Hepatitis E
Yesterday, Today and Tomorrow

Ellie Barnes
Disclosures

- Nothing to disclose
Presentation outline

The Virus and Epidemiology
Clinical disease
Vaccine development
Immune control
Conclude with the mysteries of hepatitis E
How it all began.....

• **In 1978**
  - 52,000 infected non-A/B hepatitis in Kashmir valley -1700 deaths

• **Unique features of the epidemic**
  - Compressed epidemic, no secondary waves infection,
  - Cholestasis in 20%
  - Young adults-pregnant women more severe disease
  - Distinct histology-bile plugs in canaliculi and pseudo-ductules.

• **Only recognised as a distinct disease in 1983**
  - Balayan et al inervirology 1983
  - Pooled stool extract from patients (soviet military in Afghanistan) with non-A/B hepatitis to a healthy volunteer (the first author)
  - 30nm virus particles in stool
India Hepatitis E outbreak kills 12 in Maharashtra

An outbreak of Hepatitis E has killed at least 12 people and left 4,089 others unwell, officials in the western Indian state of Maharashtra said.

The outbreak happened in Ichalkaranji, a city of 300,000 people.

 Officials say several hundred patients have been treated and discharged since 15 May, while another 200 patients are under medical care in hospitals across the city.

More than 70 pregnant women have also been hit by jaundice so far, they said.

HEV still kills 10,000 people/year
Hyper endemic regions

Khuroo et al Virus Research 2011
HEV genomic map; three open reading frames

- 7 more years to sequence the virus (Reyes et al Science 1990)
- Positive strand RNA virus, 7.3kb, non-enveloped virus

<table>
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<th>ORF 1</th>
<th>0 nucleotides - 5353 nucleotides</th>
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- ORF1 encodes methyltransferase, protease, helicase and RdRp
- ORF2 encodes capsid protein (involved in virion assembly)
- ORF3 encodes small protein which may act as viral accessory protein and associates with cells cytoskeleton

Adapted from Ahmed I et al Virus research 2011
Viral Genotypes

- Four dominant genotypes (80% homology) and subtypes
- **Genotypes 1 and 2; cause of epidemics in Asia and Africa**
  - developing countries-waterborne
- But......increasing awareness that HEV also a disease of the West (several hundred cases/year in UK)
- **Genotypes 3 and 4;** locally acquired in USA, Asia, Europe
  - Zoonosis-meat poorly cooked, or contact with animals.
  - HEV; 10% pork sausages available to the public in the UK
  - Sewage and rivers Europe and Asia
- **Prevalence depends on assay used to detect antibodies...**
# Prevalence of HEV exposure

<table>
<thead>
<tr>
<th>Country of study</th>
<th>Seroprevalence %</th>
<th>Year of publication</th>
<th>Subjects studied (n)</th>
<th>Sex ratio m/f</th>
<th>Median Age</th>
<th>Laboratory test used</th>
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*na*, data not available.

*Table adapted from Kaufmann et al Plos One June 2011*
France many of the best things in life.......but

at a cost........

- **Toulouse (Midi-Pyrenees)**
  - 52.5% adult blood donors HEV IgG+ (Wantai assay)
  - 80% hunters, 70% people over 58 years old
  - 44% Toulouse sausages-HEV RNA+
    - Mansuy et al; Emerging Infectious Diseases-Dec 2011
  - Higher here also in the liver transplant population
Hepatitis E viral genotypes and animal species

Raw sequence data courtesy of Prof. Peter Simmonds, Edinburgh
Clinical presentation

- Incubation 3-8 weeks; average 40 days
- From asymptomatic to fulminant liver failure
- Acute infection
  - High mortality- (25%) in pregnant women
  - High mortality in those with chronic liver disease
    - Hamid S et al Hepatology 2002
HEV in pregnancy

- Studies assessed acute hepatitis and fulminant liver failure in pregnant versus non-pregnant women
  - Pregnancy associated with a higher HEV viral load
  - Prolonged period of HEV viraemia (day 15; 88.3% pregnant vs 27.6% non-pregnant viraemic \( P<0.001 \))
    - Begum, N. International journal of gynaecology and obstetrics 2010
- Foetal outcomes also poor
- Mechanism of severe disease;
  - TH2 response in pregnant women (altered immune profile)
  - Hormones of pregnancy-facilitate viral replication
HEV—a new disease

