Hepatitis E:

E is for Elusive

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- Harvey Alter
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- Ray Kim

Lecture

- Cases
- Clinical Presentation
- Genotypes
- Human and animal hosts
- Testing and epidemiology
- Treatment

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 Chronic renal dysfunction
  - DM type 2 Hypercholesterolemia
  - HTN Hypothyroidism
  - GERD Gout
- 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

Time After starting	Time After stopping	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Other
7 wks	3 wks	235	210	10.6	*Admission*
	3.3 wks	167	165	9.1	Discharge
	3.5 wks	130	159	8.6	
8 wks	4 wks	92	202	8.5	
9 wks	5 wks	53	139	6.1	–
10 wks	6 wks		153	3.4	
12 wks	8 wks		134	1.7	
14 wks	10 wks	43	121	1.1	
8 mos	7 mos	55	85	0.7	–
Normal		*<35*	*<125*	*<1.2*	

- Dx Highly likely DILI from allopurinol.

Davern Gastroenterology 2011;141:1665-1672

Hepatitis E Case 1

- Serologic testing After Discharge

Time After starting	Time After stopping	ALT (U/L)	Alk P (U/L)	Bilirubin (mg/dL)	Other
7 wks	3 wks	235	210	10.6	*Admission*
	3.3 wks	167	165	9.1	
	3.5 wks	130	159	8.6	
8 wks	4 wks	92	202	8.5	
9 wks	5 wks	53	139	6.1	**HEV Anti IgM(+)/IgG(+) RNA (-)**
10 wks	6 wks		153	3.4	
12 wks	8 wks		134	1.7	
14 wks	10 wks	43	121	1.1	
8 mos	7 mos	55	85	0.7	**HEV anti IgG(+) IgM(-)/RNA (-)**
Normal		*<35*	*<125*	*<1.2*	

- Diagnosis HEV
- FU 6 months later no sxs 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

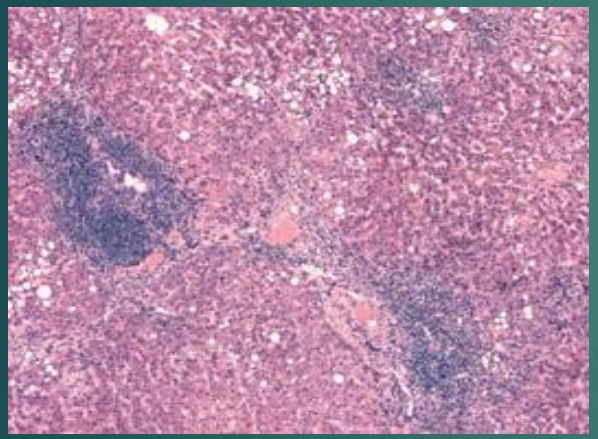
	Baseline	Peak
ALT(iu/l)	112	2328
Bili(mg/dl)	2.5	35
INR	1.1	2.0

- Laboratory testing on admit
  - HEV IgM(+), IgG (-), RNA (+) Genotype 3
- Hospitalized 14 days with recovery

De Silva Digestive and Liver Disease 2012;44:930-934

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 (+)

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

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

Halleux Trans Infectious Disease 2012;14:99-102

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
  - include 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

Hoofnagle NEJM 2012, Davern Gastro 2011

*au·toch·tho·nous ôˈtäkTHənəs/ *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 under-cooked 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

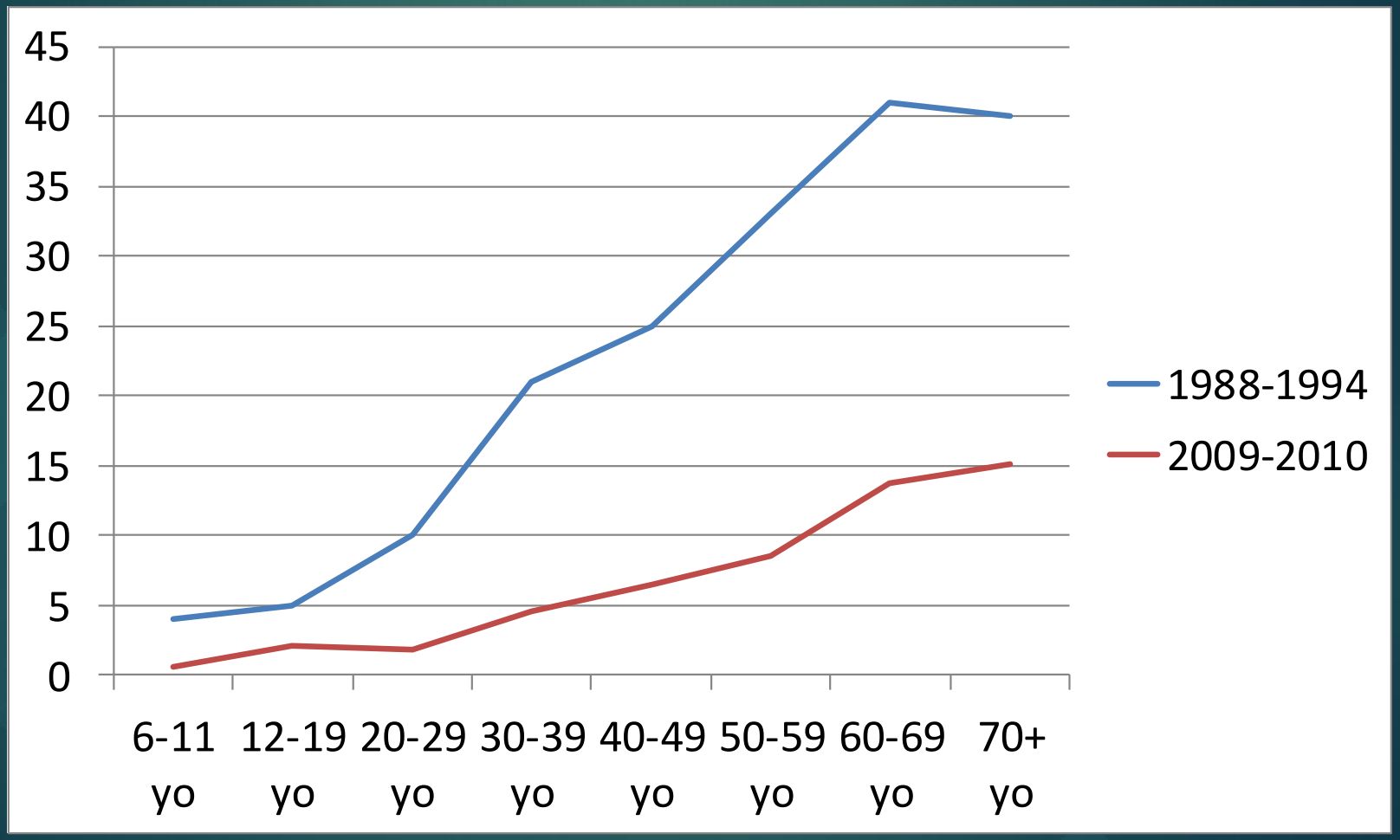
- Clinically indistinguishable

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

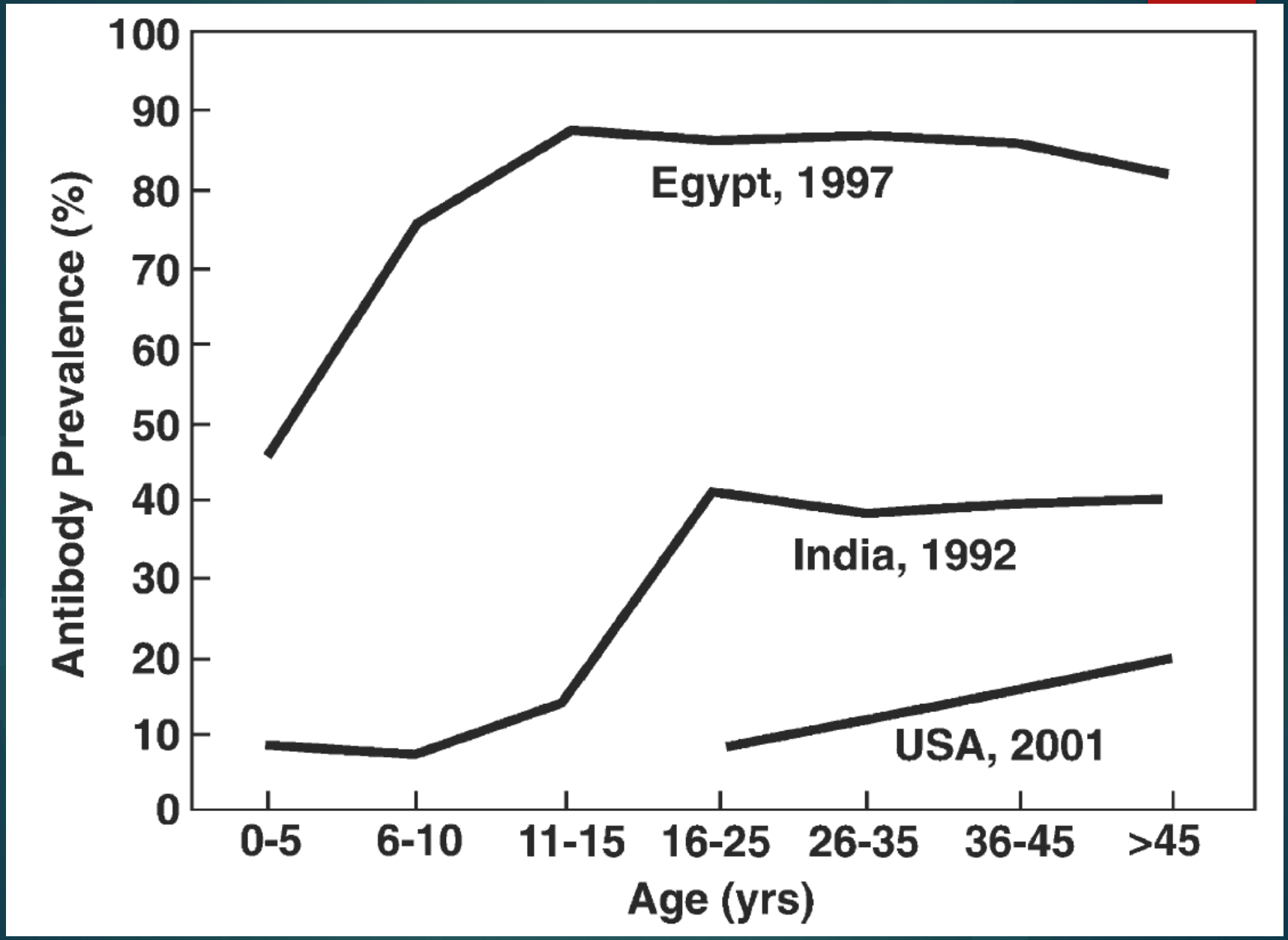
- Virological characteristics:
  - HAV much more stable in the environment
  - >x100 higher fecal titer

Emerson. NEJM 2004;351:23, Percell. J Hep 2008;48:494

Comparison of NHANES III and IV
1988-94 (N= 18,695)
2009-2010 (N= 7885)



HEV Prevalence: 3 countries



Percell. J Hep 2008;48:494

HEV in NHANES Data

- Overall Anti-HEV Positivity: 21.0%

Variable		Prevalence
Sex	Female	20.4%
	Male	21.6%
Race/Ethnicity	Whites, NH	22.1%
	Black, NH	14.5%
	Mexican	20.3%
Country of Birth	US	20.1%
	Mexico	30.9%
	Other	26.2%
Region	South	14.7%
	Northeast	20.8%
	Midwest	26.6%
	West	25.0%

Kuniholm. JID 2009;200:49

HEV Epidemiology in the US

- NHANES III
  - Cross-sectional sample representative of general civilian household (1988-1994)
  - 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

Kuniholm. JID 2009;200:49

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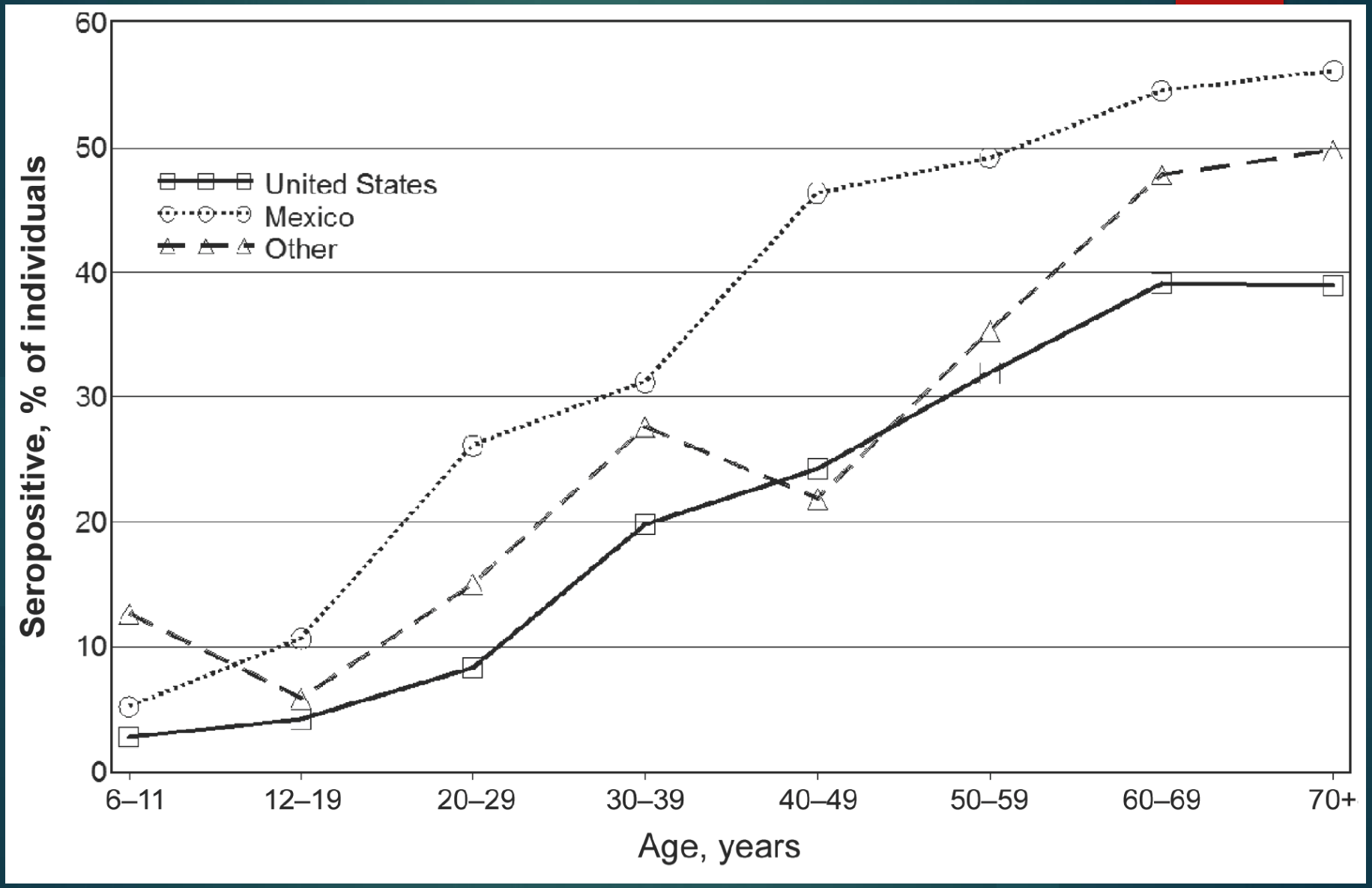
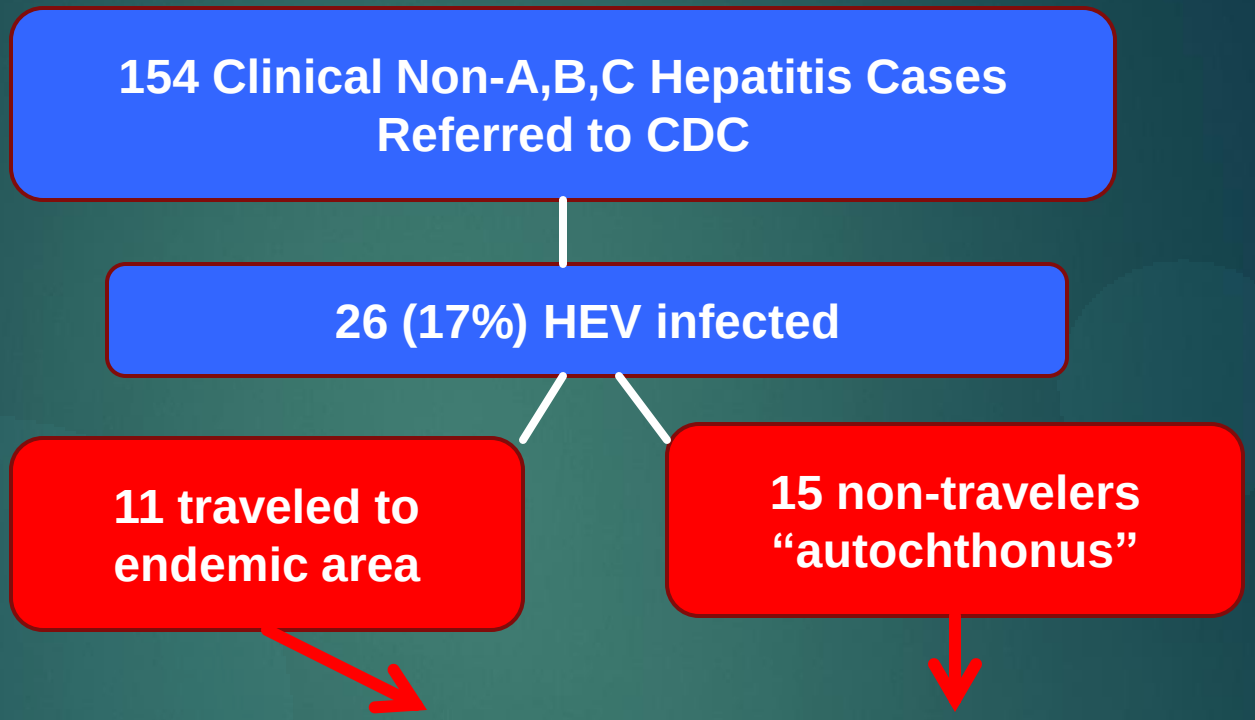
Kuniholm. JID 2009;200:49

