EUROPE’S NEW HEPATITIS PROBLEM

Many get infected with hepatitis E, and a few get very sick. How can the virus be stopped?

Thomas Vanwolleghem, MD PhD
Hepatoloog UZA, Antwerpen
Onderzoeker, Erasmus MC
• HEV virology
• HEV (sero)-epidemiology

• HEV clinical presentation – treatment
  • HEV clinical cases- diagnosis

• HEV animal reservoirs- zoonotic risk
• HEV experimental models
Hepatitis E virus and the global disease burden

- Nonenveloped +sense single stranded RNA virus (27-34 nM)
- Family *Hepeviridae*, Genus *Orthohepevirus*
- 3 ORF

Aggarwall R, Hepatol 2011; Debing Y. J Hepatol 2016
Hepatitis E virus and the global disease burden

- 4 major genotypes:
  - 1+2 restricted to humans
  - 3+4 broad host range (zoonotic)

Hepatitis E virus is emerging

- **Seroprevalence:**
  - Overall ↓ until 2011 (Germany and the Netherlands)
  - ↑ young (largely unexposed) adults

- ↑ HEV RNA positive blood donations in the Netherlands
  - Oct 2012 – Mar 2013 1:2742
  - Apr 2014 – Sep 2014 1:611

HEV Transmission

Transmission mainly via fecal-oral route
Sero-epidemiological surveys in Europe/US

Acute Fulminant HEV

US: zeldzaam (0,4%), totale ALF cohorte n=681
Meer frequent in HEV gt 1 infection

Acute on Chronic LF/ Decompensated LF

Prospective UK/French series: 3,2%
8 yr Retrospective Single Center (Toulouse): n=7 (age >65 yrs), alcohol+

Acute alcoholic hepatitis: 3,6% (total, n=84)

Chronic liver disease: 21% HEV IgG+

DILI: 3% HEV IgM+ (total n= 318)
Scheme: HEV Zoonosis

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Classical acute HEV (gt 1/2/3/4)

Chronic HEV genotype 3

Chronicity rate = 65.9% in SOT recipients (n=65/85)

HIV
SOT
BMTx
Cancer chemotherapy

“Immunocompetent”: immune suppressive R/undefined CD4 defect

-> Rapid fibrosis progression

NEJM 2012, Blood 2013;122:1079
GASTROENTEROLOGY 2011;140:1481 ; Hepatology 2014,60 (3).
Extrahepatic manifestations

Neurological: (~100 cases)
Guillain-Barre
Brachial neuritis
Meningo-encephalitis

Kidney disease:
glomerulonephritis
± cryoglobulinemia

Replication vs HEV RNA Detection?
Animal models
Seldom HEV negative strand PCR (Placenta)

## Treatment for chronic HEV

Reduction of immune suppression $\rightarrow$ successfull in 32.1%

<table>
<thead>
<tr>
<th>Drug</th>
<th>In vitro effect</th>
<th>In vivo effect</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribavirin</td>
<td>Inhibition of HEV replication</td>
<td>HEV clearance in chronic hepatitis E; occasional cases of treatment failure</td>
<td>Intracellular GTP depletion through inosine 5'-monophosphate dehydrogenase inhibition</td>
</tr>
<tr>
<td>PegIFNa</td>
<td>Inhibition of HEV replication</td>
<td>HEV clearance in chronic hepatitis E</td>
<td>Immune activation</td>
</tr>
<tr>
<td>Sofosbuvir</td>
<td>Inhibition of HEV replication</td>
<td>Unknown</td>
<td>Nucleotide analog; inhibition of the viral RNA-dependent RNA polymerase</td>
</tr>
<tr>
<td>Mycophenolic acid (including prodrug mycophenolate mofetil)</td>
<td>Inhibition of HEV replication</td>
<td>Unclear, possibly associated with HEV clearance in chronic hepatitis E</td>
<td>Intracellular GTP depletion through inosine 5'-monophosphate dehydrogenase inhibition; immune suppression</td>
</tr>
<tr>
<td>mTOR inhibitors (rapamycin, everolimus)</td>
<td>Stimulation of HEV replication</td>
<td>Higher HEV RNA levels in patients with chronic hepatitis E on mTOR inhibitors</td>
<td>Inhibition of an eIF4E binding protein 1-dependent antiviral signaling pathway downstream of mTOR</td>
</tr>
<tr>
<td>Calcineurin inhibitors (cyclosporin A, tacrolimus)</td>
<td>Stimulation of HEV replication</td>
<td>Unknown; tacrolimus use associated with increased risk of viral persistence</td>
<td>Inhibition of cyclophilin A and B</td>
</tr>
</tbody>
</table>

Treatment PegIFN?

