Hepatitis E:
E is for Elusive

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Medical Director Hepatitis B Foundation Doylestown PA
Thank you to:

- Ken Sherman
- Heiner Wedemeyer/Michal Manns
- Carrie Frenette
- Rollie Dickson
- Harvey Alter
- Saleem Kalilli
- Ray Kim
Disclosures

- None
Hepatitis E Case 1

- 80 yo male presents with jaundice
  - 6 weeks prior started allopurinol 300 mgs per day
  - 3 week prior fatigue, nausea, abd discomfort, dark urine. Allopurinol DC
  - No history of liver disease, ETOH viral risk factors.

PMH
- CAD
- DM type 2
- HTN
- GERD

Medications (all for several years)
- Colchicine
- Promethazine
- Simvastatin
- L-thyroxine
- Metformin

Davern Gastroenterology 2011;141:1665-1672
Hepatitis Case 1

- Admit: Jaundiced, no fever, rash, stigmata CLD
  - HAV, HBV, HCV serologies (-), SmAb 1:320,
  - ANA (-) . US, CT abd no path findings

Dx Highly likely DILI from allopurinol.
Hepatitis E Case 1

- **Serologic testing After Discharge**

<table>
<thead>
<tr>
<th>Time After starting</th>
<th>Time After stopping</th>
<th>ALT (U/L)</th>
<th>Alk P (U/L)</th>
<th>Bilirubin (mg/dL)</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 wks</td>
<td>3 wks</td>
<td>235</td>
<td>210</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>8 wks</td>
<td>4 wks</td>
<td>92</td>
<td>202</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>9 wks</td>
<td>5 wks</td>
<td>53</td>
<td>139</td>
<td>6.1</td>
<td>Normal</td>
</tr>
<tr>
<td>10 wks</td>
<td>6 wks</td>
<td>43</td>
<td>121</td>
<td>1.1</td>
<td>HEV Anti IgM(+) IgG(-) RNA (-)</td>
</tr>
<tr>
<td>12 wks</td>
<td>8 wks</td>
<td>43</td>
<td>121</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>14 wks</td>
<td>10 wks</td>
<td>55</td>
<td>85</td>
<td>0.7</td>
<td>HEV anti IgG(+) IgM(-) RNA (-)</td>
</tr>
<tr>
<td>8 mos</td>
<td>7 mos</td>
<td>&lt;35</td>
<td>&lt;125</td>
<td>&lt;1.2</td>
<td></td>
</tr>
</tbody>
</table>

- **Diagnosis HEV**
- **FU 6 months later no sx liver disease**

Davern Gastroenterology 2011;141:1665-1672
Hepatitis Case 2

50 y male with well compensated ESLD due to HCV/ETOH

- Presents with jaundice, new onset ascites requiring hospitalization
- No recent travel

Labs

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (iu/l)</td>
<td>112</td>
<td>2328</td>
</tr>
<tr>
<td>Bili (mg/dl)</td>
<td>2.5</td>
<td>35</td>
</tr>
<tr>
<td>INR</td>
<td>1.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Laboratory testing on admit

- HEV IgM(+), IgG (-), RNA (+) Genotype 3

Hospitalized 14 days with recovery
Hepatitis Case 3

- 44 yo female, Living donor Renal Transplant 9-2003
  - 2004 ALT 76, AST 36
  - 2006 4x ULN persisted
  - HAV, HIV, HBV, CMV, EBV, Autoimmune testing (-)

- 4-2009 HEV anti-IgG (+) RNA (+)

- Bx : lymphocytic portal infiltrate with piecemeal necrosis, stage 2 fibrosis

- Lowering immunosuppression
  - ALT/AST WNL
  - RNA (-)
  - Fibroscan 1 yr later F0-F1
Hepatitis E Clinical

- Incubation time 2-8 weeks
  - Peak viremia is during incubation period and early phase of subclinical or symptomatic disease

- Initial symptoms of usually nonspecific
  - Flu-like symptoms, malaise, myalgia, arthralgia, weakness, and vomiting.
  - May have jaundice, itching, uncolored stools, and darkened urine

- May be misdiagnosed as DILI
  - 3% in US, 12% in UK

- Asymptomatic infections 2-4 x greater than symptomatic infections

- Features different in autochthonous* (gen 3,4) and Genotypes 1 and 2

*au·toch·tho·nous ˈô-täkTHənəs/ adjective
1. (of an inhabitant of a place) indigenous rather than descended from migrants or colonists.
Occurrence of HEV Infection

- **Source:** Human or zoonotic
- **Factors:** Contaminated water or undercooked pork or game meat
- **Route of transmission:** Fecal-oral
- **Liver:** main target organ
- **Mechanism of delivery and basis of tropism remains unclear**
  - Extra-hepatic tropism unlikely, but not excluded
# HEV versus HAV

- Clinically indistinguishable

<table>
<thead>
<tr>
<th></th>
<th>HAV</th>
<th>HEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation</td>
<td>~ 30 days</td>
<td>~ 40 days</td>
</tr>
<tr>
<td>Dose-dependent severity</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Mortality in general</td>
<td>0.1-2%</td>
<td>1-4%</td>
</tr>
<tr>
<td>Mortality in pregnancy</td>
<td>No difference</td>
<td>Up to 20%</td>
</tr>
<tr>
<td>Bimodal disease</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Chronicity</td>
<td>No</td>
<td>No/yes Transplant patients</td>
</tr>
<tr>
<td>In developed region</td>
<td>Epidemic, endemic</td>
<td>Ab+, but rare disease</td>
</tr>
<tr>
<td>In developing region</td>
<td>Ab+, but rare disease</td>
<td>Epidemic, endemic</td>
</tr>
<tr>
<td>Age</td>
<td>Older children, young adults</td>
<td></td>
</tr>
</tbody>
</table>

- Virological characteristics:
  - HAV much more stable in the environment
  - >x100 higher fecal titer

Comparison of NHANES III and IV 1988-94 (N= 18,695) 2009-2010 (N= 7885)
HEV Prevalence: 3 countries

- Egypt, 1997
- India, 1992
- USA, 2001

Percell. J Hep 2008;48:494
HEV in NHANES Data

- Overall Anti-HEV Positivity: 21.0%

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>20.4%</td>
</tr>
<tr>
<td>Male</td>
<td>21.6%</td>
</tr>
<tr>
<td><strong>Race/Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Whites, NH</td>
<td>22.1%</td>
</tr>
<tr>
<td>Black, NH</td>
<td>14.5%</td>
</tr>
<tr>
<td>Mexican</td>
<td>20.3%</td>
</tr>
<tr>
<td><strong>Country of Birth</strong></td>
<td></td>
</tr>
<tr>
<td>US</td>
<td>20.1%</td>
</tr>
<tr>
<td>Mexico</td>
<td>30.9%</td>
</tr>
<tr>
<td>Other</td>
<td>26.2%</td>
</tr>
<tr>
<td><strong>Region</strong></td>
<td></td>
</tr>
<tr>
<td>South</td>
<td>14.7%</td>
</tr>
<tr>
<td>Northeast</td>
<td>20.8%</td>
</tr>
<tr>
<td>Midwest</td>
<td>26.6%</td>
</tr>
<tr>
<td>West</td>
<td>25.0%</td>
</tr>
</tbody>
</table>
HEV Epidemiology in the US