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



Mean Age	32	61
Icteric	92%	47%
Organ Transplant	0	47%
Gt3	0/4	8/8
Fulm. Hep Failure	0	3

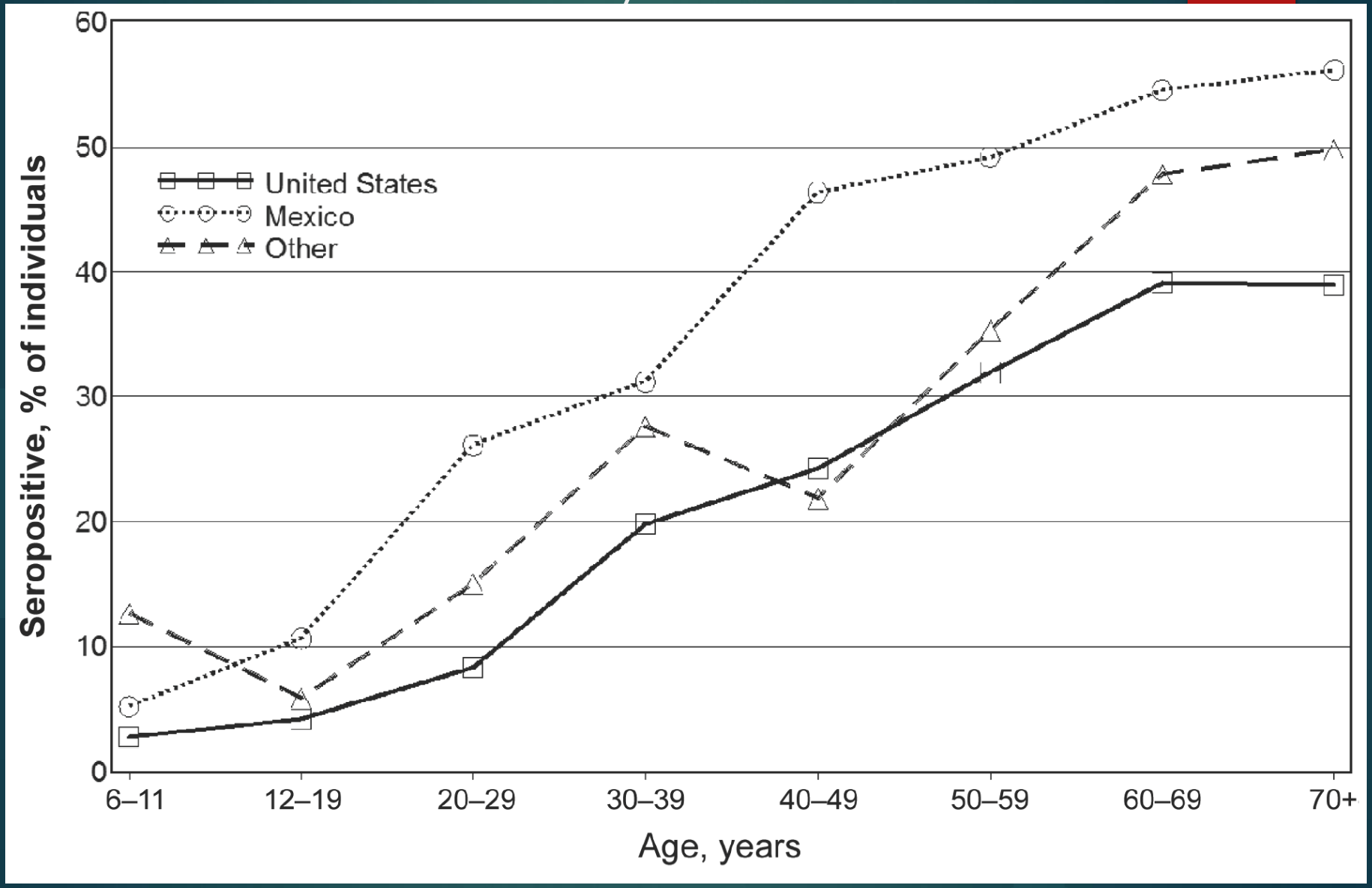
What is the National Seroprevalence of anti-HEV?

- 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

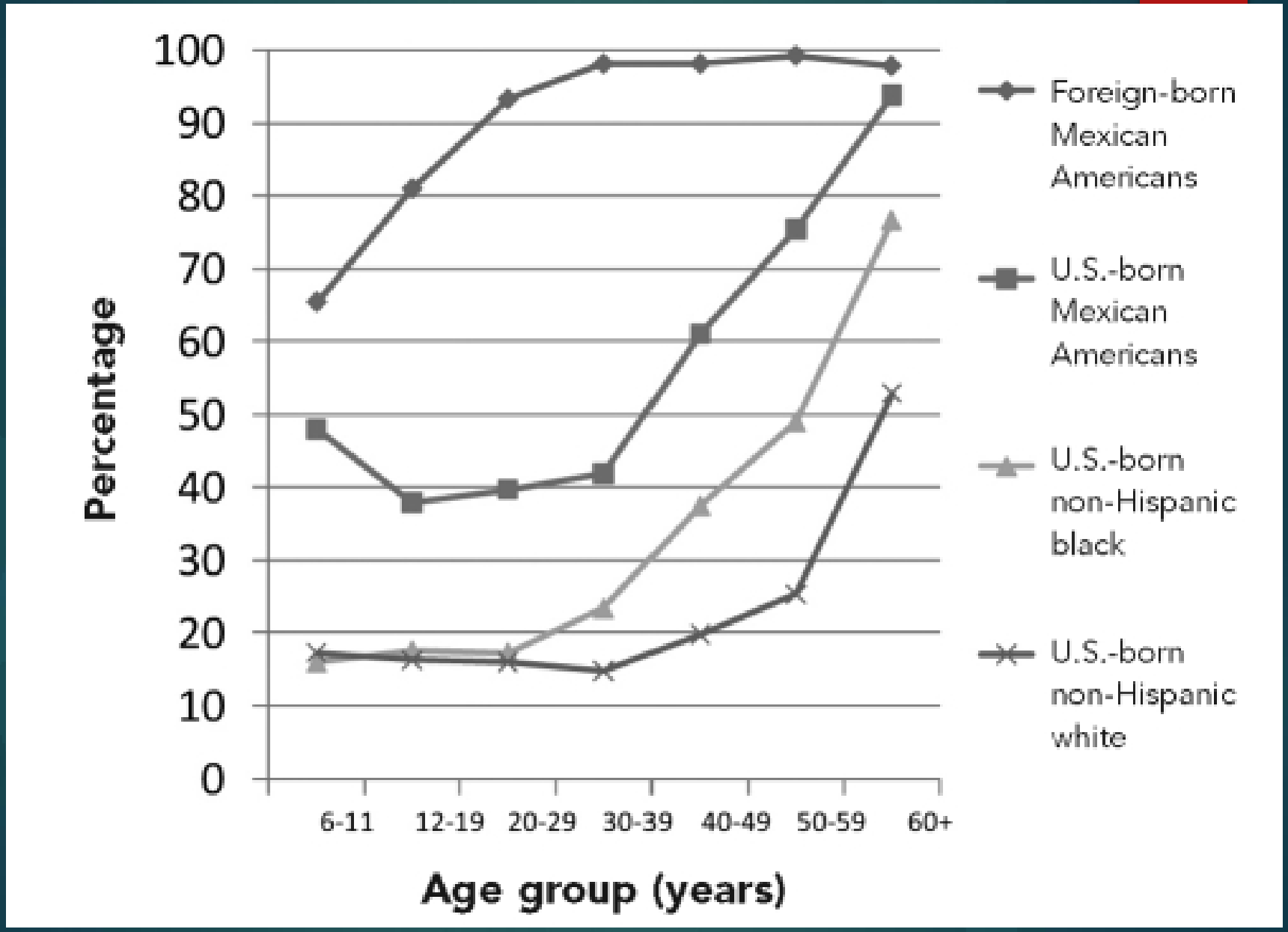
** Kuniholm et al, J Infect Dis 2009; 200:48-56*

Prevalence of HEV by Place of Birth



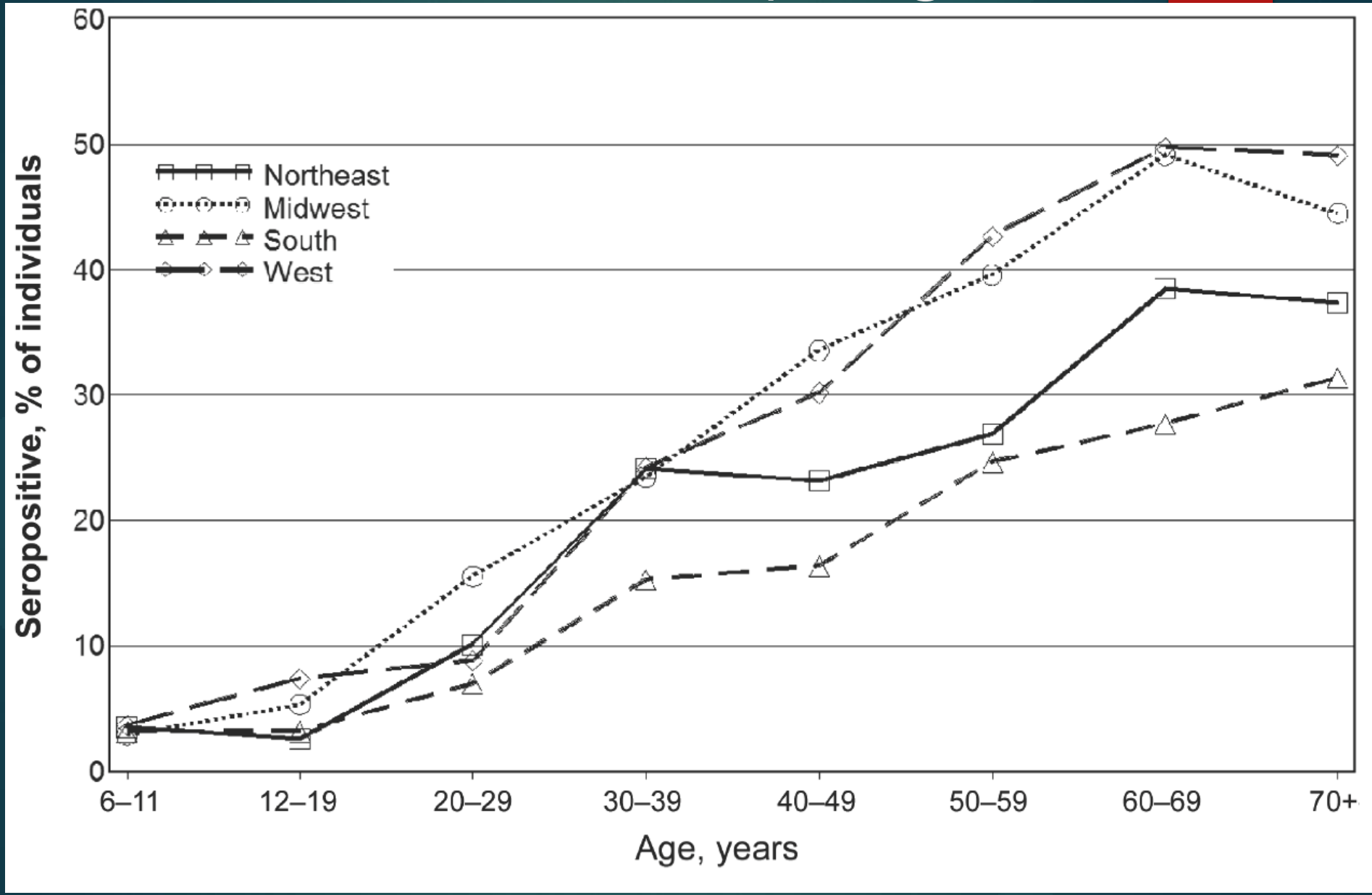
Kuniholm. JID 2009;200:49

Prevalence of HAV by Place of Birth



Klevens. Public Health Rep 2011;126:522

Prevalence of HEV by Region



Kuniholm. JID 2009;200:49

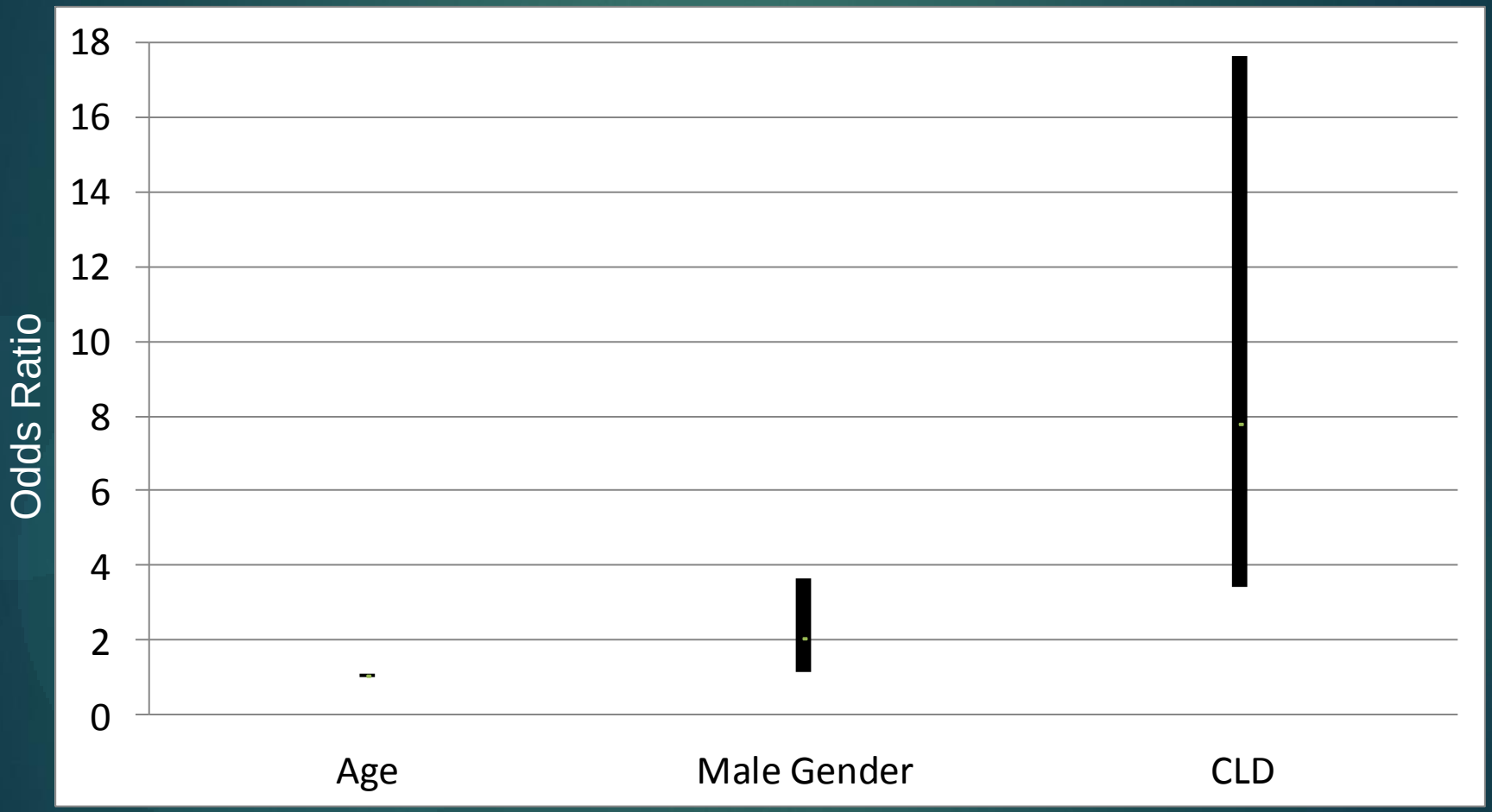
Risk Factors for HEV*

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

*US-born subjects only

Kuniholm. JID 2009;200:49

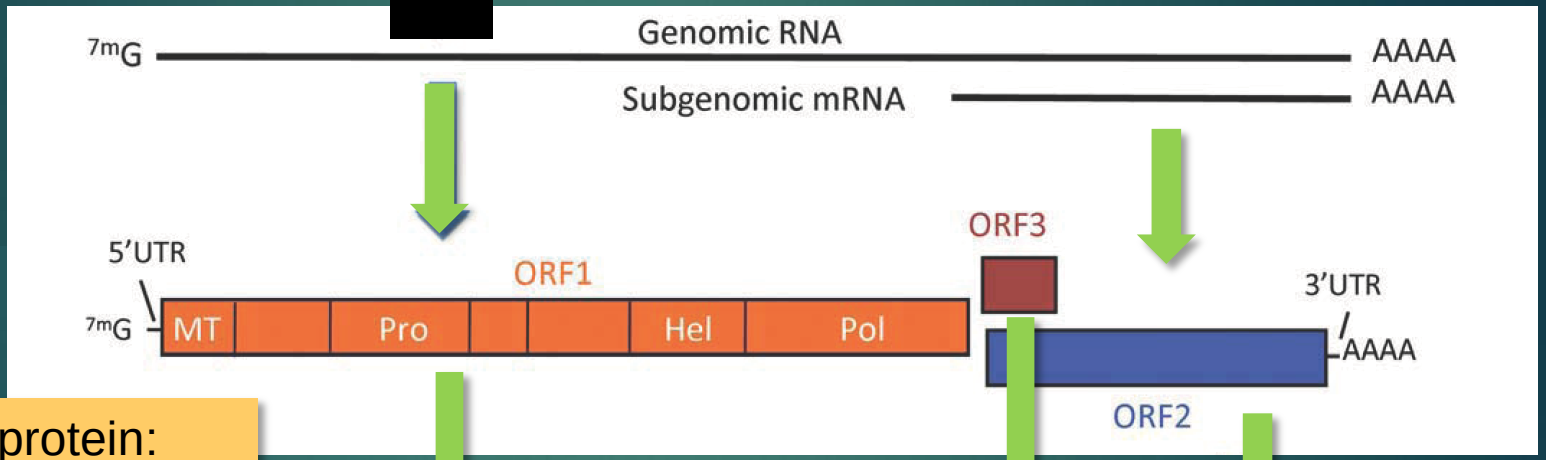
HEV SEROPREVALENCE
in patients with Chronic Liver Disease