3-month course
LTX n=3
135 µg/week
2 clear
1 relapse

Leukemia n=1
PegIFNα2b 1 µg/kg BW/week
2 rapid response
2 slow response

Not in KTX

Ann Int Med 2010
RBV for chronic HEV

Retrospective series (n=59)

- Median 3 months
- Median dose: 600 mg per day (upto 1200mg), ~ 8.1mg/kg
- EOT= 95%
- “SVR24 wks” =78%

~ weight based RBV (12 mg/kg): 1000 mg vs 1200 mg (anemia!)

Prediction of response: monitor HEV RNA in stool

- + @ 1 month in 100% of relapsers
- + @ 3 months in 66% of relapsers vs 0% of responders

### UZA case 1: HTx recipient; feb 2014

<table>
<thead>
<tr>
<th>HEV IgG -</th>
<th>HEV IgG +</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEV IgM -</td>
<td>HEV IgM +</td>
</tr>
</tbody>
</table>

#### ALT (U/L)

- **Baseline**: 20
- **After meal**: 180
- **2 Mar 2014**: 2.04E+06
- **3 Mar 2014**: 2.89E+06
- **4 Mar 2014**: 2.6E+06
- **TREATMENT START**: 14.8
- **6 Mar 2014**: 14
- **9 Mar 2014**: 10.1
- **12 Mar 2014**: 13.9
- **13 Mar 2014**: 12.6

#### HEV RNA Quantitative (IU/mL)

- **10 Mar 2014**: 1.02E+06
- **11 Mar 2014**: (negative)
- **12 Mar 2014**: (negative)

#### Hb (mg/dL)

- **2 Mar 2014**: 14.8
- **3 Mar 2014**: 14
- **4 Mar 2014**: 10.1
- **5 Mar 2014**: 13.9
- **6 Mar 2014**: 12.6

#### Mycophenolate (mg)

- **10 Mar 2014**: 0
- **11 Mar 2014**: 0
- **12 Mar 2014**: 0

#### Methylprednisolone (mg)

- **10 Mar 2014**: 0
- **11 Mar 2014**: 0
- **12 Mar 2014**: 0

#### Tacrolimus (mg)

- **10 Mar 2014**: 10
- **11 Mar 2014**: 10
- **12 Mar 2014**: 10
### UZA case 2: HTx + KTx recipient; 2016

#### HEV PCR (WIV) + : 1/7 ; 23/8 en 26/9

#### HEV serology : IgG+ en IgM+ 1/7

Start RBV on 10/10

- HEV RNA 1,30 E6 IU/mL (2/11) < 4,76 E7 IU/mL (7/10)
- RBV monitoring (Nijmegen): 1.01 mg/L. (2-3 mg/L)
- RBV monitoring (Nijmegen): 1.07 mg/L

**HEV infectie april-juni ’16**

**HEV PCR-Junii 2015**

**Start RBV**
HEV PCR (WIV) + : 11/8 en 12/10
HEV serology : IgG- en IgM+ 11/8

HEV RNA 5,32 E+6 IU/ml (12/10)

HEV infectie mei-juli ’16
HEV PCR- Jan 2016
Immune compromised patient with consistent ALT rise >1 month:

- HEV PCR (qualitative) (WIV)
  → Voorgedefinieerde DOTS test
  (ism Veerle Matheeussen, Microbiologie UZA)

- HEV serology: (WIV Recomline --> Wantai)
  low NPV, to be combined with HEV PCR
Voedingsadviezen voor transplant pt

Merendeel leverworsten HEV RNA+
Cfr Figatellu Corsica/Zuid-Frankrijk
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- HEV animal reservoirs - zoonotic risk
- HEV experimental models
# Zoonotic Risk? Animal Reservoirs?