- NHANES III
  - Serum samples available in 18,695 out of 24,713 participants

- HEV Testing
  - No ‘gold standard’
  - ‘Homegrown’ EIA for anti-HEV IgG
  - Confirmatory testing for Ab (antigen blocking assay)
  - No testing for RNA
HEV in NHANES Data

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</table>
Prevalence of antibody to HEV by Place of Birth

Kuniholm. JID 2009;200:49
CDC Lab Based Surveillance for HEV Infection in the US 2005-2012

154 Clinical Non-A,B,C Hepatitis Cases Referred to CDC

26 (17%) HEV infected

11 traveled to endemic area
15 non-travelers “autochthonous”

<table>
<thead>
<tr>
<th>Mean Age</th>
<th>32</th>
<th>61</th>
</tr>
</thead>
<tbody>
<tr>
<td>Icteric</td>
<td>92%</td>
<td>47%</td>
</tr>
<tr>
<td>Organ Transplant</td>
<td>0</td>
<td>47%</td>
</tr>
<tr>
<td>Gt3</td>
<td>0/4</td>
<td>8/8</td>
</tr>
<tr>
<td>Fulm. Hep Failure</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>
What is the National Seroprevalence of anti-HEV?

- Kuniholm et al* tested a nationally representative sample of 18,695 serum samples from the US population for anti-HEV IgG antibodies using a research (non-commercial) enzyme immunoassay.

- Serum from NHANES, 1988-1994, showed, overall, 21% anti-HEV IgG.

* Kuniholm et al, J Infect Dis 2009; 200:48-56
Prevalence of HAV by Place of Birth

Klevens. Public Health Rep 2011;126:522
Prevalence of HEV by Region

Kuniholm. JID 2009;200:49
### Risk Factors for HEV*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adj. Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Military service</td>
<td>1.21 (0.99-1.48)</td>
</tr>
<tr>
<td>Source of water (well vs. city)</td>
<td>0.78 (0.63-0.97)</td>
</tr>
<tr>
<td>Lifetime sex partners (&gt;10)</td>
<td>0.91 (0.73-1.14)</td>
</tr>
<tr>
<td>MSM, ever</td>
<td>1.09 (0.68-1.74)</td>
</tr>
<tr>
<td>Any pet</td>
<td>1.19 (1.01-1.40)</td>
</tr>
<tr>
<td>Dog</td>
<td>1.22 (1.04-1.43)</td>
</tr>
<tr>
<td>Cat</td>
<td>1.12 (0.90-1.38)</td>
</tr>
<tr>
<td>Pork products consumption</td>
<td>0.78 (ham) – 0.89 (bacon)</td>
</tr>
<tr>
<td>Liver/organ meat consumption</td>
<td>1.38 (1.01-1.88)</td>
</tr>
<tr>
<td>Anti-HCV (+)</td>
<td>1.71 (1.07-2.74)</td>
</tr>
<tr>
<td>Anti-HBc (+)</td>
<td>1.37 (1.00-1.86)</td>
</tr>
<tr>
<td>Anti-HAV (+)</td>
<td>0.80 (0.70-0.92)</td>
</tr>
</tbody>
</table>

*US-born subjects only*
HEV SEROPREVALENCE in patients with Chronic Liver Disease

Atiq et. al. EMERGING INFECT DIS 2009
HEV

RNA virus (family Hepeviridae)

Polyprotein:
- Methyltransferase
- Protease
- Helicase
- Polymerase

Regulatory (viral egress?)

Capsid

Aggarwal. Hepatology 2011;54:2218
Cell Culture Systems for HEV

- Cell culture systems and infectious cDNA clones has been developed for genotypes 3 and 4
  - Human lung cells line A549
  - Human hepatoma cells lines
    - HepG2/C3A
    - PLC/PRF/5 h
  - Swine kidney cells
    - Okamoto H et al., 2011, Rev Med Virol 2011; 21
1978: Water-borne epidemic in Kashmir caused 20,000 icteric cases; 700 FH; 600 deaths; not HAV
1980: Epidemic hepatitis among Russian soldiers in Afghanistan; not HAV related
1983: Russian volunteer swallows fecal extract from 9 acute cases in the Afghan epidemic and recovers 27-30nm VLP from his acute phase stool (Balayan)
CDC recovers identical VLP from macaques inoculated with acute phase stool; serial passage
1990: Bile from cyno macaques used in differential hybridization to clone HEV (Reyes, G:Gene Labs)
Historical Aspects ~1950s

- Retrospective serologic testing of stored sera confirmed enteric non-A-non-E hepatitis in New Delhi (1955-1956)
- November 1955: Flooding of Yamuna river and contamination of city water
- 29,000 icteric cases
  - Highest attack rate in adults
  - Wang DC et al., Lancet 1980; 2
... end of 1970s - 1980s

- Kashmir Valley, India
  - Nov 1978-April 1979
  - 275 clinical cases, 11-40 years old in villages with common water source, among 16,620 inhabitants
  - Rate of fulminant hepatitis was 4.4%
  - Khuroo MS. Am J Med 1980; 68

- Former soviet republics of Central Asia- Turkmenistan, Kyrgyzstan, Uzbekistan, 1980 and 1986
  - Ketiladze / Favorov / Shahgildyan

Transmission Studies

- Confirmation of new hepatitis agent was demonstrated by Dr. Michael Balayan in a volunteer self-inoculation with pooled fecal material
- 12 August 1981
- Day 36: Acute hepatitis
- Duration: 3 weeks
- Days 28-45: in IEM aggregates of 27-30 nm VLP from stool with sero-conversion sera, but not hep A, B or PT NANB

Two Cy macaques inoculated with stool suspension from the experiment showed excretion of same VLP, LT elevation and histological changes in liver.
Dedicated to Dr Michael Balayan, who at much risk to his life undertook a self-inoculation experiment to prove the infectious and transmissible nature of the enteric non-A, non-B hepatitis agent (Balayan MS, et al. Intervirology; 20:23-31, 1983)
Hepatitis E Virus (HEV)- Breakthrough of 1990s

- 1990: Reyes isolated a nucleic acid clone representing part of hepatitis E viral genome from bile of an experimentally-infected animal.

- 1991-1992: Tam and Huang sequenced entire HEV genome showing heterogeneity of Asian and Mexican isolates - genotypes 1 and 2, respectively.