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*

HEV: HISTORY

- 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)

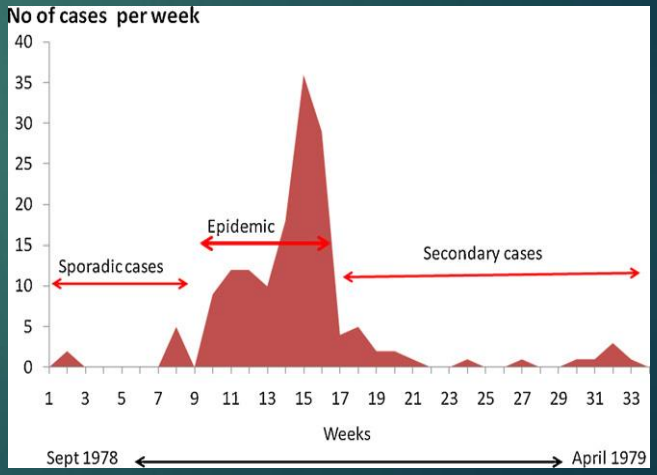
Historical Aspects ~1950s

- 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
- Smaller outbreaks: India (1982), Nepal (1984), Algeria (1985), Mexico (1986)



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

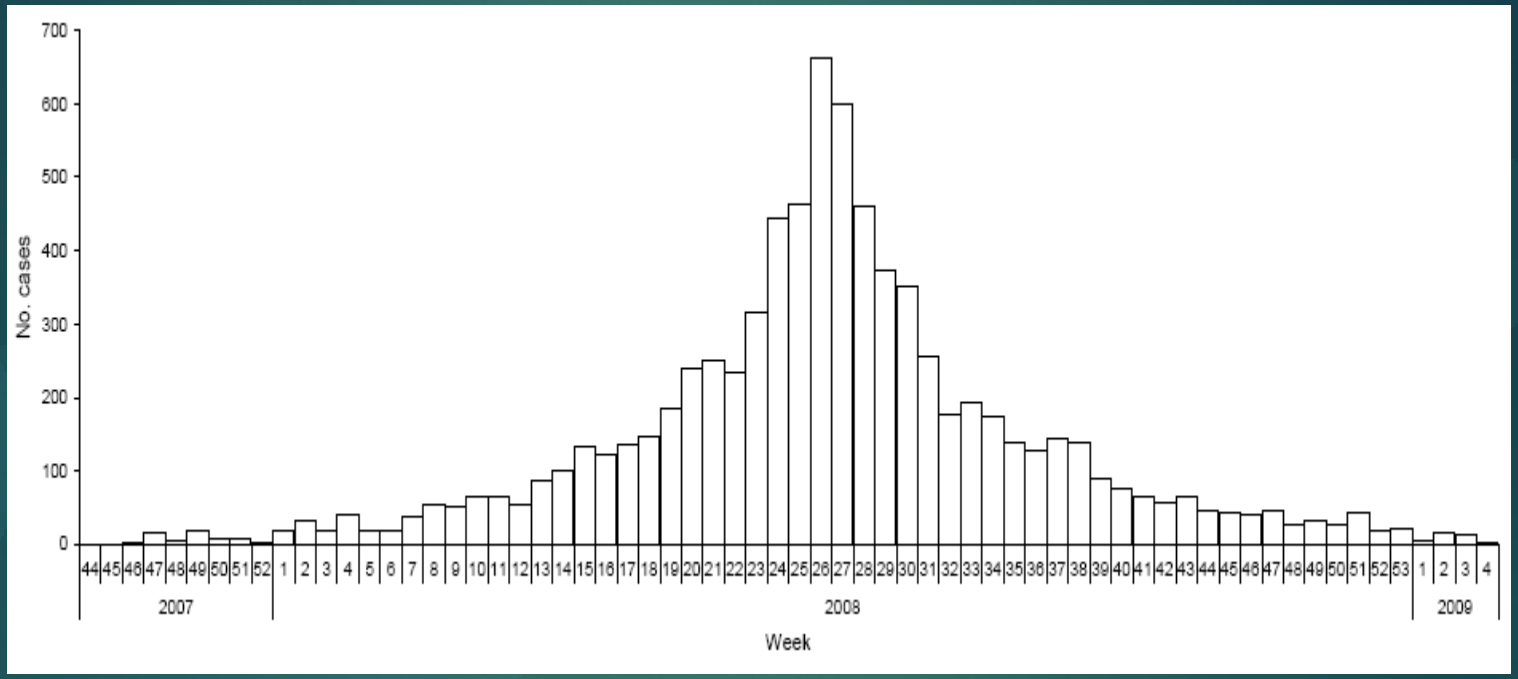
Two Cy macaques inoculated with stool suspension from the experiment showed excretion of same VLP, LT elevation and histological changes in liver

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**

Teshale, et al., Emerg Infect Dis. 2010;16:126-9

HEV Prevalence and Disease Pattern

	Highly Endemic	Less endemic
Human Disease	Common, sporadic and epidemic	Infrequent, sporadic
Transmission*	Contaminated water	Undercooked meat, ?Animal contact
Reservoir	Primarily human	?Zoonotic
Host characteristics	Young, healthy	Elderly, comorbid
Pregnant women	Fulminant in ~20%	Not reported
Chronic infection	None	Immunosuppression

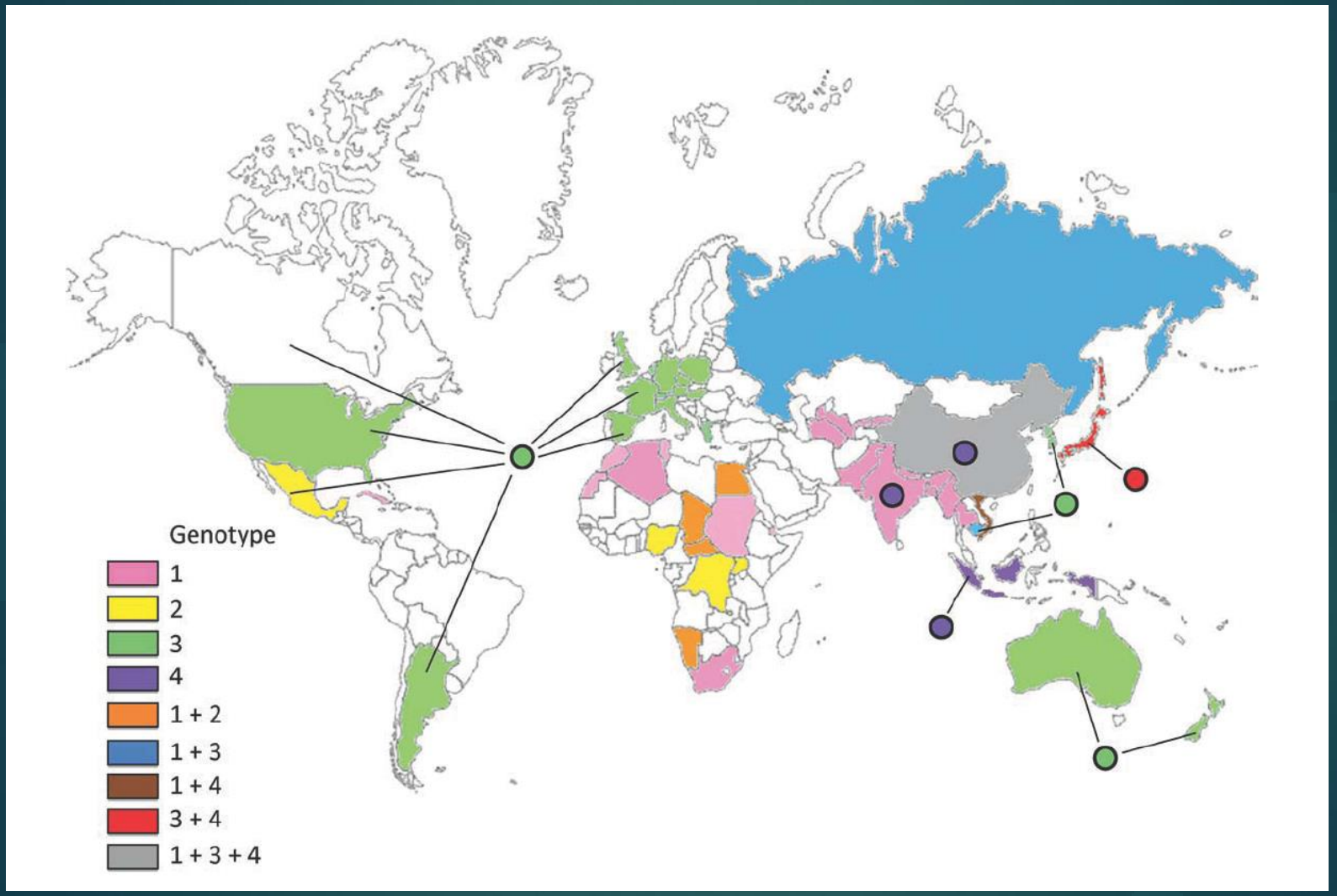
* Other modes of transmission (less frequent):

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)



Aggarwal. Hepatology 2011;54:2218

HEV: Clinical Differences Genotypes

Characteristics	Genotypes 1 and 2	Genotypes 3 and 4
Occurrence in U.S.	Travel-related, imported	Autochthonous
Rate of icteric illness	High	Low
Infection		Infection in majority of young healthy patients is asymptomatic
Disease		
Age distribution	Rates highest adolescents and young adults	Rates highest among older adults (ave 60 yrs), co morbidities
Sex distribution	Similar rates men/women	Higher rates men >3:1
Mortality	High among pregnant women(10-25%) 3rd trimester	High older adults Chronic liver dz up to 70%
Extra hepatic features	Few	Neurologic complications, glomerulonephritis
Chronic infection	None	Immunosuppressed (common)

Hoofnagle NEJM 2012;367:1237-44; Kamar Lancet 2012;379:2477-2488

Genotype 1 vs 3 Hepatitis E

Feature	Genotype 1 (Epidemic)	Genotype 3 (Endemic)
Sex (M:F)	1:1	3:1
Age	20-45 yrs	40-80 yrs
2nd Spread	Uncommon	Not known
Source	Water	Food
Agent	Human	Swine
Seasonality	Yes	Usually not
Fatality rate	Pregnancy	Elderly
Extrahepatic	Yes (Pancreas)	Yes (CNS)
Chronicity	No	Yes, immune deficient

HEV Genotypes

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

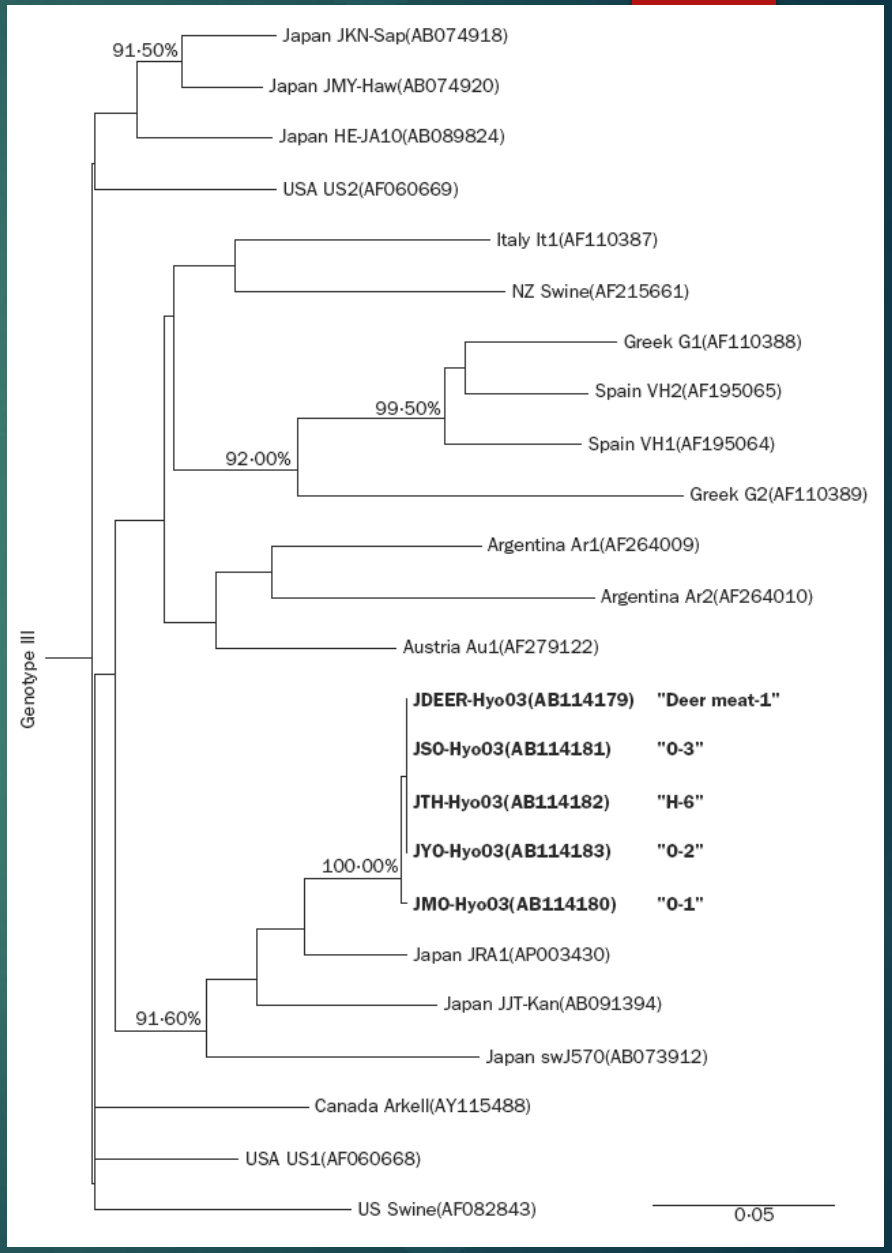
Hoofnagle. NEJM 2012;367:1237

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%

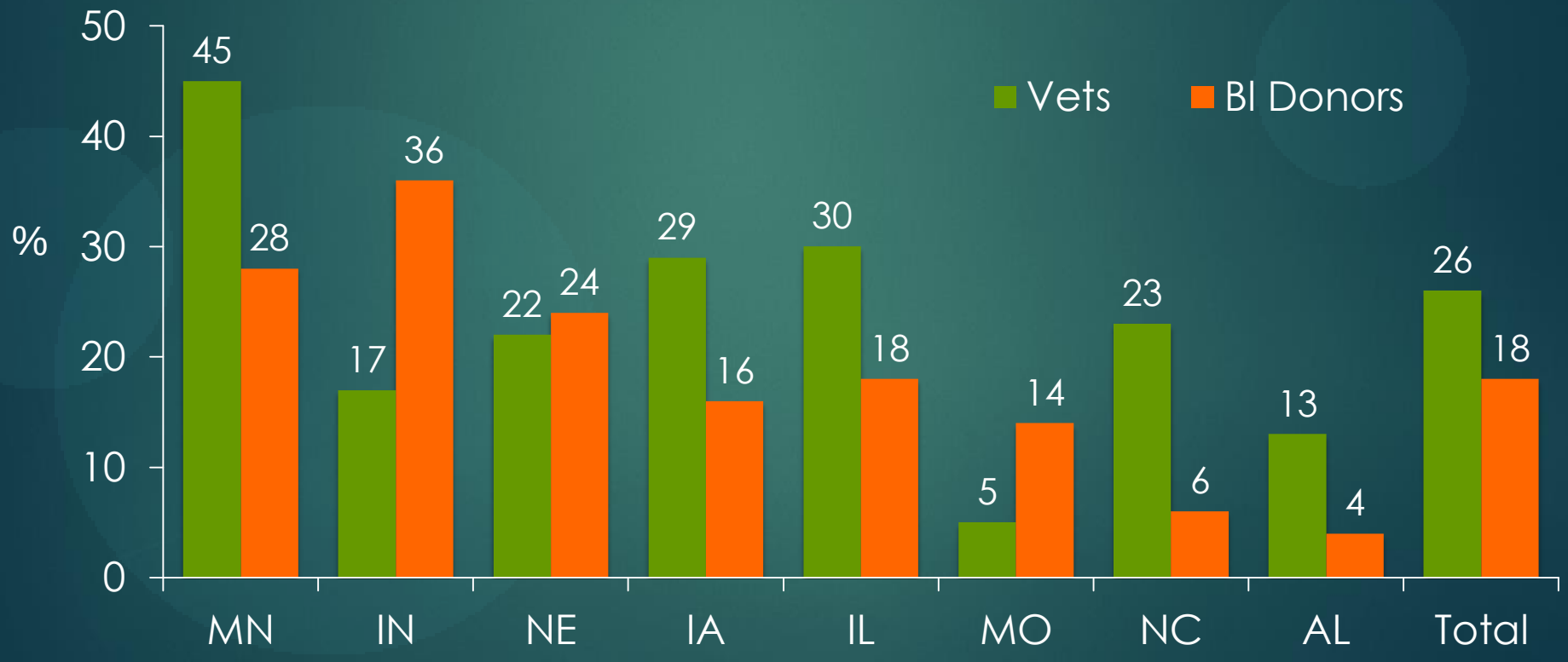
(326 nt in ORF 1)