<table>
<thead>
<tr>
<th>Natural animal host</th>
<th>Classification (genus/species, genotypes [gt])</th>
<th>Experimental hosts for cross-species infection</th>
<th>Zoonotic infection in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td><em>Orthohepevirus A</em> gt 1, 2, 3, 4</td>
<td>Non-human primates, pigs (gt 3, 4), rabbits (gt 1, 4), lambs (gt 1), Wistar rats (gt 1)</td>
<td>Yes</td>
</tr>
<tr>
<td>Domestic swine</td>
<td>gt 3, 4</td>
<td>Non-human primates, rabbits, Mongolian gerbils (gt 4), Balb/C mice (gt 4)</td>
<td>Yes (gt 3, 4), likely (gt 5, 6)</td>
</tr>
<tr>
<td>Wild boar</td>
<td>gt 3, 4, 5, 6</td>
<td></td>
<td>Yes (gt 3, 4), likely (gt 5, 6)</td>
</tr>
<tr>
<td>Deer</td>
<td>gt 3</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit</td>
<td>gt 3</td>
<td>Pigs</td>
<td>Likely</td>
</tr>
<tr>
<td>Mongoose</td>
<td>gt 3</td>
<td></td>
<td>Likely</td>
</tr>
<tr>
<td>Camel</td>
<td>gt 7</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Moose</td>
<td>unknown</td>
<td></td>
<td>Not known</td>
</tr>
<tr>
<td>Chicken</td>
<td>Avian HEV gt 1, 2, 3</td>
<td>Turkeys</td>
<td>No</td>
</tr>
<tr>
<td><em>Orthohepevirus B</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td>Unlikely</td>
</tr>
<tr>
<td>Ferret</td>
<td></td>
<td></td>
<td>Unlikely</td>
</tr>
<tr>
<td>Greater bandicoot</td>
<td></td>
<td></td>
<td>unlikely</td>
</tr>
<tr>
<td>Asian musk shrew</td>
<td></td>
<td></td>
<td>unlikely</td>
</tr>
<tr>
<td>Mink</td>
<td></td>
<td></td>
<td>unlikely</td>
</tr>
<tr>
<td>Bat</td>
<td><em>Orthohepevirus D</em></td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Cutthroat trout</td>
<td><em>Piscihepevirus</em></td>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>
CDC study
N= 4936 dieren (35 genera)

457 HEV IgG+:
Bizon (4,3%), Runderen (15%), Honden (0,9%), Ratten (0,6%)
Varkens (41,2%), Wilde zwijnen (2,9%)

Alleen hoge titers bij varkens (HEV gt3)
Zoonotic Risks in Belgium: “Pig Belt”

- Overall 70% of fatteners HEV RNA+
- serum HEV RNA- within 1 month, but ongoing fecal secretion
- infection of newborn pigs

→ True pig reservoir

Belgium (2010): slaughterhouse
→ 5/23 farms HEV RNA+
→ 8/115 (7%) HEV RNA+

Number of sows by region (2013) - Source: Eurostat

BMC Res Notes, 2012. PLOS one 2011
Zoonotic Risks in Belgium: … and Wildlife

- Wild Boar: 34% HEV IgG+
- Deer: 1-3% HEV IgG+

Wild boar density shot per region (2009-2013)

Transboundary and Emerging Diseases. 2015
HEV experimental models

- In vitro, infection of various cell lines

- In vivo, acute HEV infection in pigs, ferrets, chimpanzee, and rhesus monkeys

- Recently, in vivo, chronic HEV infection in human-liver chimeric mice
  - uPA+/+NOG (van de Garde et al. JVI 2016)
  - uPA+/+SCID/beige (Alweiss et al. J Hepatol 2016)
  - uPA+/+SCID (Sayed et al. Gut 2016)
Principle of human liver chimeras

- Genetic defined liver disease
  - Overexpression of uPA induces ER stress related apoptosis
- Immune deficient UPA+/+NOG

HEV gt3 infection of human-liver chimeric mice

- Infection with HEV gt3 derived from feces and liver but not from plasma/serum
Conclusion: HEV

- HEV gt 3 emerging in Europe
- Acute, mostly asymptomatic in immunocompetent
- Possible chronic in immunocompromised
- Viral hepatitis serology may be negative --> PCR
- Treat with RBV
- Uncooked pork meat, seafood, leverworst/paté
  - In Belgium: Deer safer than Wild Boar?
  - Many issues on food safety remaining

- Preclinical models for infectivity and antiviral studies
Acknowledgements

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Het congres

Als laatste presenteerde David de uitkomsten van zijn onderzoek naar aandachtscurves van congresgangers.

thomas.vanwolleghem@uza.be
tel 3853
Excretion of Infectious Hepatitis E Virus Into Milk in Cows Imposes High Risks of Zoonosis

HEV gt 4 RNA in Feces (37.1%), bloed en melk (Yunnan, China)

Transmissie nr Rhesus monkey (gavage):
Pasteurisatie (30' 62°C of 72°C): onvoldoende
Koken (3' 100°C) = sterilizatie
Rural China: Mixed Farming