- 2008-chronic disease in solid organ transplant recipients
  - New disease for an old virus
  - Poster lost amongst the many at AASLD......(!)
HEV causes persistent infection and liver fibrosis

- Chronic Hepatitis E in solid organ transplant recipients
  - Kamar N et al; NEJM 2008 (Toulouse group)
  - 215 elevated LFTs screened
  - Livers, kidneys, kidneys/pancreas transplants
  - 14 patients with acute HEV infection (6.5%)-8/14 chronic
  - Lower CD4 counts in those that became chronic

- Since described in heart transplantation
  - S Pischke et al Am J Transpl July 2012

- Treatment-reduce immunosuppression, ribavirin monotherapy

- HIV + people
  - Single case HEV RNA+, HIV+, cirrhosis.
    - Dalton HR et al; NEJM 2009
  - Higher seroprevalence, (123 men, 6 HEV IgG+) nil RNA +
    - Pischke J V Hepatitis 2010
Factors associated with HEV persistence

- 17 centres retrospective European study

- 85 solid organ transplant patients infected with HEV, (52 from Toulouse)
  - HEV persistence in 56 patients (66%)
    - Kamar N, et al Gastroenterology 2011;140:1481-1489

- Factors associated with persistence:
  - Tacrolimus rather than Cyclosporin A
  - Low platelet count at the time of diagnosis with HEV
  - Lower ALT at time of infection

- 18 (32.1%) achieved viral clearance after the dose of immunosuppressive therapy was reduced

- No HEV reactivation was observed after HEV clearance

- Serology: high false negative rate

- ALT typically 1-300 IU/mL
Occult HEV-a silent chronic carrier state?

- Chronic carrier state-HEV Abs negative, HEV RNA positive

- HEV prevalence studies in wild boar
  - Anti-HEV seroprevalence 25-30%
  - HEV RNA was found to be positive (bile) 68% of the animals.

- Humans; case report OLT NASH-cirrhotic within 5/12
  - Donor HEV RNA neg in blood, RNA positive in liver
    - B. Schlosse et el; J hepatology Jan 2012

- Reactivation of HEV in acute lymphoblastic leukaemia stem cell transplant patient
  - le Coutre P et al GUT 2009
Vaccines for HEV

- Two vaccines for HEV
- Recombinant proteins based on HEV capsid
- Phase-II/III clinical studies

ORF 2 - 660aa

5395 7375
Vaccines for HEV (1)

- rHEV vaccine
  - M Shrestha et al NEJM 2007
  - 1794 Nepalese soldiers
  - 3 i.m. vaccine doses months 0, 1 and 6 vs placebo
  - 69 acute HEV; 66 in placebo group
  - Efficacy of 96%

- Development is not being pursued - Why?

- Developed as a military vaccine
  - Recently in Afghanistan - where soldiers drank bottled water only 1 case HEV.
Vaccines for HEV (2)

- HEV vaccine 239-antigen adsorbed onto Al Hydroxide
  - Zhu et al Lancet 2010-tested in large phase III study
  - genotype-1 HEV strain-produced in bacterial cells
  - 10,000 Chinese adults (phase II safe and immunogenic)
  - Placebo controlled
  - 3 i.m. injections (at 0, 1, and 6 months vs. placebo)

- 15 cases in placebo gp 13 months after the last vaccine
  - (12 genotype-4)
- No infections in vaccine group
- 100% efficacy!
So isn’t it all sorted? -not really……

- No data in anyone but healthy adults
  - pregnant women, children, people with chronic liver disease

- Kinetics Ab response-long term protection?
  - Which titres are protective.
  - In phase-II study Shrestha et al- 40% lost anti-HEV abs within 600 days of last vaccine

- Long term safety-link between hepatitis E and AI hepatitis (?)