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



Meng. J Clin Microbiol 2002;40:117

Hépatite E : une histoire de bêtes Francaise

Hollande

- Prélèvement de sang, de foie et de muscle, cerf,sanglier, chevreuil
- PCR «conventionnelle » et temps réel « maison »
- Sérologie (ELISA)
- Séquençage : génotype 3c

PACA

3 familles, 17 malades

Les malades (pas les sains) avaient mangé du figatellu

PCR et microscopie électronique dans 12 figatelli

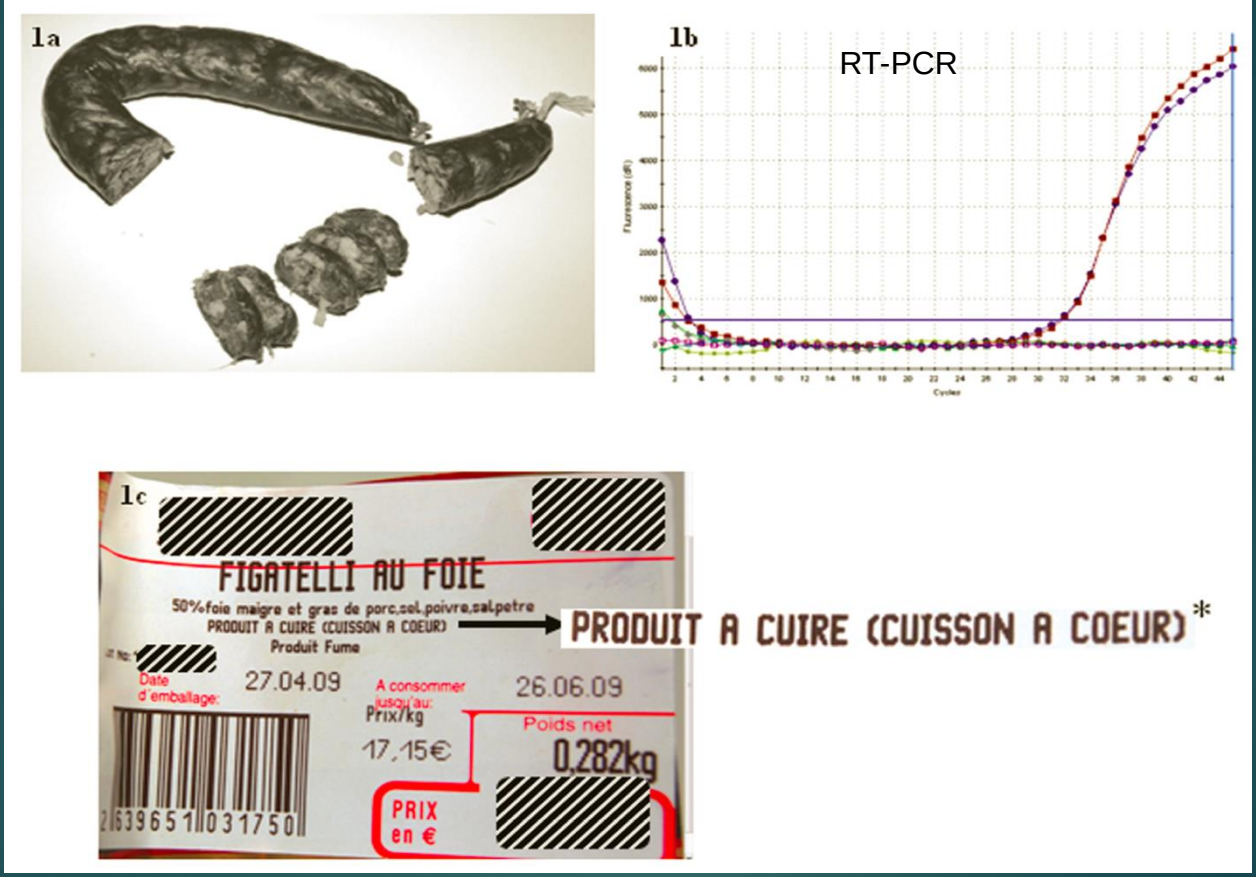
Séquençage : génotype 3f

➔ Conclusion : attention à la viande crue ou peu cuite !

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

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

How Might Non-Swine Handlers be Exposed to Contaminated Pork? [Caution: "This slide is not for the queasy" Dr Harvey Alter]

- 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

ASIAN SEAFOOD RAISED ON PIG FECES APPROVED FOR US CONSUMERS (Bloomberg News)

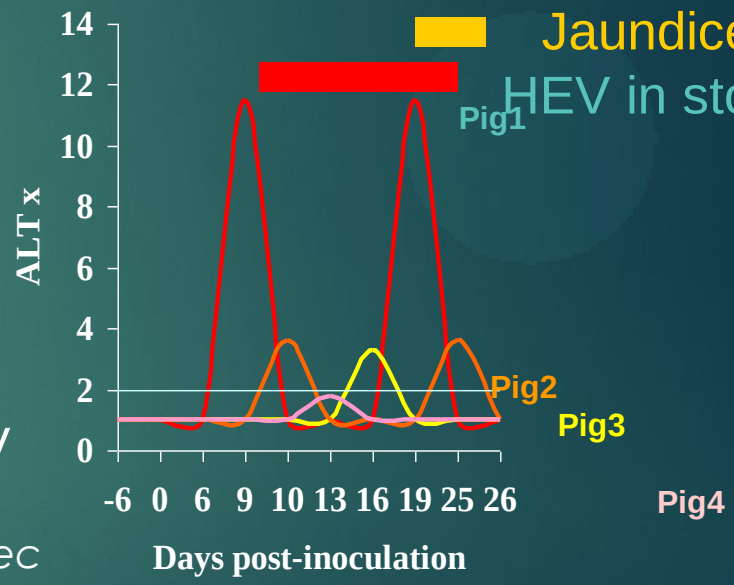
Vietnam: “Nguyen Van Hoang packs shrimp headed for US in dirty plastic tubs. He covers them with ice made from tap water that the Health Ministry says should be boiled before drinking because of the risk of contamination.” Pig farms abundant and water run-off possible.

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
  - *Balayan M et al., J Med Virol 1990, 32:58-9*
- *CDC confirmed HEV genotype 3 in historical experiment samples*
  - *Lu L. et al, J Med Virol 2002*
- 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
  - *Drobeniuc J, et al. J Infect Dis. 2001 Dec 15*
- 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

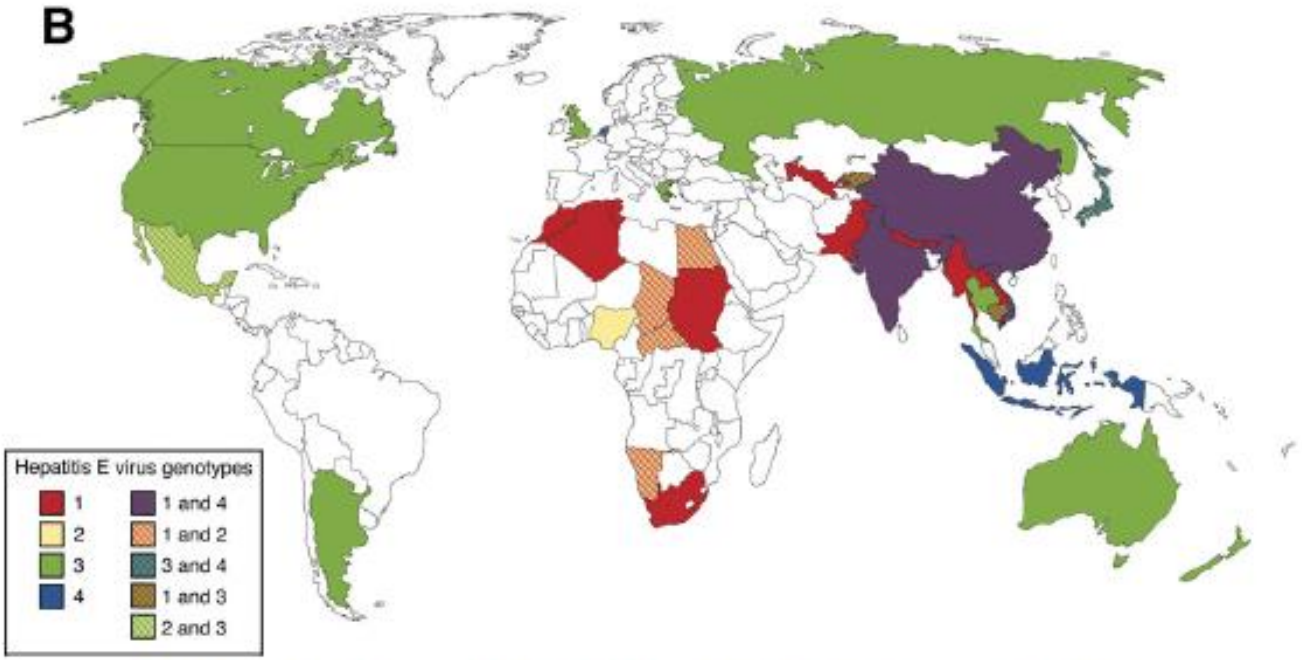


Figure 2. (*A*) Worldwide prevalence of HEV and (*B*) the geographic distribution of the different HEV genotypes.

Wedemeyer Gastro 2012

Hepeviridae- Proposed Classification and Host Range*

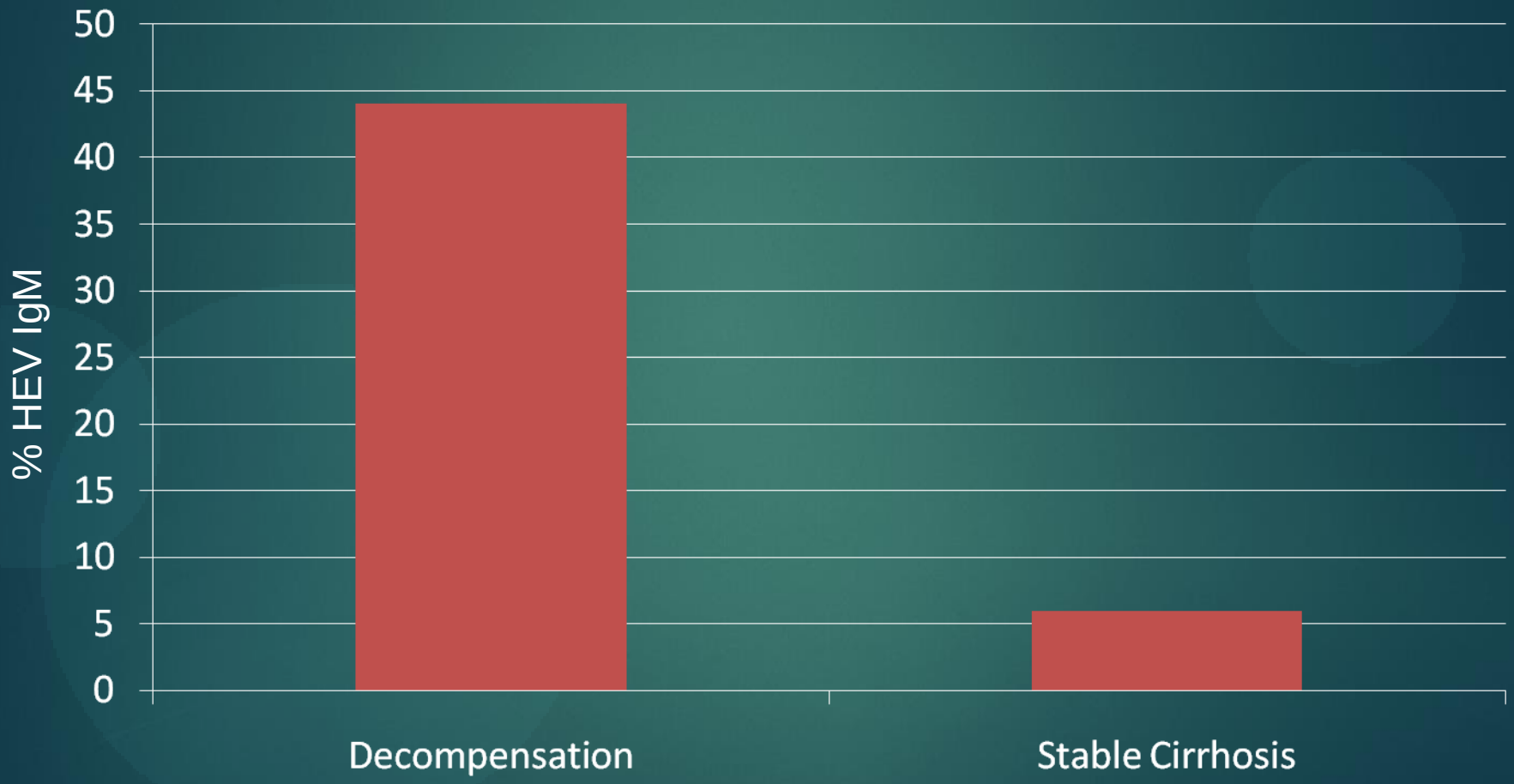
HEV	Natural Host
Genus *Hepevirus*	
Genotype 1	human
Genotype 2	human
Genotype 3	human, pig, deer, mongoose, rabbit
Genotype 4	human, pig
Putative Gt 5	rats
Putative Gt 6	Wild boar
Putative Genus *Avihepeviridae*	
Genotype 1	chicken (Australia)
Genotype 2	chicken (USA)
Genotype 3	chicken(Europe and China)
Putative Genus *Piscihepevirus*	
Cutthroat throut virus	fish

**XJ 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 document 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

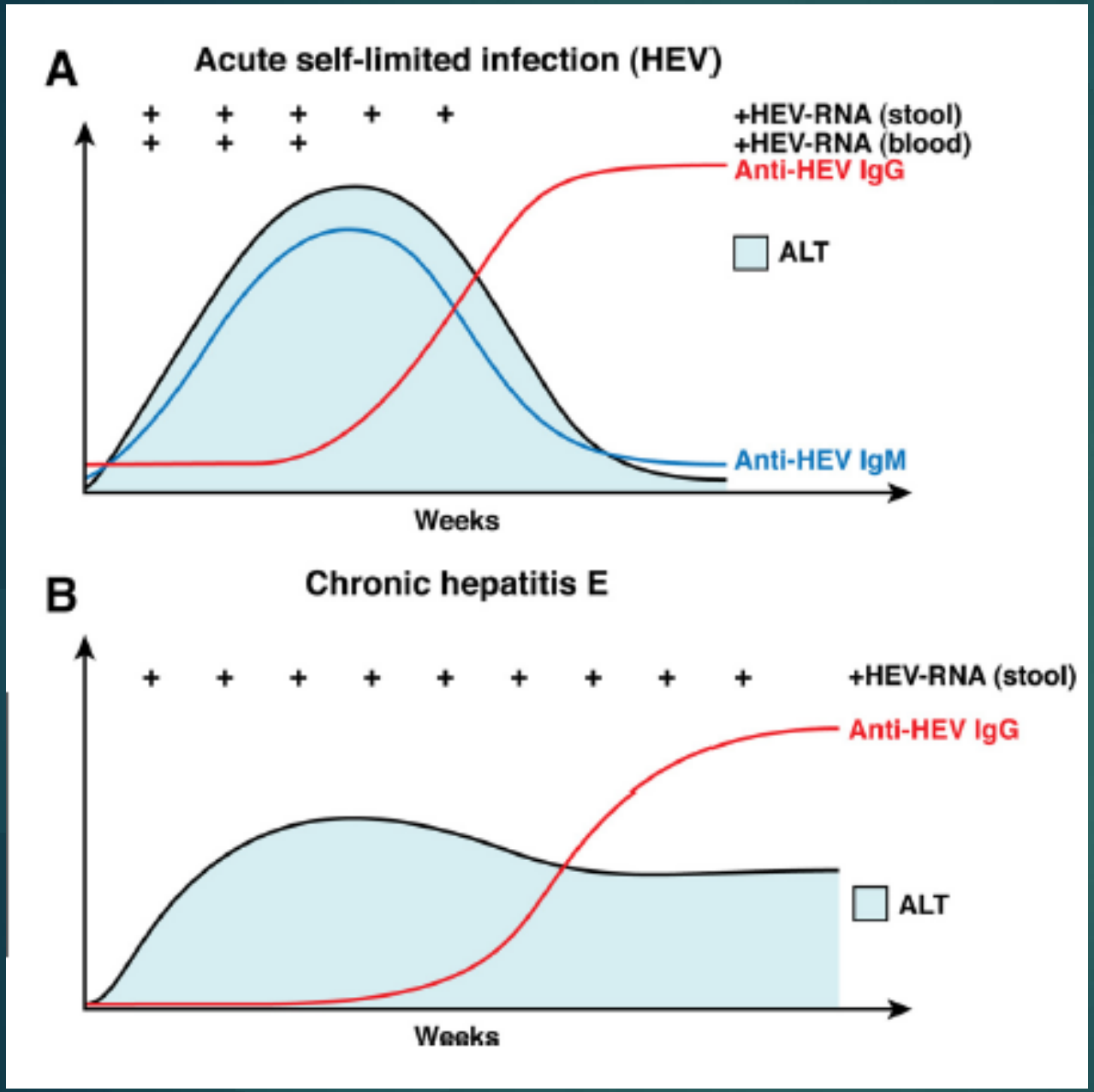
Anti-HEV IgG among HALT-C cases and controls

	Cases N=89	Controls N=267	P value	Odds Ratio
Seroprevalence #(%)	20 (22.5)	55 (20.6)	0.71	1.12 (0.63-1.99)
Seroconverters #(%)	5 (5.6%)	5 (1.8%)	0.064	3.12 (0.88-11.04)