- 1992: Dawson developed first anti-HEV EIA showing that IgM is a short-lived marker of recent infection

- 1992-2000: Improvement of serologic assays and development of molecular tests
A Modern Outbreak of Hepatitis E, Uganda 2007-2009

Distribution of cases of jaundice during an epidemic of hepatitis E in Kitgum District, Uganda (N = 7,919), by week of report, October 2007 through January 2009

## HEV Prevalence and Disease Pattern

<table>
<thead>
<tr>
<th></th>
<th>Highly Endemic</th>
<th>Less endemic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human Disease</strong></td>
<td>Common, sporadic and epidemic</td>
<td>Infrequent, sporadic</td>
</tr>
<tr>
<td><strong>Transmission</strong>*</td>
<td>Contaminated water</td>
<td>Undercooked meat, Animal contact</td>
</tr>
<tr>
<td><strong>Reservoir</strong></td>
<td>Primarily human</td>
<td>Zoonotic</td>
</tr>
<tr>
<td><strong>Host characteristics</strong></td>
<td>Young, healthy</td>
<td>Elderly, comorbid</td>
</tr>
<tr>
<td><strong>Pregnant women</strong></td>
<td>Fulminant in ~20%</td>
<td>Not reported</td>
</tr>
<tr>
<td><strong>Chronic infection</strong></td>
<td>None</td>
<td>Immunosuppression</td>
</tr>
</tbody>
</table>

*Other modes of transmission (less frequent):
1. Person-to-person: household transmission (1-2%)
2. Materno-fetal
3. Transfusion

Aggarwal. Hepatology 2011;54:2218
HEV Genotypes

- 5 genotypes: 1/2 (human), 3/4 (human, swine) and 5 (avian)
## HEV: Clinical Differences Genotypes

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Genotypes 1 and 2</th>
<th>Genotypes 3 and 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occurrence in U.S.</td>
<td>Travel-related, imported</td>
<td>Autochthonous</td>
</tr>
<tr>
<td>Rate of icteric illness</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Infection</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td>Infection in majority of young healthy patients is asymptomatic</td>
</tr>
<tr>
<td>Disease</td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
<td><img src="https://via.placeholder.com/150" alt="Image" /></td>
</tr>
<tr>
<td>Age distribution</td>
<td>Rates highest adolescents and young adults</td>
<td>Rates highest among older adults (ave 60 yrs), co morbidities</td>
</tr>
<tr>
<td>Sex distribution</td>
<td>Similar rates men/women</td>
<td>Higher rates men &gt;3:1</td>
</tr>
<tr>
<td>Mortality</td>
<td>High among pregnant women (10-25%) 3rd trimester</td>
<td>High older adults Chronic liver dz up to 70%</td>
</tr>
<tr>
<td>Extra hepatic features</td>
<td>Few</td>
<td>Neurologic complications, glomerulonephritis</td>
</tr>
<tr>
<td>Chronic infection</td>
<td>None</td>
<td>Immunosuppressed (common)</td>
</tr>
</tbody>
</table>

## Genotype 1 vs 3 Hepatitis E

<table>
<thead>
<tr>
<th>Feature</th>
<th>Genotype 1 (Epidemic)</th>
<th>Genotype 3 (Endemic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td>1:1</td>
<td>3:1</td>
</tr>
<tr>
<td>Age</td>
<td>20-45 yrs</td>
<td>40-80 yrs</td>
</tr>
<tr>
<td>2nd Spread</td>
<td>Uncommon</td>
<td>Not known</td>
</tr>
<tr>
<td>Source</td>
<td>Water</td>
<td>Food</td>
</tr>
<tr>
<td>Agent</td>
<td>Human</td>
<td>Swine</td>
</tr>
<tr>
<td>Seasonality</td>
<td>Yes</td>
<td>Usually not</td>
</tr>
<tr>
<td>Fatality rate</td>
<td>Pregnancy</td>
<td>Elderly</td>
</tr>
<tr>
<td>Extrahepatic</td>
<td>Yes (Pancreas)</td>
<td>Yes (CNS)</td>
</tr>
<tr>
<td>Chronicity</td>
<td>No</td>
<td>Yes, immune deficient</td>
</tr>
</tbody>
</table>
## HEV Genotypes

<table>
<thead>
<tr>
<th></th>
<th>Genotype 1/2</th>
<th>Genotype 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic distribution</td>
<td>Developing countries</td>
<td>Developing and developed countries</td>
</tr>
<tr>
<td>Pattern of spread</td>
<td>Epidemic and sporadic</td>
<td>Sporadic</td>
</tr>
<tr>
<td>Secondary spread</td>
<td>uncommon</td>
<td>Extremely rare</td>
</tr>
<tr>
<td>Icteric illness</td>
<td>Common</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Extrahepatic</td>
<td>Few</td>
<td>Neurologic</td>
</tr>
<tr>
<td>Treatment</td>
<td>None</td>
<td>Ribavirin, Interferon</td>
</tr>
</tbody>
</table>

Hoofnagle. NEJM 2012;367:1237
HEV as Zoonosis

- 4 Japanese developed acute hepatitis 6 weeks after sharing deer meat sashimi

- Patient sera: HEV RNA+

- Frozen left-over deer meet: HEV RNA $10^5$ copies/g

- Sequence homology: 99.7%
  (326 nt in ORF 1)

Tei Lancet 2003;362:371
HEV Antibody Prevalence in Swine Veterinarians

- 109 of 468 swine vets were anti-HEV human strain (+)
  - 95 were also (+) for swine HEV
- No association with time spent with pigs, history of needle sticks/cuts, or industry/academic employment

Hépatite E : une histoire de bêtes Francaise

<table>
<thead>
<tr>
<th>Holland</th>
<th>PACA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prélèvement de sang, de foie et de muscle, cerf, sanglier, chevreuil</td>
<td>3 familles, 17 malades</td>
</tr>
<tr>
<td>PCR «conventionnelle » et temps réel « maison »</td>
<td>Les malades (pas les sains) avaient mangé du figatellu</td>
</tr>
<tr>
<td>Sérologie (ELISA)</td>
<td>PCR et microscopie électronique dans 12 figatelli</td>
</tr>
<tr>
<td>Séquençage : génotype 3c</td>
<td>Séquençage : génotype 3f</td>
</tr>
</tbody>
</table>

**AASLD 2009 – Reesink HW, Hollande, Abstract 907 actualisé ; Colson P, France, Abstract 912 actualisé**

> Conclusion : attention à la viande crue ou peu cuite !
Figatellu, Traditional French Pig liver sausage

▶ Cause acute HEV (gen 3) in 7/13 who ate it raw

▶ “product to cook (cook thoroughly)”

Colson, JID 2010:202
Gastro Elitism Movement: wild boar pappardelli, pigs feet Milanese

Figatelli (raw pork sausage): favorite in Southern France

Liver slime from pig poop is pooled and used to irrigate soil and plants

(don’t forget to eat your veggies)

Dunkin Donuts sells pork donuts in China

Scrapple made from pig heads and liver

11% of raw pig liver in US markets tested HEV RNA+

USDA recommendations:
- cook pork meat to 145F; organ meats to 160F
Vietnam: “Nguyen Van Hoang packs shrimp headed for US in dirty plastic tubs. He covers them with ice made from tap water that the Health Ministry says should be boiled before drinking because of the risk of contamination.” Pig farms abundant and water run-off possible.