- Chinese FDA approved Jan 2012 (Hecolin)
  - no approval in Europe and USA
  - unlikely when above issues not addressed. GSK planning development?
CD4+ T cell count in immunosuppressed patients - associated with persistence

Rapid loss of Ab responses over time in natural infection (and after vaccination).
- 26-67% undetectable Abs 2 years after infection
- 4.5% detectable Abs after 30 years
  - Khuroo et al virus research 2011

? T cell immunity and viral control
Collecting samples in Cornwall

- Cohort 20 patients
- 12 men, 8 women
- Age range 38-92 years (Mean 68.6)
Identifying HEV T cell targets

ORF 1 - 1771aa

ORF 2 - 660aa

ORF 3 - 116aa

Negative control  HEV Ags  FEC
T cell immunity to HEV-associated with viral control

Within 12 months of primary infection

Structural proteins dominant T cell targets (not true for HCV)

Time since date of clinical presentation with primary infection
T cell immunity to HEV-associated with viral control

Within 12 months of primary infection

More than 12 months after primary infection

0 months 12 months 12 years

Time since date of clinical presentation with primary infection

Structural proteins dominant T cell targets associated with viral control
(not true for HCV)
Wane over time - partially
T cell function and HEV

**CD8s**

- HEV101 (pool M peptide)

<table>
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<tr>
<th></th>
<th>MIP-1-B</th>
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**CD4s**

- HEV103 CD4 response to pool M

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Polyfunctional CD8+ T cell response

CD4+ T cells rarely detected
The mysteries of hepatitis E (at least 7)

- Prevalence and incidence unknown
  - Diagnostic tests?

- Source in developed countries often unclear?

- High mortality in pregnant women
  - Why?

- Paediatric paradox; children <10 years old spared infection

- Causes persistent infection
  - In whom, how and why?

- Repeated epidemics in exposed regions
  - ? long term immune protection

- Efficacious vaccine available for use?
Summary - Yesterday, Today and Tomorrow

• Yesterday
  • Like hepatitis A-disease in developing countries

• Today
  • Persistent disease-failure of T cell immunity
  • ? Occult infection
  • Vaccination possible

• Tomorrow
  • Vaccine-at risk populations West and Asia/Africa
  • Screen organ transplant-donors and recipients
  • Better understanding of disease susceptibility
Acknowledgements

Team at Truro
- Harry Dalton
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- Peter Simmonds-Edinburgh

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- Abby Harrison
- Leo Swaddling
- Annie Lissington
- Denise O’Donnell
- Lizzie Stafford
- Paul Kleenerman
- Jane Collier
- Katie Jefferies
- Stephen Jubb
HEV and autoimmunity? watch this space

- HEV and autoimmune hepatitis
  - recent posters
- HEV and other autoimmune diseases.....
37 male. Referred to A/E by GP
Tired and lethargic for 8 days
Aches in biceps and cramps in arms
PMH –nil
ETOH-minimal
Holiday in Rhodes 1 month previously-with partner
Examination unremarkable
Investigations

- US abdomen; normal-spleen slightly enlarged.
- Hepatitis A IgG pos, IgM neg.
- HIV, Hep C, EBV, CMV, and HBV negative.
- LFTs steadily normalised
- Hep E sent by micro...+
- Diagnosis – Type-I diabetes and acute Hepatitis E
- ?? coincidence
Former Canadian prime minister Brian Mulroney diagnosed with diabetes

Globe and Mail newspaper

Mulroney's health took a turn after returning from Istanbul....

During the trip, he contracted hepatitis E after consuming something, which affected his liver's processing of natural insulin and ultimately led to the diabetes, he told the newspaper.

"In my case, it (the liver!) stopped feeding insulin," he said.....I was a full-blown diabetic.”

Mulroney is reportedly giving himself three daily injections of insulin, but he added: "I feel terrific."
• Anti-HEV IgG assays vary: Seroprevalence estimates vary widely

• 1998; 5 human cases- assays varied widely

• WHO standard-established in 2002

• anti-HEV IgG drop out

• Common assay underestimates prevalence x4.
  • Using more sensitive assay show that anti-HEV prevalence is much higher than previously recognised