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

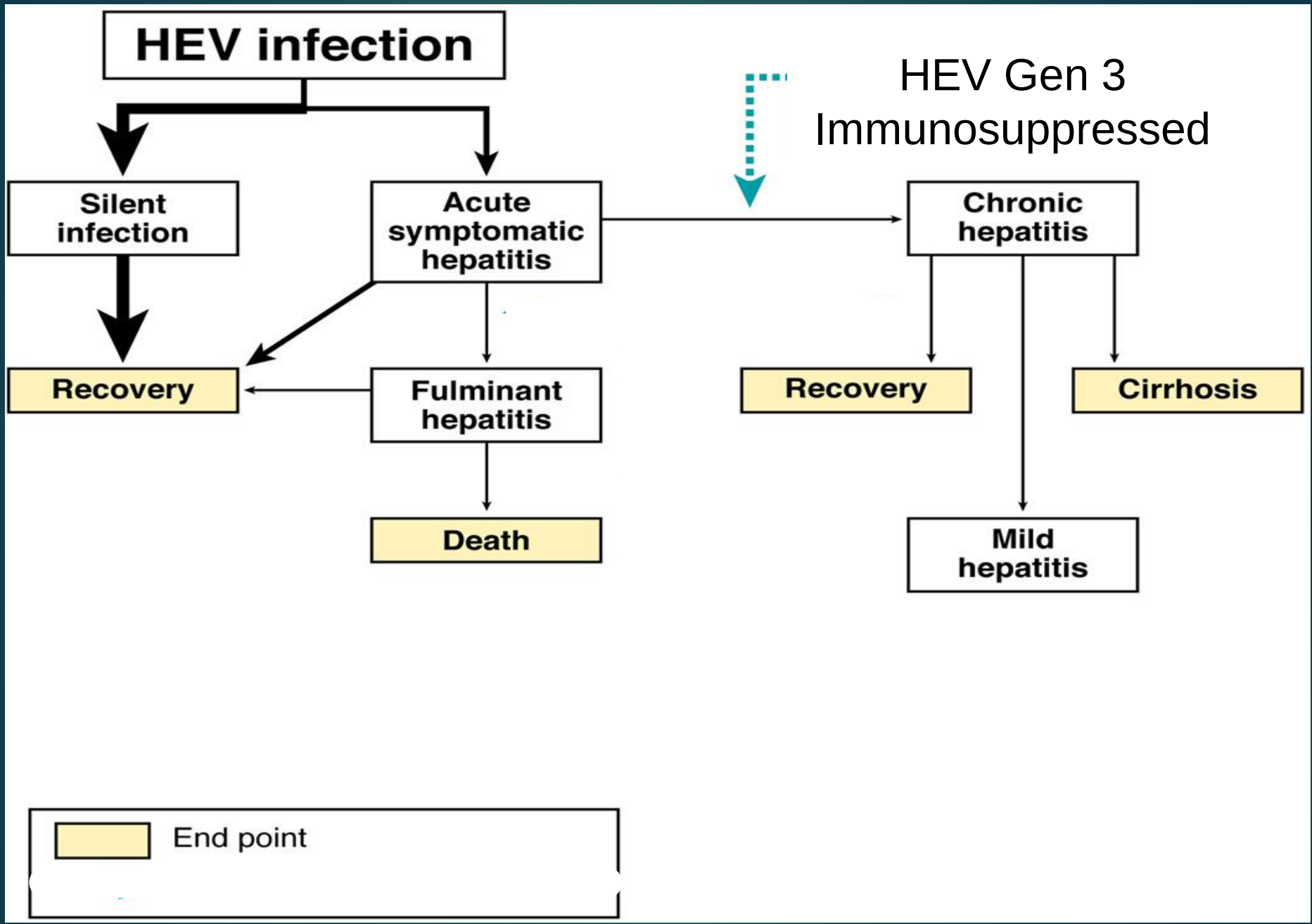
*Data from NIH HEV Scientific Workshop, Bethesda, 26 March 2012



Wedemeyer Gastro 2012

CLINICAL OUTCOMES

	ACUTE DISEASE	CHRONIC DISEASE	MORTALITY
Immunocompetent	YES	NO	LOW
Pregnancy	YES	NO	VARIABLE
Chronic Liver Disease	YES	NO	HIGH
Immunosuppressed -HIV -Post-Transplant -Cancer Chemotx	YES	YES	VARIABLE



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)
  - High HEV seroprevalence in nonendemic countries (Thomas et al., 1997)
- Different prevalence rates using different assays

Khudyakov Virus Research 2011;161:84-92

Variability of anti HEV IgM assays

Assay	Sensitivity	Specificity
NIH *	98%	78.5%
CDC*	98%	93.4%
International Immuno-Diagnostics (Foster City CA)	82.4%	91.7%
MP Biomedicals (Singapore	72.5%	93%
Diagnostic Systems (Russia)	98%	96.6%
Mikrogen GmbH (Germany)	92.2%	96.1%

*Not commercially available

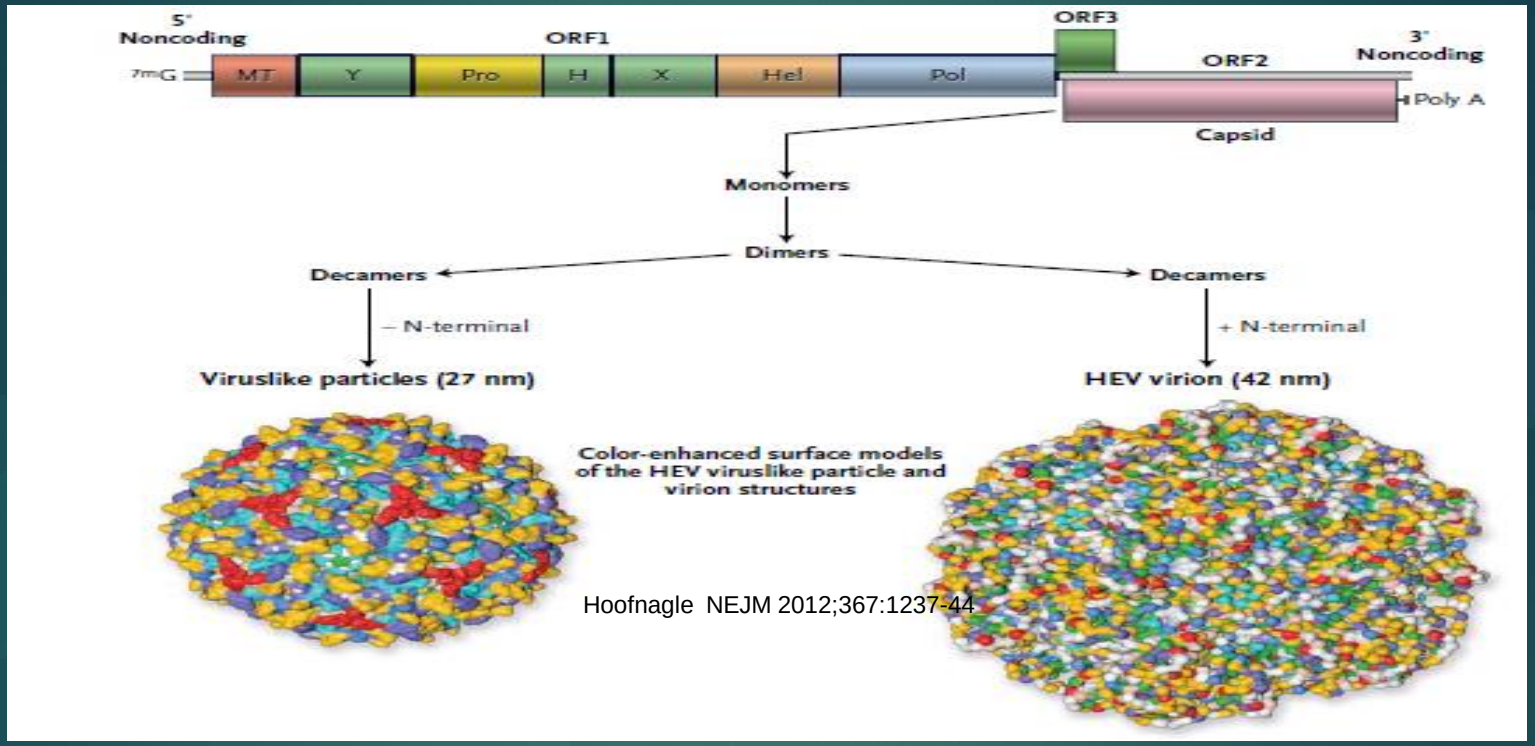
All samples in sensitivy panel wth acute jaundice, (-) ABC and HEV RNA+

All HEV RNA (+) with well defined genotypes

Drobenuic Clin Infect Dz 2010 ;51:e24-27

HEV: Antibody Testing

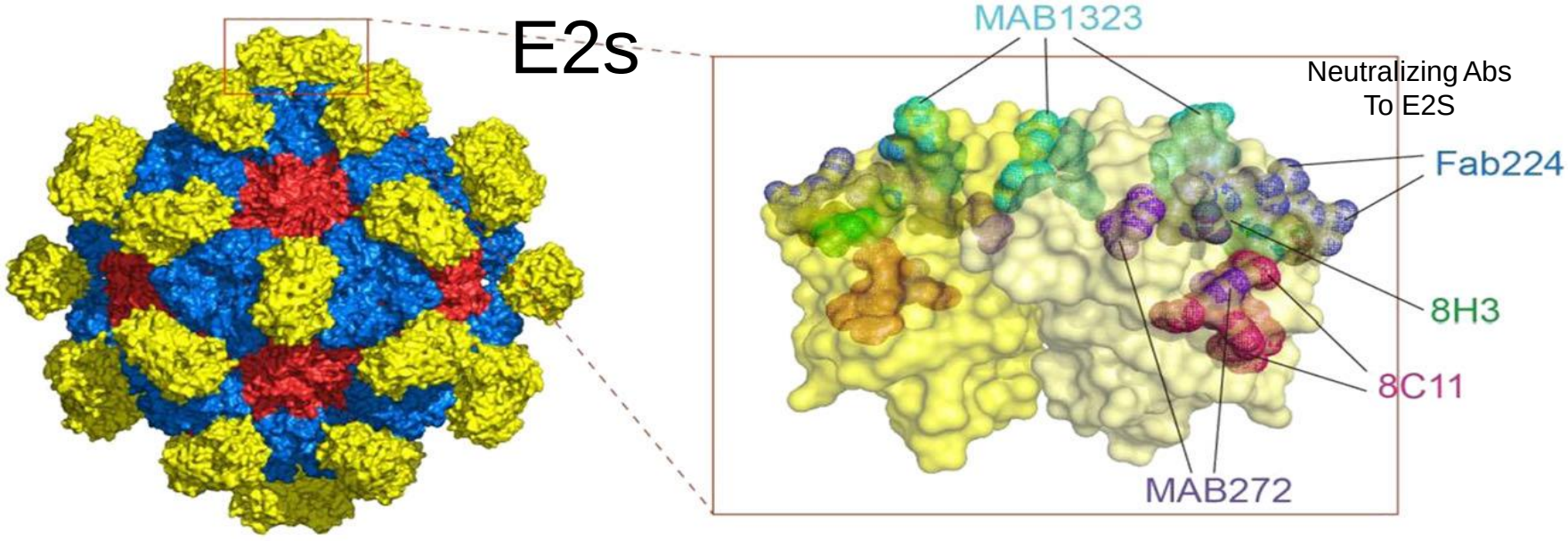
- Synthetic peptides derived from ORF2 and ORF3 proteins are major targets used for HEV diagnostic assays



- Differences in peptides used accounts for differences in sensitivity

Khudyakov Virus Research 2011;161:84-92

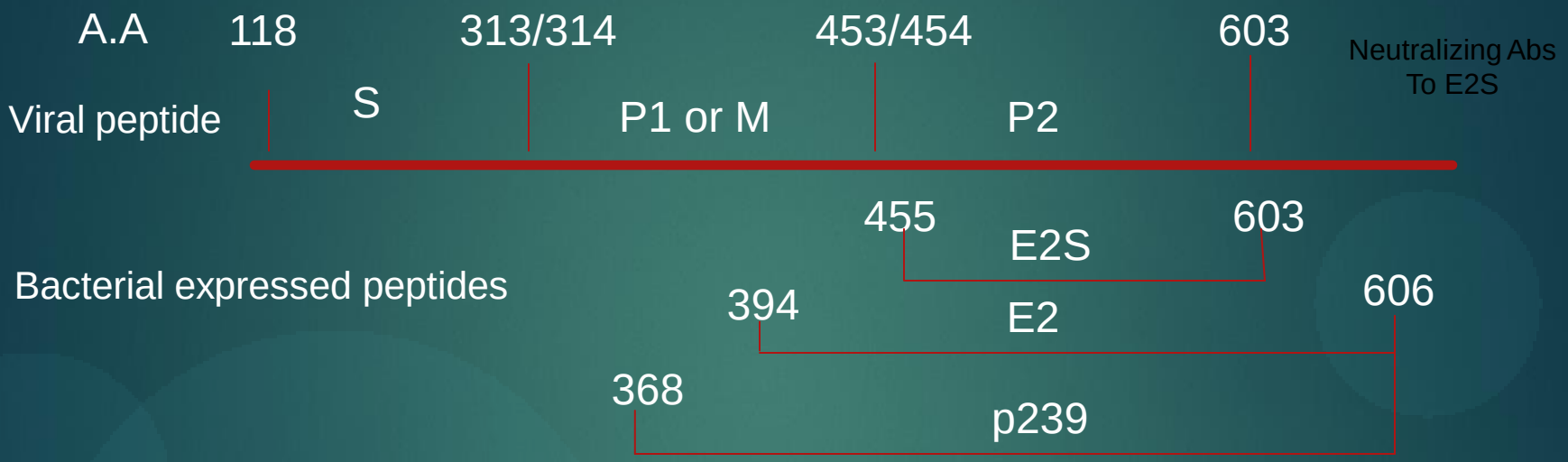
Crystal structure of HEV capsid protein and neutralizing sites



- HEV capsid has three domains
  - Shell (red above) AA 118-313
  - Middle domain (P1 blue) AA 314-453
  - Protruding domain (P2 yellow) AA 454-606
- E2s domain is bacterially expressed Peptide (= to P2 domain) contains all identified neutralizing epitopes

Zang Rev Med Virol 2012;22:339-349, Kamar Lancet 2012;379:2477-2488

Bacterial Expressed Peptides from Viral P2 Domain used for Anti-HEV Testing



- Peptide pE2 contains 66 additional AA with extension into P1
  - appears to stabilize dimeric structure, making it a useful dx agent
- p239 adds 26 more AA in P1 domain.
  - Additional of 26 AA results in formation of a Virus like particle, enhancing immunogenicity
- The antigenicity of these peptides is virtually similar

Zang Rev Med Virol 2012;22:339-349,

HEV: Differences Main anti-IgG assays

- The GL assay (IgG kit by Genlabs [Singapore])
  - Used to be most commonly used assay world wide
  - Serum Ab directed to recombinant peptides ORF2(negative terminal) and ORF3 (complete protein)
  - Lack of E2s domain (On ORF2)

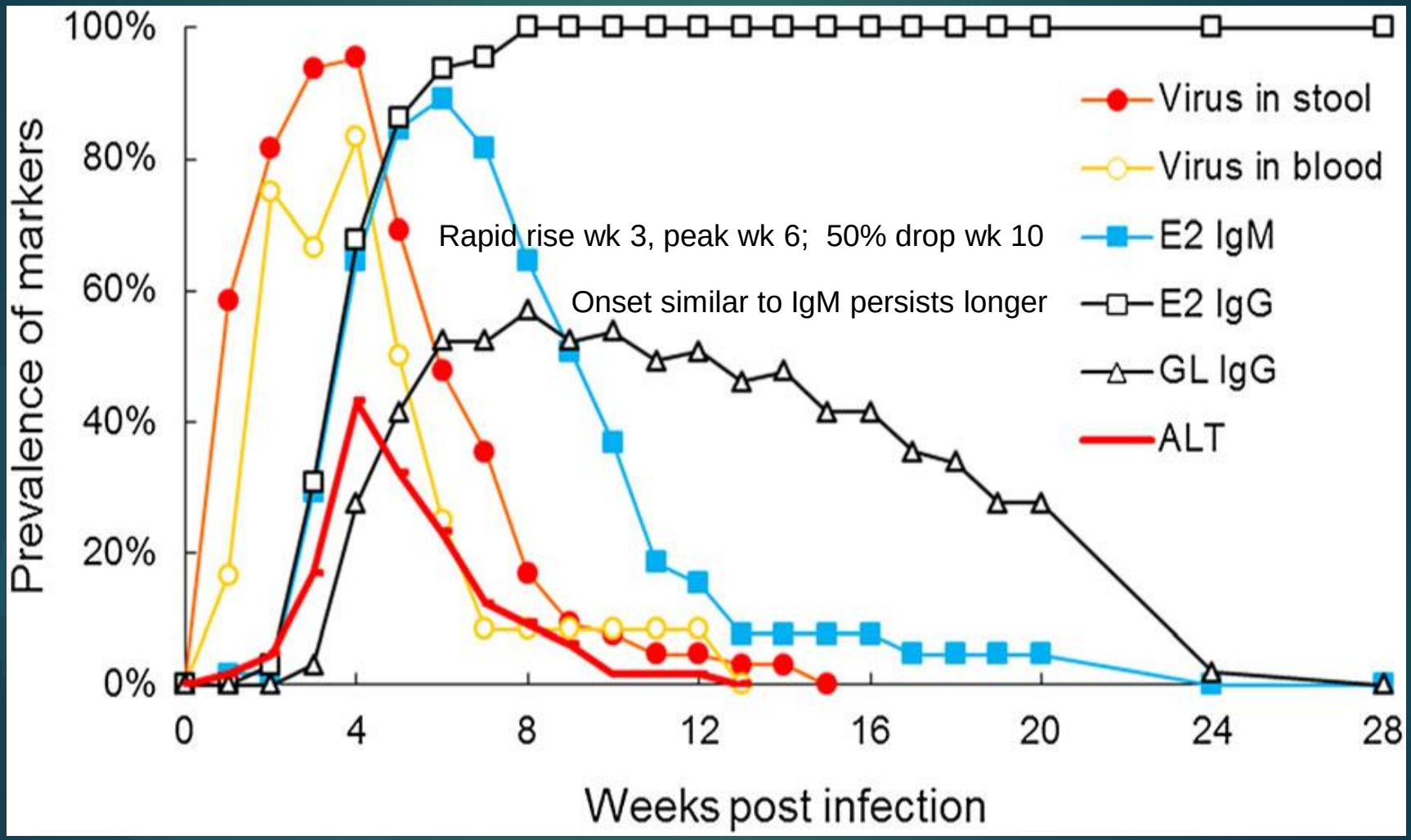
Bendall J Med Virol 2010;82:799-805
Zang Rev Med Virol 2012;22:339-349Park BMC Inf Disease 2012;12:1-6