Vietnam ships 100 million pounds of shrimp per year to the U.S.; 8% of the shrimp Americans eat

“At a tilapia farm in China’s Guangdong provence, Chen feeds the fish partly with feces from hundreds of pigs and geese”....about 27% of seafood Americans eat comes from China; FDA inspects only 2.7% of imported food.
Hepatitis E as a Zoonosis - A Historical Outlook

- Primarily was proposed by Dr M. Balayan by experimenting on piglets
- CDC confirmed HEV genotype 3 in historical experiment samples
- In nature Swine HEV was first characterized from pigs in the US
  - Meng XJ, et al., PNAS USA 1997; 94
- Swine workers have higher anti-HEV prevalence than general population
- Small outbreaks after consumption of raw boar and deer liver in Japan
Hepatitis E as a Zoonosis-Current Status*

- Various animal strains of HEV were genetically characterized from pig, chicken, rabbit, deer mongoose, fish.

- At least 4 recognized and 2 putative new genotypes have been identified

*Data from NIH HEV Scientific Workshop, Bethesda, 26 March 2012
Figure 2. (A) Worldwide prevalence of HEV and (B) the geographic distribution of the different HEV genotypes.
**Hepeviridae- Proposed Classification and Host Range**

<table>
<thead>
<tr>
<th>HEV</th>
<th>Natural Host</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genus Hepeivirus</strong></td>
<td></td>
</tr>
<tr>
<td>Genotype 1</td>
<td>human</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>human</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>human, pig, deer, mongoose, rabbit</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>human, pig</td>
</tr>
<tr>
<td>Putative Gt 5</td>
<td>rats</td>
</tr>
<tr>
<td>Putative Gt 6</td>
<td>Wild boar</td>
</tr>
<tr>
<td><strong>Putative Genus Avihepeviridae</strong></td>
<td></td>
</tr>
<tr>
<td>Genotype 1</td>
<td>chicken (Australia)</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>chicken (USA)</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>chicken (Europe and China)</td>
</tr>
<tr>
<td><strong>Putative Genus Piscihepevirus</strong></td>
<td></td>
</tr>
<tr>
<td>Cutthroat throat virus</td>
<td>fish</td>
</tr>
</tbody>
</table>

*XM Meng, Hepatitis E in US/An NIH Research Workshop, March 26, 2012*
Now back to the human story

- Who is at risk?
- Who needs testing?
HEV Acute on Chronic Decompensation
A problem in India?: not clearly documented in other regions

Kumar et al., IND J GASTRO, 2004
RELATIONSHIP OF HEV TO HEPATIC DECOMPENSATION IN THE HALT-C TRIAL

1050 in Randomized phase HALT-C

314 had a clinical event during follow up in HALT-C

89 met criteria of decompensation over a 24 week period

N. Samala: AASLD 2013
Anti-HEV IgG among HALT-C cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases N=89</th>
<th>Controls N=267</th>
<th>P value</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seroprevalence</td>
<td>20 (22.5)</td>
<td>55 (20.6)</td>
<td>0.71</td>
<td>1.12 (0.63-1.99)</td>
</tr>
<tr>
<td>(#(%))</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Seroconverters</td>
<td>5 (5.6%)</td>
<td>5 (1.8%)</td>
<td>0.064</td>
<td>3.12 (0.88-11.04)</td>
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Hepatitis E as a Cause of Acute Liver Failure*

- The US ALF Study Group has enrolled >1800 adults since 1998
- Final analysis was conducted on 699
  - 3/699 (0.4%) tested IgM anti-HEV +
  - 2 had high titer of IgG anti-HEV
  - No HEV RNA detected
- Conclusion: Acute HEV infection is rare cause of ALF in the United States

*Data from NIH HEV Scientific Workshop, Bethesda, 26 March 2012
## Clinical Outcomes

<table>
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<th>Acute Disease</th>
<th>Chronic Disease</th>
<th>Mortality</th>
</tr>
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<tbody>
<tr>
<td>Immunocompetent</td>
<td>YES</td>
<td>NO</td>
<td>LOW</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>YES</td>
<td>NO</td>
<td>VARIABLE</td>
</tr>
<tr>
<td>Chronic Liver Disease</td>
<td>YES</td>
<td>NO</td>
<td>HIGH</td>
</tr>
<tr>
<td>Immunosuppressed - HIV - Post-Transplant - Cancer Chemotx</td>
<td>YES</td>
<td>YES</td>
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HEV infection

- Silent infection
  - Recovery
- Acute symptomatic hepatitis
  - Fulminant hepatitis
    - Death
  - Recovery
- Chronic hepatitis
  - Recovery
  - Cirrhosis
  - Mild hepatitis

HEV Gen 3
Immunosuppressed

End point

Adapted Wedemeyer Gastro 2012;142:1388-1397
HEV Problems with serologic assays

- Sensitivity/Specificity complicated by lack of understanding of underlying HEV prevalence
  - Detection of anti-HEV among “negative” controls (Goldsmith et al., 1992)
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All HEV RNA (+) with well defined genotypes

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- Differences in peptides used accounts for differences in sensitivity.

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  - Recombinant protein pE2 encoded by protruding domain of the ORF-2
  - Presents the dimerized form of E2s domain with conformational epitopes preserved as in virion
    - vs linear monomeric ags used in GL assay
  - Protruded E2s domain is region w/ immune dominant epitopes

HEV infection of Rheus monkeys

- Rapid rise wk 3, peak wk 6; 50% drop wk 10
- Onset similar to IgM persists longer

Hepatitis E 86 Rheus Monkeys

E2 based assay higher sensitivity 98% vs 62%
Higher level and longer positive than GL

Wantai E2 IgG assay vs GL IgG assay (no E2s domain)

- WHO anti-HEV reference serum (UK acquired gen 3)
  - Lower limit of detection is 0.25 versus 2.5 Wu/mL
  - (+) in more sera from PCR-confirmed cases (98% vs. 56%)
  - Remained (+) longer post infection;

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- The assay used should incorporate the Recombinant protein pE2 encoded by protruding region of ORF-2

- Testing for anti-HEV IgG is not helpful for active infection given >/=15% adults in West countries (+)

- Exposure to HEV can be documented with Anti HEV IgG testing with use of an appropriate assay pE2 assay
Course of Acute HEV Infection

- Initial ALT rise IgM and HEV RNA present
- Titers of anti-HEV IgG can be detected early in infection and persist

HEV in Pregnant Women

- More frequently affected
  - 2nd and 3rd trimester
- 20-50% of HEV infected pregnant women develop fulminant hepatitis
- Mortality rate 20% in India and Pakistan
- Mortality rates 15-25% if infected in 3rd trimester
- Increased frequency of spontaneous abortion, stillbirth, and neonatal deaths

Aggarwal R. J Gastro and Hepato 2000
Khuroo, J Viral Hep 2003
Diagnosis Acute HEV Infection in Immunocompetent Pts

- HEV anti-IgM: best test for acute HEV.
  - HEV RNA detected for Ave 2wks serum, 4 wks stool
  - Serum RNA (+) during incubation and early illness but may be (-) by time of jaundice or clinical sx
    - (-) 34-50% tested at or near onset of illness (IgM+)
    - Dx by serum RNA may be of limited value

