</td>
<td>++++</td>
<td>28-38</td>
</tr>
<tr>
<td>4B2</td>
<td>IgG1</td>
<td>ORF2.1, conformational</td>
<td>+</td>
<td>52-59</td>
</tr>
<tr>
<td>2E2</td>
<td>IgG1</td>
<td>ORF2.1, conformational</td>
<td>+/-</td>
<td>60-76</td>
</tr>
</tbody>
</table>
Genelabs HEV IgM ELISA

Blood donors
HEV epidemic
Burnet/Select HEV IgM ELISA

Blood donors
HEV epidemic
### Burnet/Select HEV IgM ELISA

<table>
<thead>
<tr>
<th>Population (n)</th>
<th>IgM reactive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australian blood donors (400)</td>
<td>1 (0.25)</td>
</tr>
<tr>
<td>Disease state sera (other hepatitis) (84)</td>
<td>1 (1.2)</td>
</tr>
<tr>
<td>Nepal, prevalence (1126)</td>
<td>16 (1.4)</td>
</tr>
<tr>
<td>Nepal epidemic (94)</td>
<td>92 (97.9)</td>
</tr>
</tbody>
</table>
IgM anti-HEV: Sporadic hep, Nepal

IgM ELISA OD

Patient number

Dr Iswar Shrestha, Kathmandu
Current status of HEV diagnostics

- HEV ELISAs based on ORF2.1 protein
  - MP Biomedicals, Asia-Pacific
  - IgM (diagnostics) ELISA 3.0
  - IgG (seroprevalence)
  - Antigen sandwich ELISA 4.0 (seroprevalence, zoonotic infections and seroprevalence)
Genelabs HEV IgM ELISA (1 - old assay)

Genelabs (1) HEV IgM ELISA

Sample/Cutoff range

GLD1 (Neg)  GLD1 (Pos)

Positive
MP Bio HEV IgM ELISA 3.0
## Genelabs new HEV IgM ELISA

<table>
<thead>
<tr>
<th>Population (n)</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors (95)</td>
<td>1 (1.1)</td>
</tr>
<tr>
<td>Other hepatitis (88)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Other disease (25)</td>
<td>1 (4.0)</td>
</tr>
<tr>
<td>Control total (208)</td>
<td>5 (97.6% spec)</td>
</tr>
<tr>
<td>Acute HEV (151)</td>
<td>149 (98.7% sens)</td>
</tr>
</tbody>
</table>

H.Y.Chen et al, CDLI 2005
IgM anti-HEV: Sporadic hep, Nepal

"Patients would arrive at the clinic in the morning, and wait until I had time to run the test so they could get the result"

Dr Iswar Shrestha, Siddhi Polyclinic, Nepal
Rapid Immunochromatographic Tests

(1) Gold conjugate (gold-MAb-antigen), rehydrated with 4 drops Rehydrating Reagent

(2) 25µl undiluted serum (or blood) added to sample pad

(3) Serum reaches limit line, card secured closed reversing flow
HEV IgM Rapid Test: Reverse Flow Technology

CONJUGATE PAD:
• Gold conj. to anti-HEV Mab, bound to HEV antigen
• reverses flow

GOLD
See red colour on Test Line

Always see red colour on Control Line

NITROCELLULOSE MEMBRANE

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HEPATITIS E IgM RAPID ASSAY

FOR RESEARCH PURPOSES ONLY

SelectVaccines
Hepatitis E IgM rapid test

Genelabs Diagnostics (MP Biomedical) Assure™
- licensed from Select Vaccines Ltd

HEV patients

Healthy
## Genelabs Assure™ HEV IgM rapid

<table>
<thead>
<tr>
<th>Population (n)</th>
<th>ELISA</th>
<th>Assure rapid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors (95)</td>
<td>1 (1.1)</td>
<td>2 (2.1)</td>
</tr>
<tr>
<td>Other hepatitis (88)</td>
<td>3 (3.4)</td>
<td>0 (0)</td>
</tr>
<tr>
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<td>1 (4.0)</td>
<td>1 (4.0) *RF</td>
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H.Y.Chen et al, CDLI 2005
Genelabs Assure™ - AFRIMS

K Myint et al, AJTMH 2005
### Genelabs Assure™ - AFRIMS

<table>
<thead>
<tr>
<th>Population (n)</th>
<th>IgM Pos (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors (100)</td>
<td>0</td>
</tr>
<tr>
<td>Other hepatitis (175)</td>
<td>0</td>
</tr>
<tr>
<td>RF positive (26)</td>
<td>0</td>
</tr>
<tr>
<td>Acute HEV (200)</td>
<td>186 (93%)</td>
</tr>
</tbody>
</table>

K Myint et al, AJTMH 2005
Hepatitis E Rapid Assay in use in refugee camp in Chad

Photo courtesy Dr Greg Armstrong, CDC, Atlanta
### MP Bio Assure™ HEV IgM rapid

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity (n)</th>
<th>Specificity (n)</th>
</tr>
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<tr>
<td>Nepal, PR China</td>
<td>96.7% (n=151)</td>
<td>98.6% (n=208)</td>
</tr>
<tr>
<td>Nepal, PRC, Indonesia</td>
<td>93.0% (n=200)</td>
<td>100% (n=275)</td>
</tr>
<tr>
<td>PR China*</td>
<td>97.0% (n=502)</td>
<td>96.5% (n=683)</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td><strong>96.0% (n=853)</strong></td>
<td><strong>97.7% (n=1166)</strong></td>
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* Two site clinical trial in China
### Alternative HEV Diagnostics

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<th>Assay</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>Baculo 62K IgM (NIH)</td>
<td>Very high</td>
<td>Very high</td>
</tr>
<tr>
<td>Quantitative IgM (AFRIMS)</td>
<td>Very high</td>
<td>High</td>
</tr>
<tr>
<td>Mosaic IgM (CDC)</td>
<td>High</td>
<td>Very high</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>High – very high</td>
<td>“Gold standard”</td>
</tr>
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</table>

RT-PCR has been invaluable in understanding transmission of indigenous/zoonotic HEV in presumed non-endemic countries, BUT it is not really suitable for routine diagnosis.
Molecular diagnostics for HEV

- Serology has specificity problems in low-incidence settings (e.g., zoonotic infections in W. Europe)

- Unsatisfactory PPV in low-incidence settings

- RT-PCR is “gold standard” for SPECIFICITY, but less sensitive than IgM serology in outbreak settings
  - Short duration and low titre of viral RNA in serum – very labile?

- Balanced approach needed – serology for returned travellers, RT-PCR for unexplained acute hepatitis?
Vaccines for hepatitis E (and A) viruses

🔹 Hepatitis A
  - Inactivated virus from cell culture
  - Antibody is protective (and easily measured)
  - Many commercially available, including Twinrix
  - Widespread use has had dramatic public health impact

🔹 Hepatitis E
  - Recombinant protein from insect cell culture or *E. coli*
  - Very effective in Phase 3 clinical trials, Nepal and China
  - Have not been commercially released – China imminent
HEV vaccines

- GSK/Novavax – HEV VLPs from insect cell culture

- Xiamen Biotech, Jiangsu – Hecolin (HEV 239)
  - Successful Phase 3 trials in Jiangsu, 2010
    » Licensed SFDA in Jan 2012

  - 30 µg *E. coli*-derived protein + Alum at 0, 1, 6 mo
  - Placebo: 15 cases HEV in 48,663 recipients
  - Vaccine: 0 cases HEV in 48,693 recipients
    » Efficacy 72-100%
Recombinant HEV antigens (4)

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