HEV: Difference 2 anti-IgG assays

- Wantai (PEG2) E2 assay
  - 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

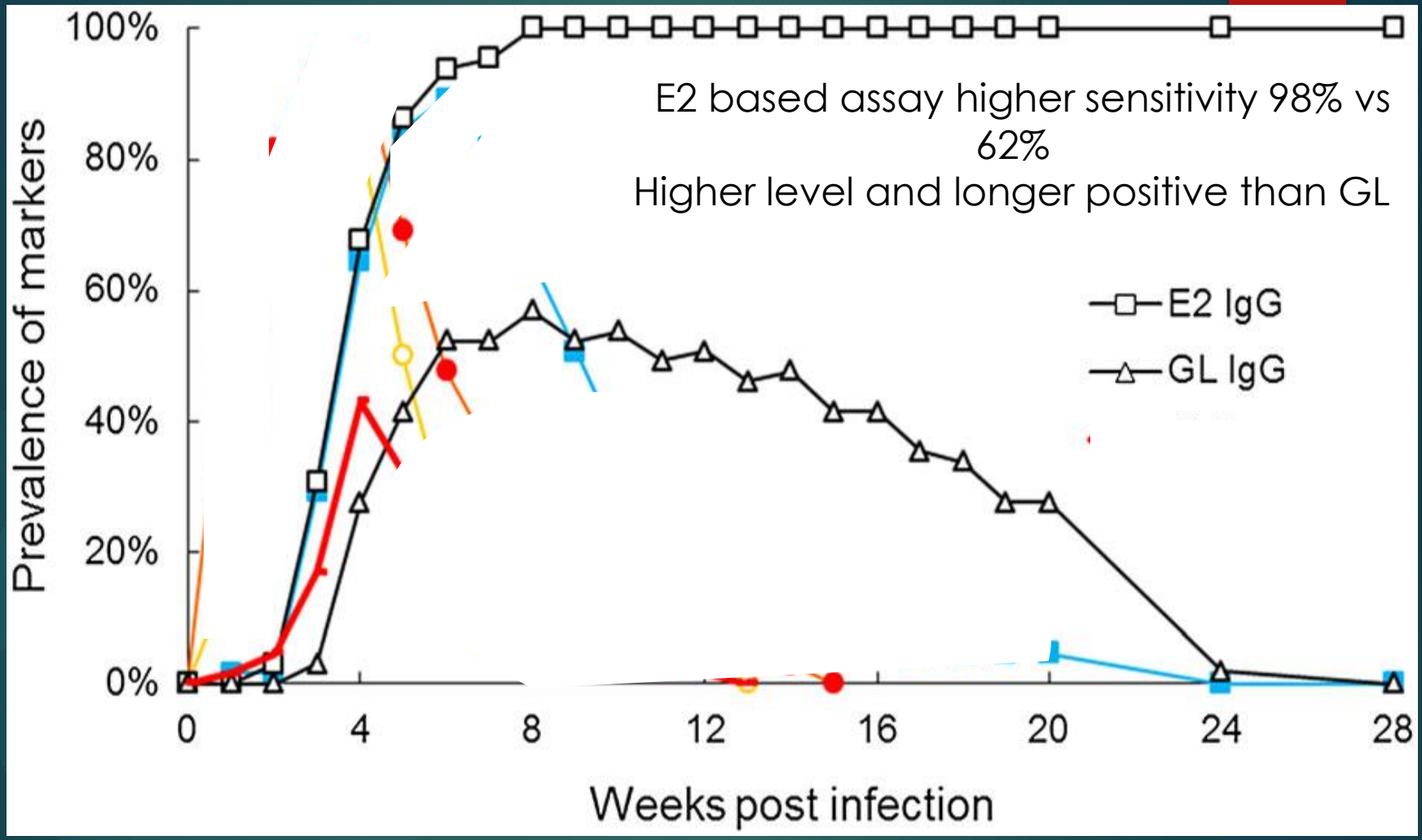
Bendall J Med Virol 2010;82:799-805
Zang Rev Med Virol 2012;22:339-349Park BMC Inf Disease 2012;12:1-6

HEV infection 86 Rheus monkeys



Zang Rev Med Virol 2012;22:339-349

Hepatitis E 86 Rheus Monkeys



Zang Rev Med Virol 2012;22:339-349

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 versus2.5Wu/mL
  - (+) in more sera from PCR-confirmed cases (98% vs. 56%)
  - Remained (+) longer post infection;

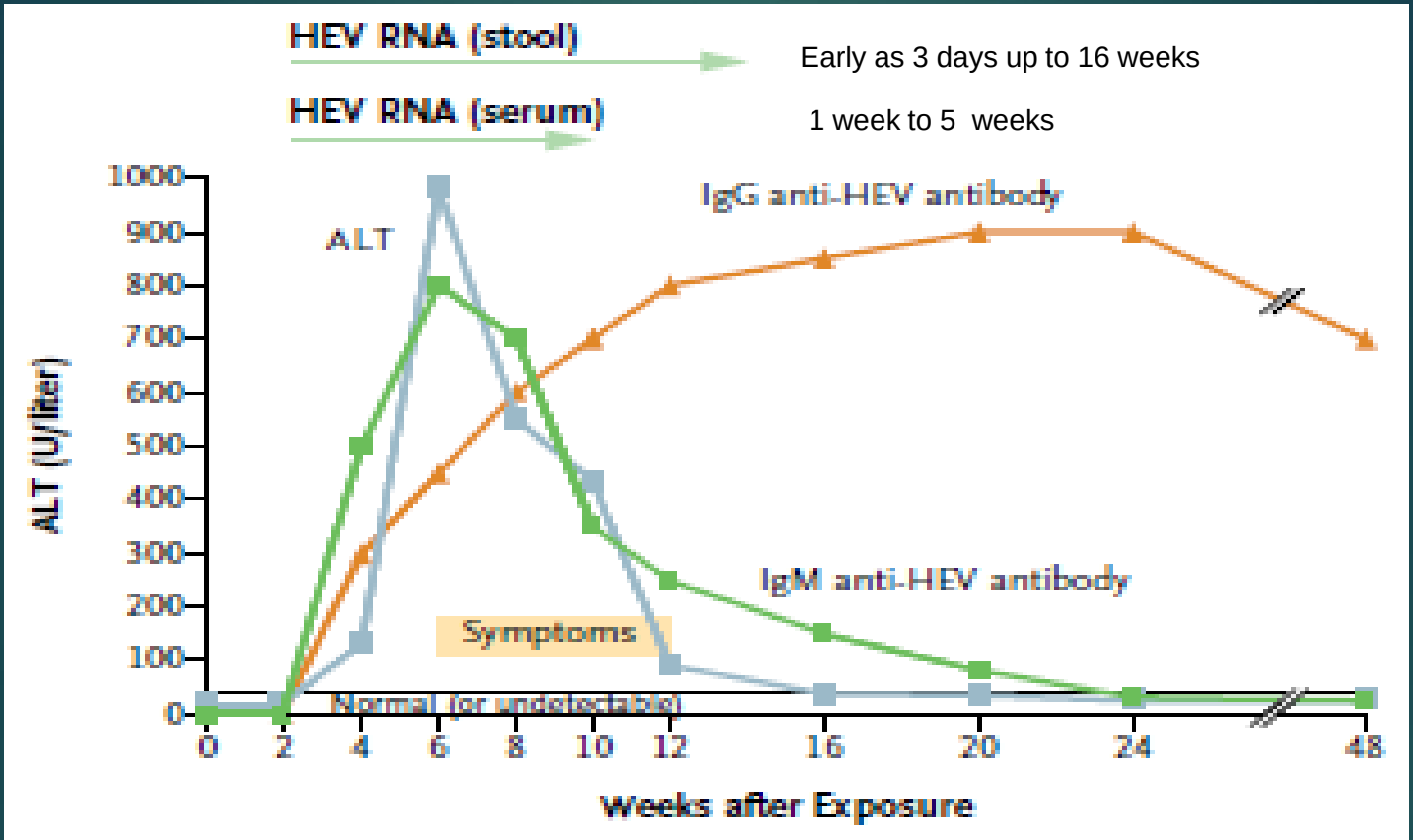
- E2 assay with higher estimate of prevalence in
  - 500 UK blood donors (16.2% vs. 3.6%)
  - *147 health check pts Korea 23.1% vs 14.3%

Bendall J Med Virol 2010;82:799-805
*Park BMC Inf Disease 2012;12:1-6

Conclusion Anti-HEV Ab Testing in Immunocompetent Patients

- Acute HEV can be accurately diagnosed using Anti-HEV IgM Ab testing
- 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

Hoofnagle NEJM 2012;367:1237-44 Khudyakov Virus Research 2011;161:84-92 Zang Rev Med Virol 2012;22:339-349

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 sxs
    - (-) 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

Hoofnagle NEJM 2012;367:1237-44 Khudyakov Virus Research 2011;161:84-92 Zang Rev Med Virol 2012;22:339-349

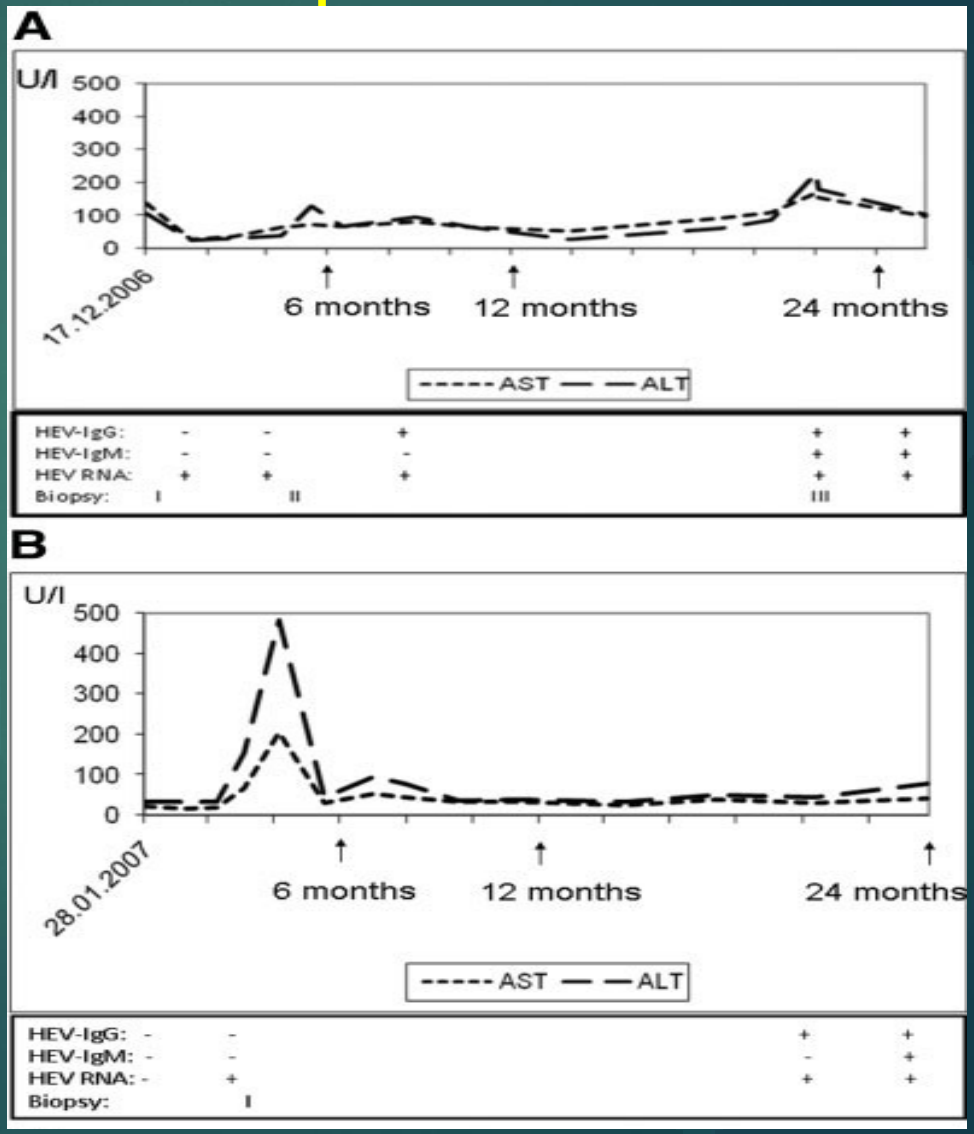
Hepatitis E: chronic

- Almost always in immunocompromised
  - Organ transplant recipients, chemotherapy, stem cell transplant and HIV[5]
- 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

Hoofnagle NEJM 2012;367:1237-44 Kamar Am J transplant 2012;2281-2287

HEV in TRANSPLANT RECIPIENTS

CHRONIC HEV INFECTION in Transplant Recipients



Pischke et. al. LIVER TRANSPLANTATION 2010

RISK AND FACTORS
HEV Chronicity in Transplant

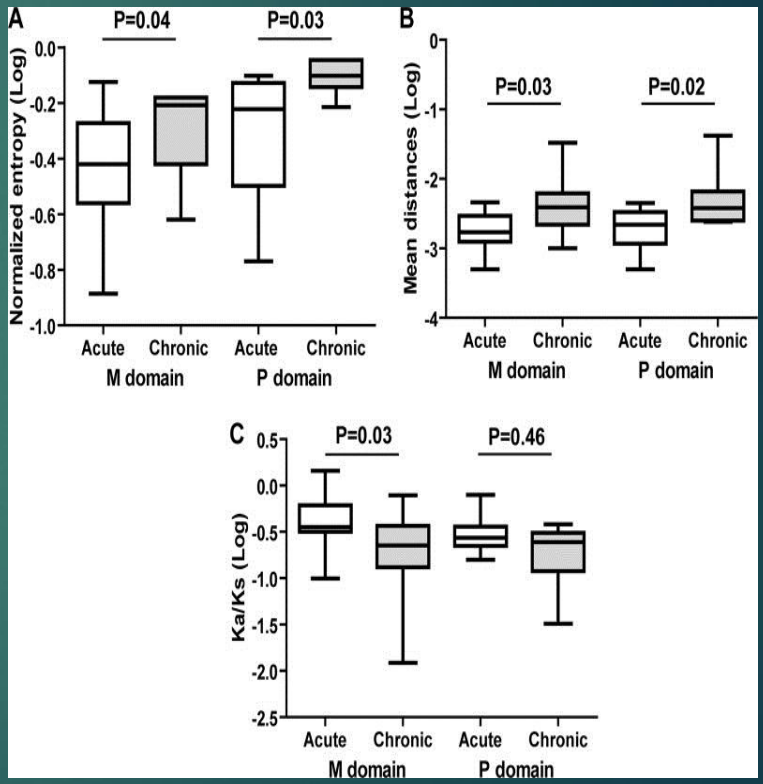
- Total with Acute HEV Post Transplant- 85
  - 17 Centers in Europe
  - 31% Liver Transplants
- Chronicity Rate- 69.5%
- Factors in Multivariate Analysis
  - Tacro > CyA
  - Low Platelet Count at Time of Diagnosis of HEV

Kamar et al., GASTROENTEROLOGY 2011

HEV VIRAL QUASIDSPECIES & CHRONICITY

Design & Results

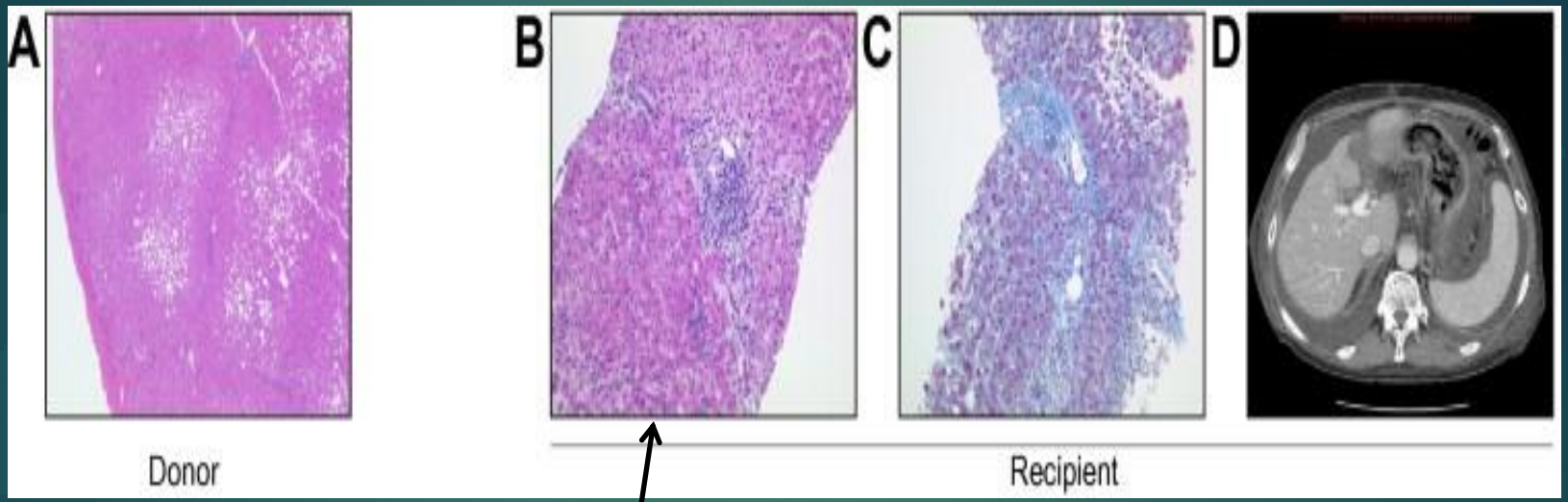
- N= 16 SOT Recipients (4 Liver/8 Kidney)
  - 8 Cleared HEV
  - 8 Developed Chronic HEV
- ORF-2 Amplified/Analyzed
  - Complexity HIGHER in those who became chronic