- HEV anti-IgG suggests exposure to HEV infection
  - May be present in acute or chronic

Hepatitis E: chronic

- Almost always in immunocompromised
  - Organ transplant recipients, chemotherapy, stem cell transplant and HIV
  - Associated with eating game, mussels, pork products and liver.
    - HEV inactivated with heating above 70 centigrade
  - HEV RNA moderate-to-high levels in serum and stool persisting for years
  - May have progressive liver disease with fibrosis or cirrhosis

HEV in TRANSPLANT RECIPIENTS
CHRONIC HEV INFECTION in Transplant Recipients

Pischke et. al. LIVER TRANSPLANTATION 2010
RISK AND FACTORS
HEV Chronicity in Transplant

- Total with Acute HEV Post Transplant: 85
  - 17 Centers in Europe
  - 31% Liver Transplants
- Chronicity Rate: 69.5%
- Factors in Multivariate Analysis
  - Tacrolimus (Tacro) > Cyclosporine (CyA)
  - Low Platelet Count at Time of Diagnosis of HEV

Kamar et al., GASTROENTEROLOGY 2011
HEV VIRAL QUASIDSPECIES & CHRONICITY

Design & Results

- N= 16 SOT Recipients (4 Liver/8 Kidney)
  - 8 Cleared HEV
  - 8 Developed Chronic HEV
- ORF-2 Amplified/Analyzed
  - Complexity HIGHER in those who became chronic

Lhomme et al, J VIROL 2012
RAPID DISEASE PROGRESSION AFTER OTLTx

Attributed to Acute Rejection
150 Days Post-Tx

1 Year Post-OTLTx

Schlosser et al., J HEPATOL 2012
PHYLOGENETIC in RECIPIENT : COMPARISON WITH DONOR

Schlosser et al., J HEPATOL 2012
HEV CHRONICITY
Liver Fibrosis After Bone Marrow Transplant for ALL

---

Halac et al, J PEDIATRICS 2013
Serologic Pre-Screen
N=328

- IgG+ 12.9%
- IgM+ 0.6%

HEV RNA in 138 with high ALT
HEV RNA+ N=1

HEV RNA Last sample
N=328
HEV RNA+ N=7

Confirmed HEV infection
8 (2.4%)

Versluis J: Blood 2013; 122:1079
Hepatitis E: Solid Organ Transplant

- 85 SOT pts from 16 Tx centers US and Europe
  - 68 men 17 women
  - 47 kidney, 28 liver, 2 liver/kidney, 6 kidney pancreas, 4 other
  - Age 23-77 med 48 years
- 32% sx at initial infection, resolved w/in a few days
  - Fatigue(20), diarrhea(5) arthralgia(4), weight loss(3)
  - Abdominal pain(2), pruritus(1), fever(1) nausea(1)
- 66% (56) developed chronic Hepatitis
  - Risks multi variate analysis FK >CSA, low plt
  - 22/26 LT pts
  - 8/56(14%) developed cirrhosis, 2 liver pts required ReLT

Kamar Gastro 2011;140:1481-1489
Multicenter review of 85 HEV-infected recipients in 17 Centers

CHRONIC HEPATITIS IN PATIENTS INFECTED WITH HEV AFTER SOLID ORGAN TRANSPLANTS

56/85 (66%) Chronic HEV

18 (32%) Cleared after dose reduction
20 (36%) Treated
18 (32%) Untreated

14 SVR
6 Viremic
13 Viremic
2 Died Cirrh.

CIRRHOSIS: 8/56 (14%)

Kamar, N: Gastro 2011;140:1481
Hepatitis E: Solid Organ Transplant

- HCV RNA testing most accurate for Diagnosis
- Lower level of ALT/AST rise vs immunocompetent
- No difference in ALT/AST rise chronic vs resolving

Kamar Gastro 2011;140:1481-1489
Hepatitis E in Organ Transplant Recipients (OTR)*

- Solid OTR are at risk for acute and chronic HEV infection.
- Overall prevalence: 1.8% - 11.3%
- Prevalence of chronic HEV infection defined by persistent viremia: 0-6.5% (median 0.8%)
- Only genotype 3 reported
- Most common risk factors: consumption of game and domestic meat

*Data from NIH HEV Scientific Workshop, Bethesda, 26 March 2012
Natural History of Hepatitis E in OTR*

- Acute hepatitis characterized by modest ALT elevation - median ~150 U/L (0.5-26 ULN)
- Spontaneous clearance occurs in ~40% cases
  - More frequently among those infected later after the transplantation
- Viral clearance not always associated with development of anti-HEV IgG
- Reactivation in persons previously exposed (IgG anti-HEV) does not occur - no need for special monitoring
- For those with chronic HEV infection cirrhosis can occur within 2-3 years in some cases

*Data from NIH HEV Scientific Workshop, Bethesda, 26 March 2012
HEV IN NIH HIV SOT COHORT

- 166 pre-transplant subjects
  - 113 awaiting liver transplant Including 10 dual organ candidates
  - 53 awaiting kidney transplant
- Adaltis and Wantai EIA
- ORF1-2 PCR Amplification
  - No positives at baseline
  - Stool not available

Sherman et al, J VIRAL HEP (in press)
HEV IN Solid Organ Transplant COHORT

Sherman et al, J VIRAL HEP (in press)
HEV IgG
Relationship to HCV Solid Organ Transplant Patients

Sherman et al, J VIRAL HEP (in press)
MULTIVARIATE ANALYSIS

- Relationship to...
  - Age in Kidney Recipients Only
- No relation to....
  - ALT
  - CD4
  - Geography
  - Gender

Sherman et al, J VIRAL HEP (in press)
HEV IN HIV-INFECTED PATIENTS
ACUTE HEV in HIV U.S. Military

- 4410 HIV positive persons followed for 32,468 person years
- 458 had ALT increase c/w acute hepatitis event
- 194 tested for HEV
- Conclusion: HEV is in the differential of acute hepatitis in HIV-infected patients

Crum-Cianflone et al, EMERG INF DIS, 2012
# HEV PREVALENCE IN HIV

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample Size (n)</th>
<th>Location</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maylin et al. 2012</td>
<td>261</td>
<td>Paris</td>
<td>1.5%</td>
</tr>
<tr>
<td>Kaba et al, 2011</td>
<td>184</td>
<td>Marseille</td>
<td>4.4% IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.6% IgM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.5% RNA chronic</td>
</tr>
<tr>
<td>Keane et al., 2012</td>
<td>138</td>
<td>SW England</td>
<td>9.4% IgG</td>
</tr>
<tr>
<td>Kenfak-Foguena et al, 2011</td>
<td>735</td>
<td>Switzerland</td>
<td>2.6% IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1% RNA chronic</td>
</tr>
<tr>
<td>Sellier et al, 2011</td>
<td>108</td>
<td>Paris</td>
<td>2.8% IgG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.9% IgM, RNA +</td>
</tr>
<tr>
<td>Renou et al, 2010</td>
<td>245</td>
<td>N &amp; S France</td>
<td>9.0% IgG South</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.0% IgG North</td>
</tr>
<tr>
<td>Fainboim et al. 1999</td>
<td>484</td>
<td>Argentina</td>
<td>6.6% IgG</td>
</tr>
</tbody>
</table>
CHRONIC HEV in HIV

Figure 1. Laboratory Data for a Patient with Coinfection with Human Immunodeficiency Virus (HIV) and Hepatitis E Virus (HEV).

Dalton et al., NEJM, 2009
CHRONIC HEV IN HIV
Progression to “Cryptogenic” Cirrhosis

Gurmit K. et al..
Journal of Infection 2011
HEV and CHEMOTHERAPY
Chronic Hepatitis After Hepatitis E Virus Infection in a Patient With Non-Hodgkin Lymphoma Taking Rituximab

TREATMENT OF CHRONIC HEV

- Pegylated Interferon
- Ribavirin
- Withdrawal of Immunosuppression
  - 18/56 Cleared HEV with reduced immunosuppression (Kamar et al, GASTRO, 2011)
HEV FOLLOWING LIVER TRANSPLANTATION IN CHILDREN

- 267 Liver Transplanted Children
- 22 With Chronic Graft Hepatitis
- 1 HEV Viremia

Ribavirin

Anti-HEV IgG
Negative- MP
Positive- Wantai

Junge et al, PED TRANSPLANT, 2013
# HEV Infection in Immunocompetent and Immunosuppressed Patients

<table>
<thead>
<tr>
<th></th>
<th>Immunocompetent</th>
<th>Immunosuppressed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Presentation</strong></td>
<td>Often symptomatic</td>
<td>Rarely symptomatic</td>
</tr>
<tr>
<td><strong>ALT at Diagnosis</strong></td>
<td>1000-3000 IU/L</td>
<td>100-300 IU/L</td>
</tr>
<tr>
<td><strong>HEV Genotype</strong></td>
<td>Genotype 1,2,3, or 4</td>
<td>Only Genotype 3 has been reported</td>
</tr>
<tr>
<td><strong>HEV Diagnostics</strong></td>
<td>Increase in IgM and IgG PCR (+) in 75%</td>
<td>Requires PCR Serologic testing unreliable seroconversion may not occur</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Resolving Hepatitis</td>
<td>Chronic infection in 60% (higher liver) and 10-15% develop cirrhosis</td>
</tr>
</tbody>
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AKamar Lancet 2012;379:2477-2488
Hepatitis E anti HEV testing

- All 4 genotypes elicit similar Antibody responses and represent a single serotype
  - One assay should cover all genotypes

- Tests for anti-HEV abs are available but not FDA approved

- Sensitivity and Specificity of assays widely variable

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- Sensitivity/Specificity complicated by lack of understanding of underlying HEV prevalence
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HEV RNA Testing

- RNA testing has great variability between assays without standardization
- No commercial assay and no assay approved by FDA

Study comparing RNA nucleic acid amplification (NAT) based assays from 20 labs from 10 different countries
  - 19/20 assays developed in house
  - Panels with all 4 genotypes and 2 negative samples

Bad news
  - 10 to 1,000 fold difference in sensitivities between majority of assays independent of virus strain

Baylis J Clinical Microbiology;2011;1234-1239
HEV Comparing RNA nucleic acid amplification (NAT) based assays from 20 labs from 10 different countries

- Good news
  - Specificity excellent
    - Except for one equivocal sample HEV RNA was not detected in any negative (control sample)
  - 18/20 assays detected RNA in all samples at highest concentration
    - Variability in assays was at lower concentrations
    - 2 assays with all (-) results primers directed at ORF1
  - RT-PCR was most sensitive assay independent of viral strain

Baylis J Clinical Microbiology;2011;1234-1239
HEV RT-PCR Conclusion

- RNA testing may be of limited value in acute infection given short duration in serum

- Real time-PCR targeting ORF2 or ORF3 should be accurate for diagnosis of chronic HEV

- Need standardization to more accurately characterize viral levels
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- No commercial assay and no assay approved by FDA
- Study comparing RNA nucleic acid amplification (NAT) based assays from 20 labs from 10 different countries
  - 19/20 assays developed in house
  - Panels with all 4 genotypes and 2 negative samples
- Bad news
  - 10 to 1,000 fold difference in sensitivities between majority of assays independent of virus strain

- Conclusion: send blood and stool to the US CDC for testing (RGG comments)

Baylis J Clinical Microbiology;2011;1234-1239
HEV Comparing RNA nucleic acid amplification (NAT) based assays from 20 labs from 10 different countries

- Good news
  - Specificity excellent
    - Except for one equivocal sample HEV RNA was not detected in any negative (control sample)
  - 18/20 assays detected RNA in all samples at highest concentration
    - Variability in assays was at lower concentrations
    - 2 assays with all (-) results primers directed at ORF1
  - RT-PCR was most sensitive assay independent of viral strain

Baylis J Clinical Microbiology;2011;1234-1239
HEV RT-PCR Conclusion

- RNA testing may be of limited value in acute infection given short duration in serum
- Real time-PCR targeting ORF2 or ORF3 should be accurate for diagnosis of chronic HEV
- Need standardization to more accurately characterize viral levels