Lhomme et al, J VIROL 2012

RAPID DISEASE PROGRESSION AFTER OTLTx

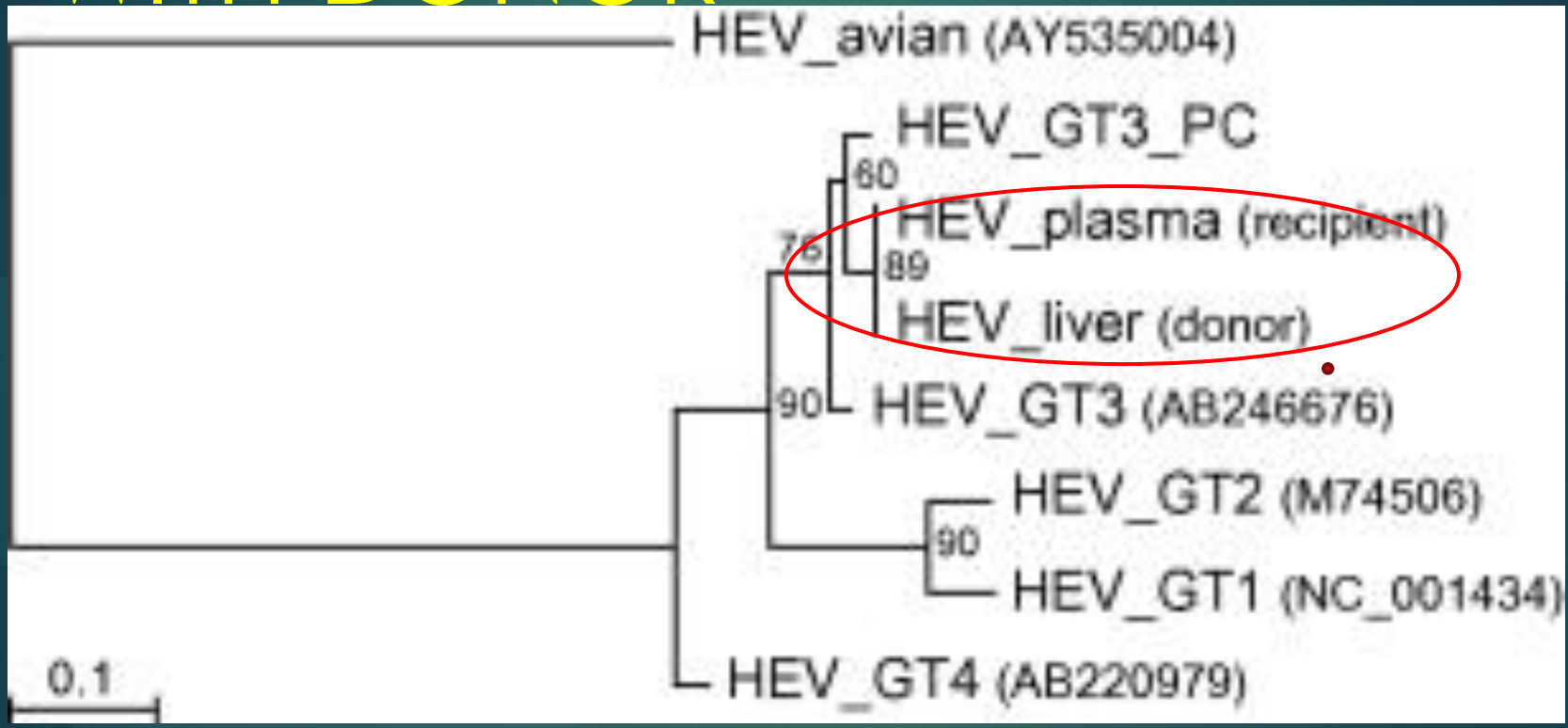
1 Year Post-OTLTx



Attributed to Acute Rejection
150 Days Post-Tx

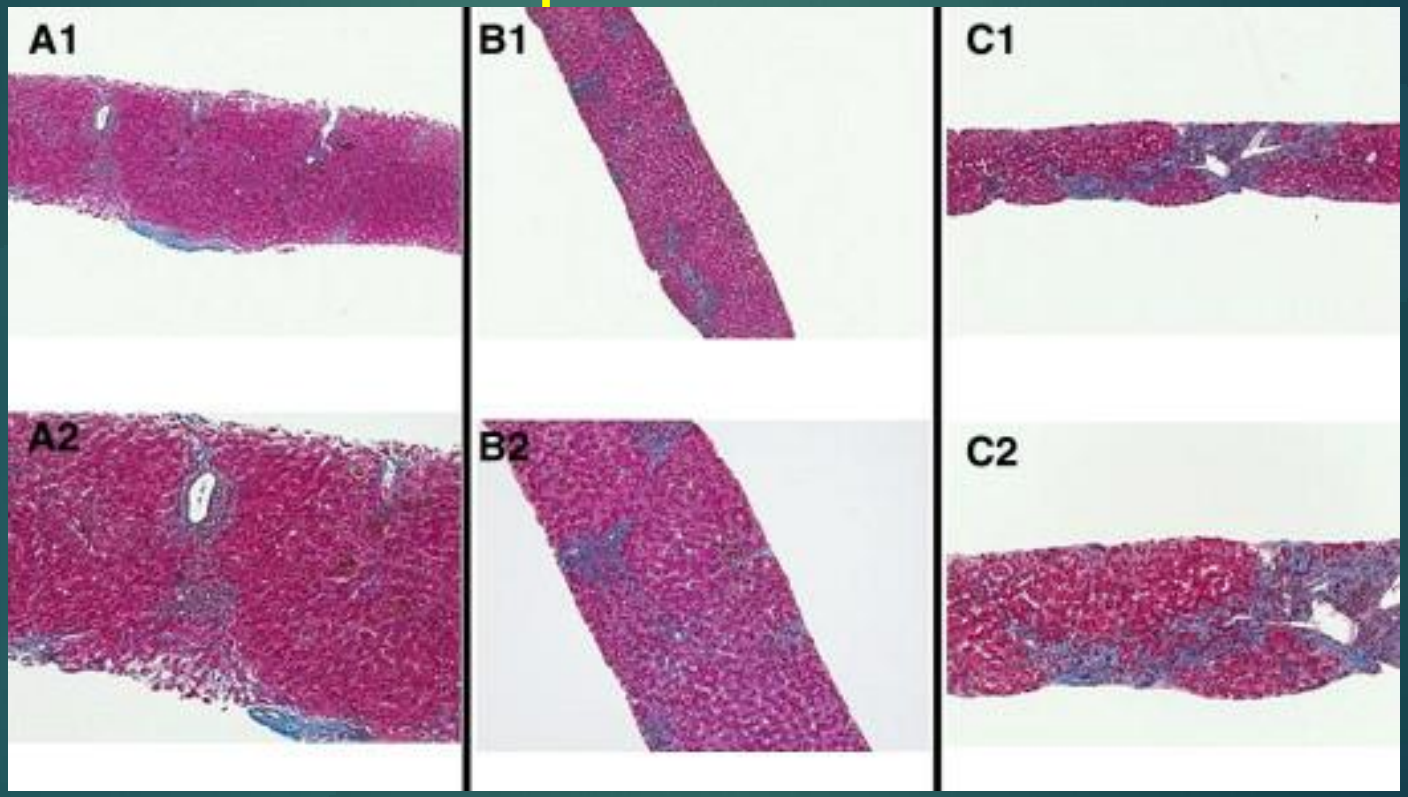
Schlosser et al., J HEPATOL 2012

PHYLOGENETIC in RECIPIENT : COMPARISON WITH DONOR



Schlosser et al., J HEPATOL 2012

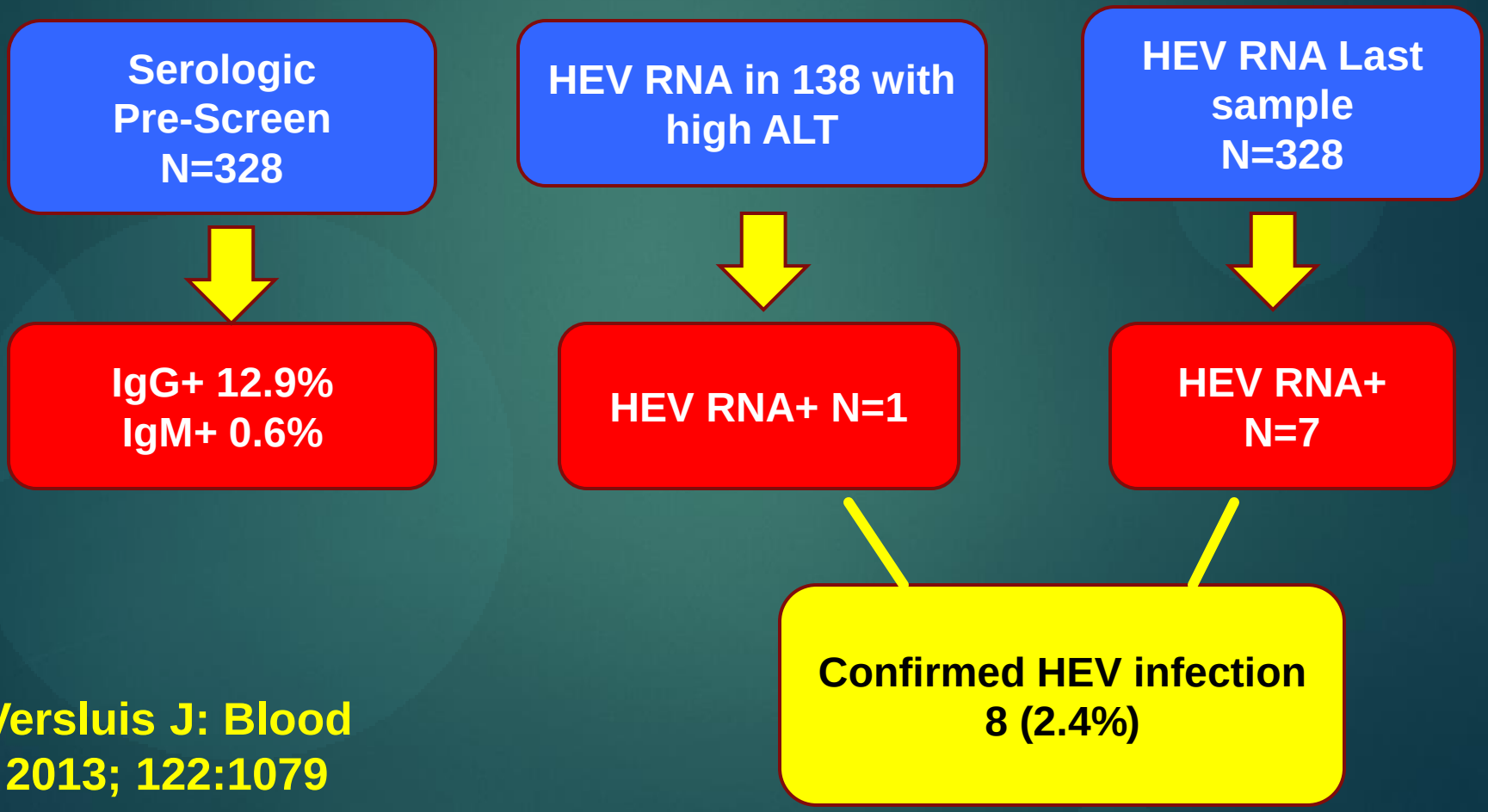
HEV CHRONICITY
Liver Fibrosis After Bone Marrow Transplant for ALL



2007 2007 2008

Halac et al, J PEDIATRICS 2013

HEV IN HEMATOPOIETIC STEM CELL TRANSPLANTATION



Versluis J: Blood 2013; 122:1079

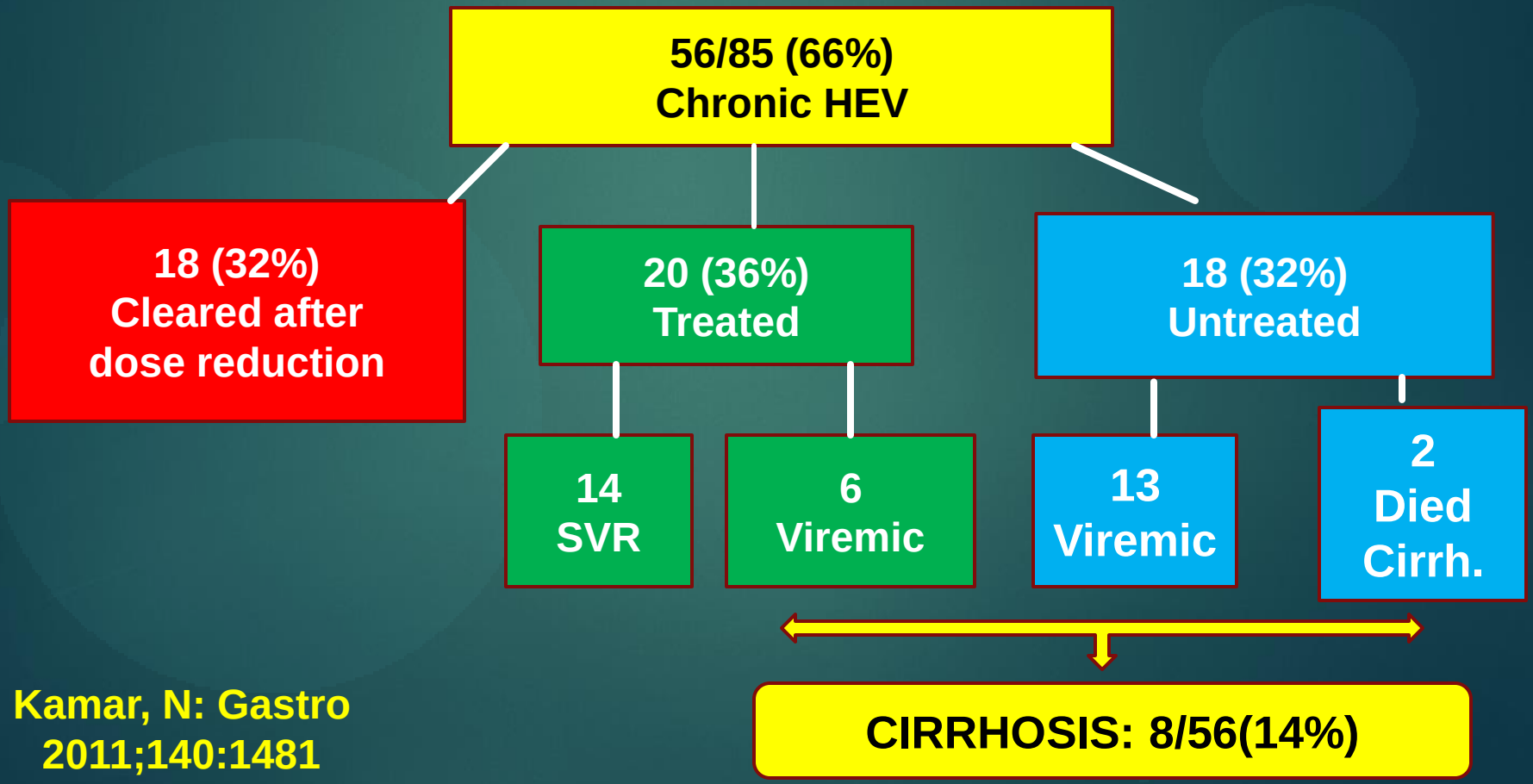
Hepatitis E: Solid Organ Transplant

- 85 SOT pts from 16 Tx centers US and Europe
  - 68 men 17 women
  - 47 kidney, 28 liver, 2 liver/kidney, 6 kidney panc, 4other
  - Age 23-77 med 48 years
- 32% sxs at initial infection, resolved w/in a few days
  - Fatigue(20), diarrhea(5) arthralgia(4), weight loss(3)
  - Abd pain(2), puritis(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

CHRONIC HEPATITIS IN PATIENTS INFECTED WITH HEV AFTER SOLID ORGAN TRANSPLANTS

Multicenter review of 85 HEV-infected recipients in 17 Centers



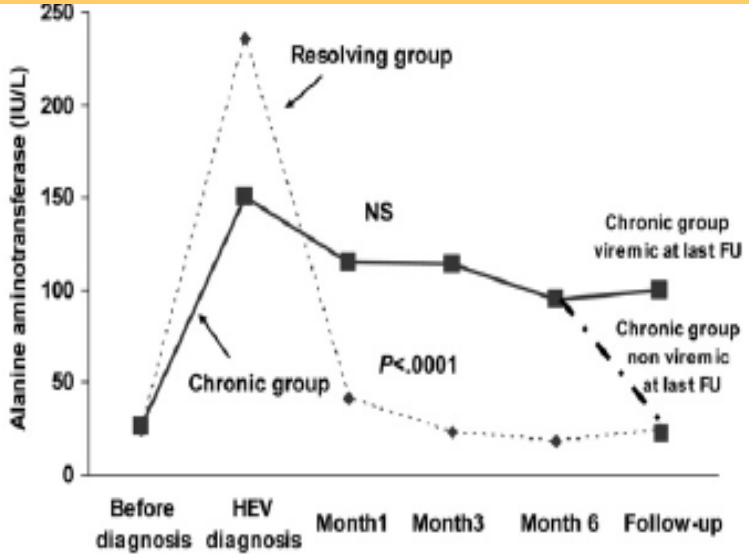
Kamar, N: Gastro 2011;140:1481

Hepatitis E: Solid Organ Transplant

HEV TESTING AT time of Diagnosis

HEV diagnostic test	No. tested	No. positive	Percent (%)
Anti-HEV IgM[a]	78	32	41.0
Anti-HEV IgG[a]	78	63	80.8
Serum HEV PCR	82[b]	82	100
HEV genotyping	64	59[c]	**All Gen 3**