Baylis J Clinical Microbiology;2011;1234-1239
Treatment

- Supportive care
- Consider ribavirin
<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Organ</th>
<th>Immunosuppression</th>
<th>Peak ALT</th>
<th>INR</th>
<th>Therapy</th>
<th>Clearance of HEV within less than</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>m</td>
<td>Liver</td>
<td>tac, mmf, decortin</td>
<td>239</td>
<td>1.3</td>
<td>Reduction of IS</td>
<td>3 months</td>
<td>SVR (follow-up &gt;2 years)</td>
</tr>
<tr>
<td>40</td>
<td>m</td>
<td>Liver</td>
<td>ciclo, mmf, decortin</td>
<td>555</td>
<td>1.0</td>
<td>Reduction of IS</td>
<td>30 months</td>
<td>SVR (follow-up &gt;2 years)</td>
</tr>
<tr>
<td>43</td>
<td>m</td>
<td>Kidney</td>
<td>tac, mmf, decortin</td>
<td>359</td>
<td>1.0</td>
<td>Reduction of IS</td>
<td>6 months</td>
<td>SVR (follow-up &gt;2 years)</td>
</tr>
<tr>
<td>65</td>
<td>m</td>
<td>Kidney</td>
<td>ciclo, mmf, decortin</td>
<td>1566</td>
<td>1.0</td>
<td>Ribavirin</td>
<td>1 month</td>
<td>SVR (follow-up &gt;2 years)</td>
</tr>
<tr>
<td>50</td>
<td>m</td>
<td>Kidney</td>
<td>ciclo, mmf, decortin</td>
<td>160</td>
<td>1.0</td>
<td>Ribavirin</td>
<td>2 months</td>
<td>SVR (follow-up &gt;5 months)</td>
</tr>
<tr>
<td>40</td>
<td>m</td>
<td>Kidney</td>
<td>ciclo, mmf</td>
<td>342</td>
<td>1.1</td>
<td>Ribavirin</td>
<td>2 months</td>
<td>SVR (follow-up &gt;4 months)</td>
</tr>
<tr>
<td>54</td>
<td>m</td>
<td>Kidney</td>
<td>ciclo, sirolimus</td>
<td>2053</td>
<td>1.1</td>
<td>Ribavirin</td>
<td>1 month</td>
<td>SVR (follow-up &gt;4 months)</td>
</tr>
<tr>
<td>50</td>
<td>f</td>
<td>Heart</td>
<td>ciclo, decortin, everolimus</td>
<td>217</td>
<td>1.0</td>
<td>Ribavirin</td>
<td>2 months</td>
<td>SVR (follow-up &gt;2 years)</td>
</tr>
<tr>
<td>66</td>
<td>f</td>
<td>Heart</td>
<td>ciclo, decortin, everolimus</td>
<td>209</td>
<td>1.1</td>
<td>Ribavirin</td>
<td>1 month</td>
<td>SVR (follow-up &gt;2 years)</td>
</tr>
<tr>
<td>57</td>
<td>m</td>
<td>Heart</td>
<td>ciclo, decortin, azathioprine</td>
<td>211</td>
<td>1.1</td>
<td>Ribavirin</td>
<td>1 month</td>
<td>SVR (follow-up &gt;2 years)</td>
</tr>
<tr>
<td>58</td>
<td>m</td>
<td>Heart</td>
<td>tac, decortin, everolimus</td>
<td>315</td>
<td>1.1</td>
<td>Ribavirin</td>
<td>No clearance</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>f</td>
<td>Lung</td>
<td>tac, mmf, decortin</td>
<td>89</td>
<td>1.4</td>
<td>Ribavirin</td>
<td>2 months</td>
<td>SVR (follow-up &gt;2 months)</td>
</tr>
<tr>
<td>56</td>
<td>f</td>
<td>Lung</td>
<td>ciclo, mmf, decortin</td>
<td>254</td>
<td>1.1</td>
<td>Ribavirin</td>
<td>2 months</td>
<td>SVR (follow-up &gt;7 months)</td>
</tr>
<tr>
<td>32</td>
<td>m</td>
<td>Lung</td>
<td>ciclo, mmf</td>
<td>270</td>
<td>1.0</td>
<td>Ribavirin</td>
<td>No clearance</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>m</td>
<td>Lung</td>
<td>tac, mmf, decortin</td>
<td>215</td>
<td>1.6</td>
<td>No therapy</td>
<td>No clearance</td>
<td>Patient died before diagnosis of HEV infection (retrospectively identified)</td>
</tr>
</tbody>
</table>

**VIRAL HEPATITIS**

**Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience**

Sven Pischke\(^1,2\), Svenja Hardtke\(^1\), Ulrike Bode\(^3\), Stephan Birkner\(^4\), Christos Chatzikyrkou\(^5\), Wolfgang Kauffmann\(^6\), Christoph L. Bara\(^7\), Jens Gottlieb\(^7,8\), Juergen Wenzel\(^9\), Michael P. Manns\(^1,2\) and Heiner Wedemeyer\(^1,2,\*)
Fig. 1. Course of bilirubin, ALT and INR in a patient with acute hepatitis E, treated with ribavirin for 6 weeks.
Fig. 2. One-year follow-up (from the beginning of ribavirin treatment) of ALT levels in transplant recipients treated with ribavirin. Patient HTR 4 suffered from viral breakthrough. Patient LuTR 3 died from non liver or therapy-associated death.

VIRAL HEPATITIS

Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience

Sven Pischke¹,², Svenja Hardtke¹, Ulrike Bode³, Stephan Birkner⁴, Christos Chatzikyrkou⁵, Wolfgang Kauffmann⁶, Christoph L. Bara⁷, Jens Gottlieb²,⁸, Juergen Wenzel⁹, Michael P. Manns¹,² and Heiner Wedemeyer¹,²,†
# Table 1. Treatment of Patients With Chronic HEV Infection

<table>
<thead>
<tr>
<th>Group of patients</th>
<th>Treatment</th>
<th>Outcomes</th>
<th>First author, year</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 Liver and kidney transplantation patients with chronic HEV infection</td>
<td>Reduction of immunosuppression</td>
<td>4 of 16 patients HEV RNA–negative</td>
<td>Kamar, 2011(^78)</td>
</tr>
<tr>
<td>56 Liver and kidney transplant recipients with chronic HEV infection</td>
<td>Reduction of immunosuppression</td>
<td>18 of 56 patients HEV RNA–negative</td>
<td>Kamar, 2011(^63)</td>
</tr>
<tr>
<td>3 Liver transplant recipients with chronic HEV infection</td>
<td>3-month course with pegylated interferon-alfa-2a</td>
<td>2 of 3 patients cleared HEV RNA, 1 relapsed after treatment</td>
<td>Kamar, 2010(^79)</td>
</tr>
<tr>
<td>2 Liver transplant recipients with chronic HEV infection</td>
<td>16 weeks or 1 year of treatment with pegylated interferon-alfa-2b</td>
<td>2 of 2 patients cleared HEV RNA</td>
<td>Haagsma, 2010(^80)</td>
</tr>
<tr>
<td>1 HIV-infected patient with chronic HEV infection</td>
<td>6 months pegylated interferon monotherapy, followed by 12 weeks of therapy with the combination of interferon and ribavirin</td>
<td>Patient tested negative for HEV RNA</td>
<td>Dalton, 2011, Ann Intern Med(^58)</td>
</tr>
<tr>
<td>7 Recipients of solid organ transplants</td>
<td>Treatment with ribavirin monotherapy for 5 months</td>
<td>6 of 7 patients cleared the virus, and 1 is still a carrier of HEV</td>
<td>Unpublished data from our group</td>
</tr>
<tr>
<td>6 Recipients of solid organ transplants</td>
<td>Treatment with ribavirin monotherapy for 3 months</td>
<td>4 of 6 patients achieved sustained virologic response, 2 relapsed</td>
<td>Kamar, 2010(^82)</td>
</tr>
<tr>
<td>9 Patients with various conditions of immunosuppression</td>
<td>Treatment with ribavirin monotherapy for 3 months</td>
<td>9 of 9 patients cleared the virus, no relapse</td>
<td>Mallet, 2010, AASLD Annual Meeting(^56)</td>
</tr>
</tbody>
</table>

AASLD, American Association for the Study of Liver Disease.
RIBAVIRIN THERAPY FOR HEV in Renal Tx recipients

CHRONIC HEV (36.5 Months) Renal Tx Recipients N=6

Ribavirin 600-800 mg/day 3 Months Total Treatment

HEV RNA Undetectable N=6

SVR N=4

RELAPS E N=2

Kamar et al, GASTROENTEROLOGY, 2010
HEV: Remember to Consider Dx

- Acute Hepatitis after Travel to under developed areas
- US or European patients with no travel History
  - Acute Hepatitis in Non A-C Hepatitis including those with possible DILI
  - Acute on Chronic Liver failure
  - Chronic or acute hepatitis in immunosuppressed patients
- Cant diagnose if you don’t Consider
Hepatitis E Vaccines*

- In animal studies, several truncated recombinant HEV capsid protein have been found to induce specific a antibodies, and to protect against liver injury following subsequent challenge with homologous and heterologous strains of the virus.

- An HEV DNA vaccine has also been shown to induce serum anti-HEV antibodies in cynomolgus macaques, and protect against a heterologous challenge.

*Aggarval R., JGH 2011; 26; Suppl. 1
Recombinant Hepatitis E Vaccines*

- The first human vaccine contained VLPs made up of a 56-kD truncated genotype 1 HEV ORF2 protein (aa 112–607) produced in Spodoptera frugiperda cells infected with a recombinant baculovirus.
  - Ph II-III: 20ug administered to 2000 Nepalese soldiers at 0, 1, 6 m.
  - Efficacy rate was dose dependent: 3-doses – 95%; 2-doses – 86%
- The second vaccine- HEV 239 vaccine, contains a more truncated HEV capsid protein (aa 368–606) expressed in Escherichia coli
  - Ph II-III: 30ug administered to 113,000 volunteers in China at 0, 1, 6 m.
  - Efficacy rate was not dose dependent: 3 and 2-doses – 100%
  - The Chinese vaccine has been shown to provide protection against genotype 4 HEV infections, even though it is based on genotype 1 virus

*Aggarval R., JGH 2011; 26; Suppl. 1
Hepatitis E Vaccine Application*

- Whether HEV vaccines should be used for the general population in highly endemic areas will depend on:
  - cost considerations,
  - the duration of protection afforded by the vaccines and
  - need for booster doses and the ability of the vaccines to interrupt transmission of infection.

- Neither vaccine has currently reached the market.

*Aggarval R., JGH 2011; 26; Suppl. 1
Prevention

- Recombinant HEV vaccine (GSK)
  - Phase 2 study in Nepalese Army units (n=2,000)
  - Vaccine/Placebo given at 0, 1, and 6 months

\[ n = 3 \ (0.3\%) \]
\[ n = 66 \ (7.4\%) \]

Shrestha NEJM 2007;356:895
Prevention

- Recombinant HEV vaccine (Innovax, China)
  - Phase 3 study in China (n=112,604)
  - Vaccine/Placebo given at 0, 1, and 6 months

- No safety concerns

Zhu Lancet 2010;376:895
Blood Donor Testing

- The next phase?
Clinical Course of Transfusion-Transmitted HEV: First case in Japan

<table>
<thead>
<tr>
<th>Donor</th>
<th>ALT</th>
<th>HEV RNA</th>
<th>IgM α-HEV</th>
<th>IgG α-HEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior Donation</td>
<td>11</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Index Donation</td>
<td>10</td>
<td>Pos*</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>F/U @ 5 months</td>
<td>8</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
</tr>
</tbody>
</table>

* Complete sequence identity with recipient (Gt 4)

6/18 donors unrelated to this case who had ALT >500 were HEV RNA+; 5/6 IgM+
Transfusion-Transmitted HEV: First Case in England

Donor: Flu Sx 14d post-donation; ALT 2050 @24d; donation IgM+ HEV RNA+ Gt3; sequence ident.

Patient: Lymphoma on chemo

AST (IUL-1)

Days since transfusion

Boxall, E. Transfusion Medicine 2006
**HEV MARKERS IN NIH VOLUNTEER BLOOD DONORS**

<table>
<thead>
<tr>
<th>No. Tested</th>
<th>Anti-HEV IgG⁺</th>
<th>Anti-HEV IgM⁺</th>
<th>HEV RNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1939</td>
<td>364 (18.8%)*</td>
<td>8 (0.4%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

* 95% confidence interval [CI], 17.0%-20.5%

* Donor HEV RNA: Scotland: 1/14,520; Sweden: 1/7986; Ger: 1/4525; Japan: 1/8185
Prevalence of anti-HEV IgG in 916 NIH volunteer blood donors by age group.

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>n</th>
<th>% Anti-HEV +</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-35</td>
<td>179</td>
<td>3.4</td>
</tr>
<tr>
<td>36-45</td>
<td>163</td>
<td>12.9</td>
</tr>
<tr>
<td>46-55</td>
<td>260</td>
<td>22.3</td>
</tr>
<tr>
<td>56-65</td>
<td>224</td>
<td>34.4</td>
</tr>
<tr>
<td>&gt;65</td>
<td>90</td>
<td>42.2</td>
</tr>
</tbody>
</table>

Apparent cumulative exposure to HEV over time.
**Blood Transmitted HEV in Endemic Area (Khuroo M. J GastroHep 2004;19:778)**

**Retrospective Study**

- **145 Multiply-Tx**
  - 13 (9%) IgM+ HEV RNA+
  - 2 (0.8%) IgM+ HEV RNA+
  - $P < .001$ OR = 12

- **250 Non-Tx**

**Prospective Study**

- **25 Tx**
  - 3/22 (13.6%) Suscept. HEV Infected
- **25 Non-Tx**
  - 0/25 Suscept. HEV Infected

**Retro: Tx <3 Mos. Pre-test**

- **13/30 (43%) HEV RNA+**
  - $P < .001$ OR = 37

- **1/50 (2%) HEV RNA+**

Traced to 4 donors HEV RNA+; IgM+
Seroprevalence and Incidence of HEV Infection in German Blood Donors

- 84/1019 (8.2%) IgG anti_HEV+
- 69 (6.8%) Confirmed WB

Archived samples (<2 yr.) available from 58

- 7 (0.7%) Anti-HEV Seroconversions; 3/7 HCV RNA+ in one sample

Juhl, D: Transfusion 2013
** Linked donor testing and serial recipient testing shows passive transfer of anti-HEV in one patient and low-level pre-existing infection in the second

** Upper bound of zero observed transmissions is 0.8%
COURSE OF ANTI-HEV IgG EVOLUTION IN A PROSPECTIVELY FOLLOWED SEROCONVERTING TRANSFUSION RECIPIENT

72U RBC/Plat; 69 tested

Recipient: No IgM anti-HEV or HEV RNA

4 days prior to last study sample, received RBC from hi-titer IgG+ donor and 2nd unit from HEV RNA+ donor
Should blood donors routinely be screened for evidence of HEV infection?
Asymptomatic Viremia

Test or Not?

Significant Clinical Disease

Proven Transfusion-Transmission
Caveats to Implementing HEV Donor Screening at This Time

- Currently no HEV standards or pedigreed panels by which to compare assay sensitivity and specificity
- HEV screening will require licensed assay for HEV RNA; no such assay in pipeline
- The frequency and duration of asymptomatic viremia in immunocompetent donors is unknown and this is main determinant in the risk equation
- The minimal infectious dose and the frequency with which that dose might be exceeded in healthy blood donors is unknown
- The frequency of clinically significant infections in immuno-competent patients not established
- Large prospective studies in recipients needed but difficult and costly
HEV

Ephemeral?