ALT in acute and chronic HEV



- 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

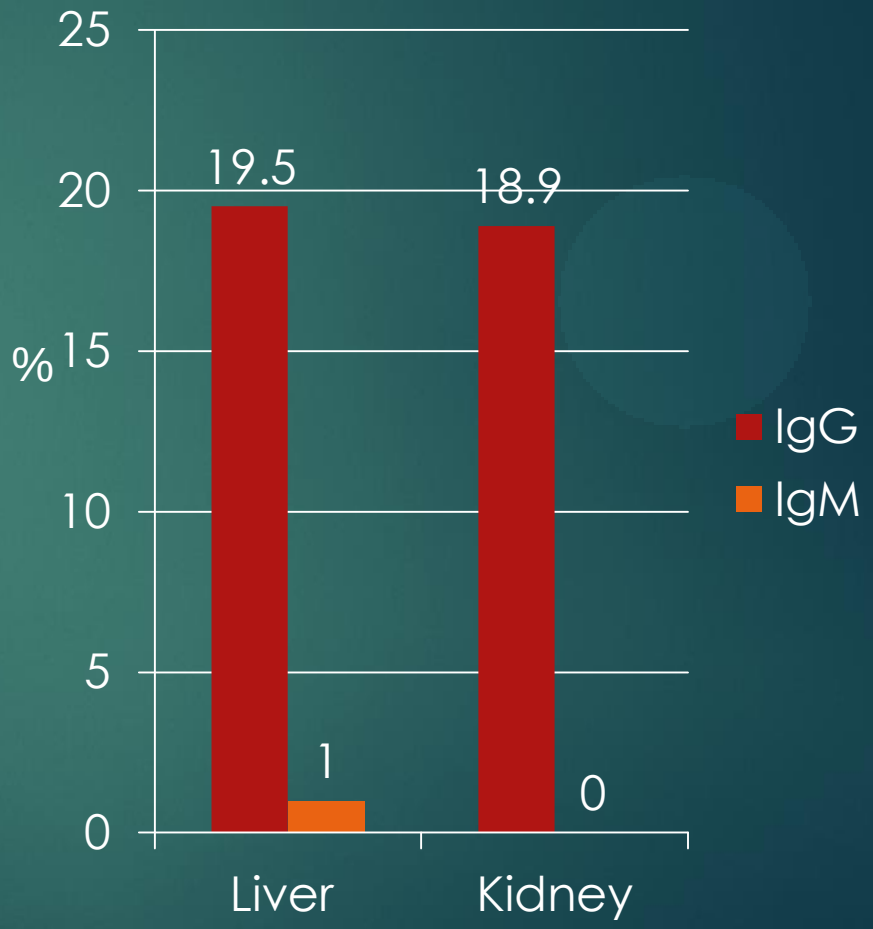
Natural History of Hepatitis E in OTR*

- 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

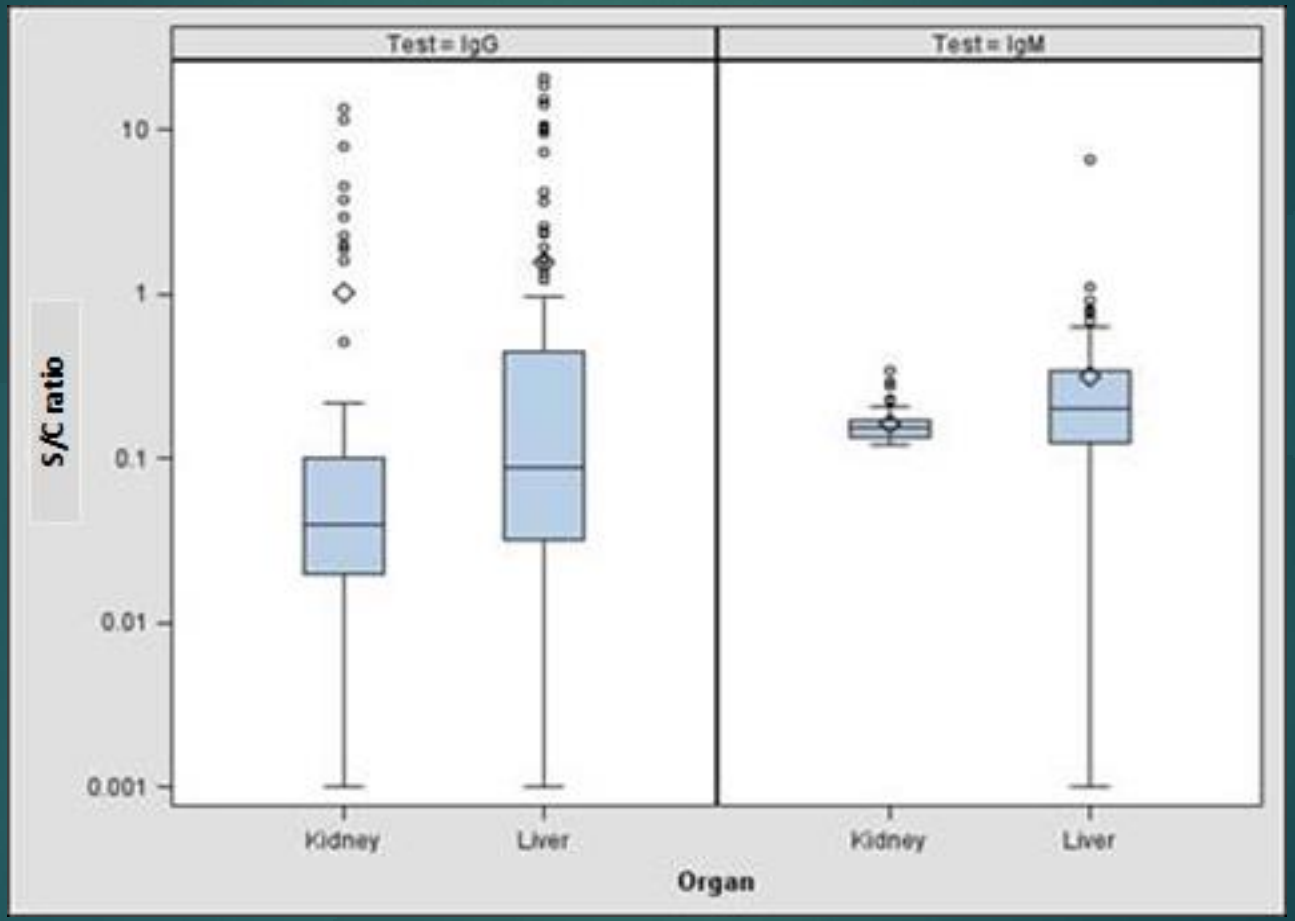
HEV IN NIH HIV SOT COHORT

- 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



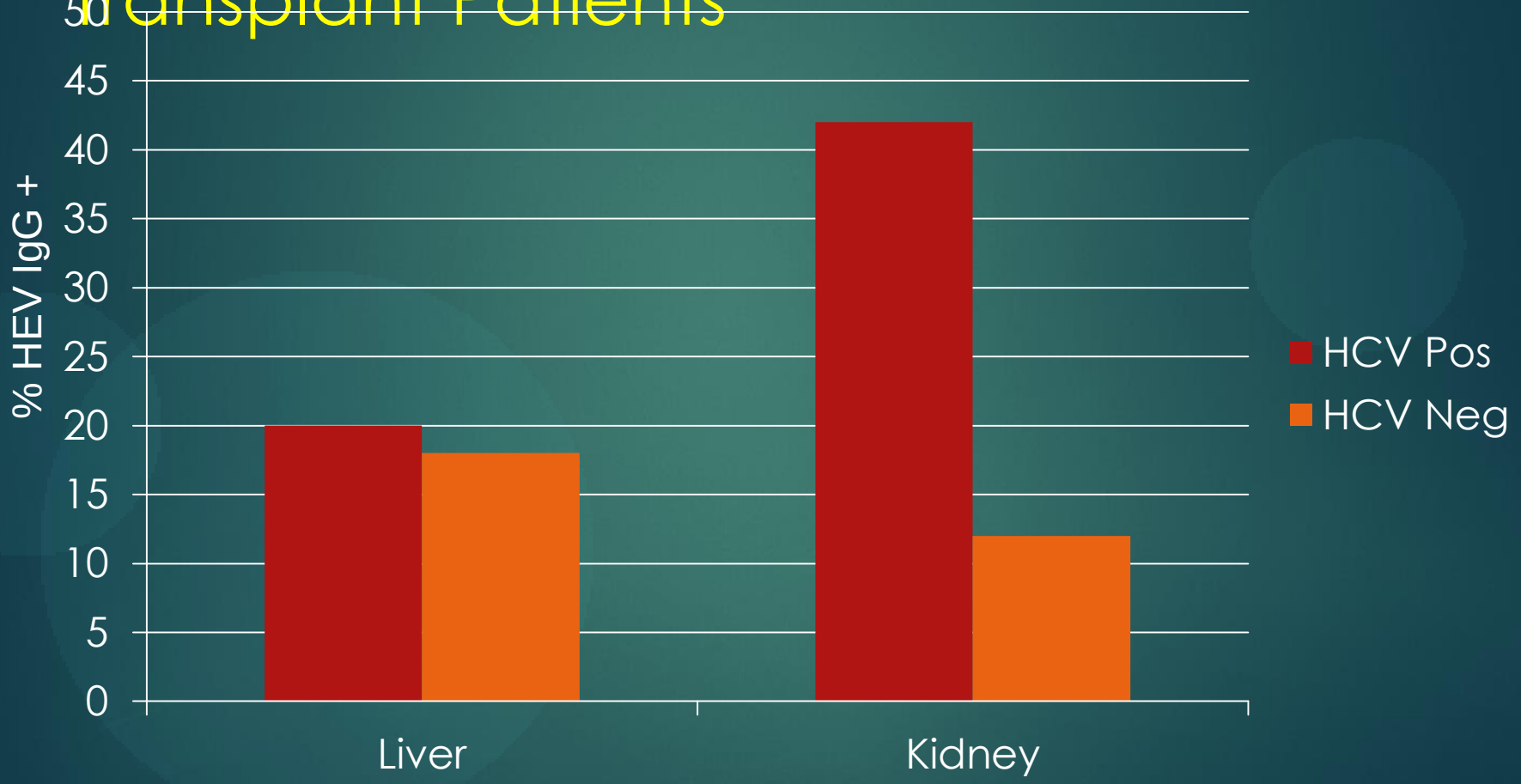
Sherman et al, J VIRAL HEP (in press)

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

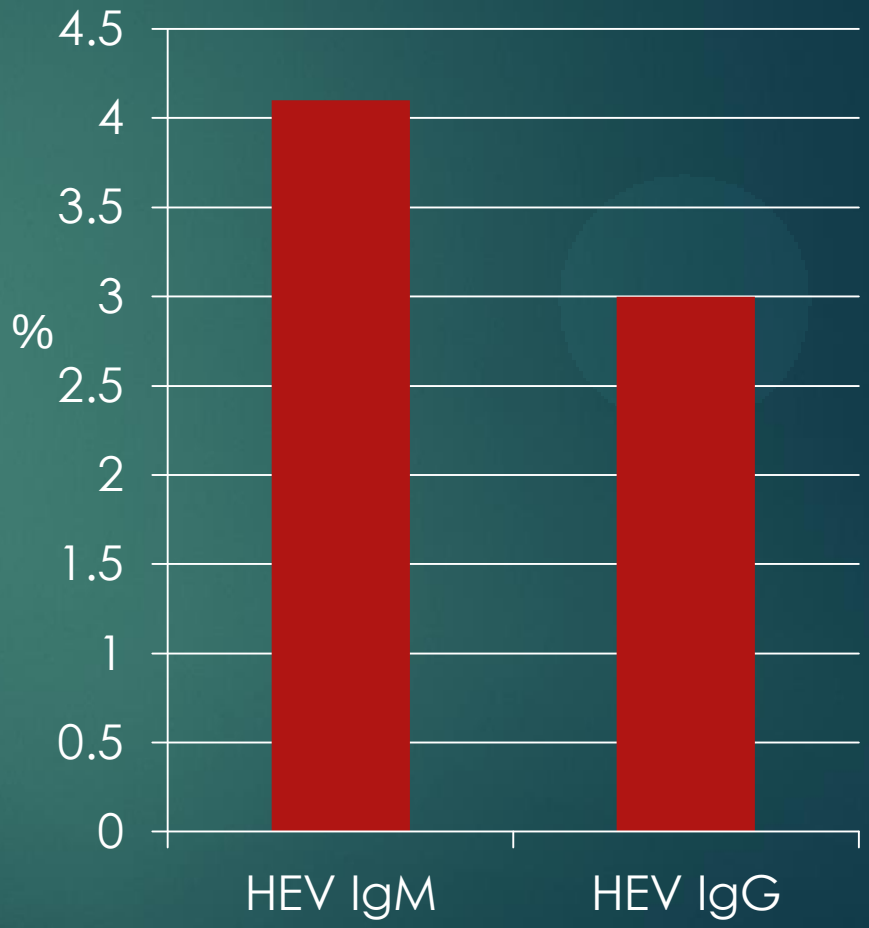
- Relationship to…
  - Age in Kidney Recipients Only
- No relation to….
  - ALT
  - CD4
  - Geography
  - Gender

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

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

CHRONIC HEV in HIV

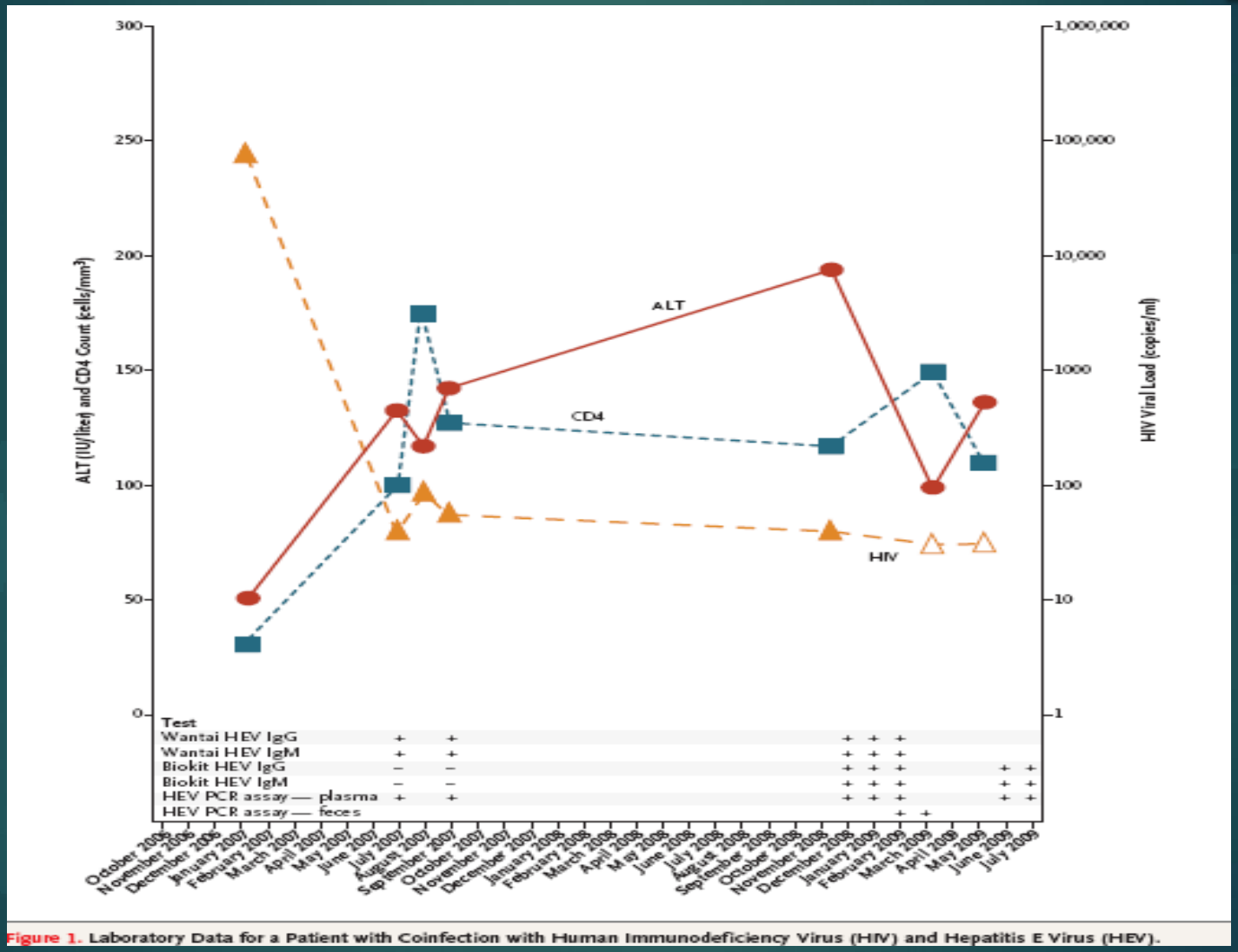
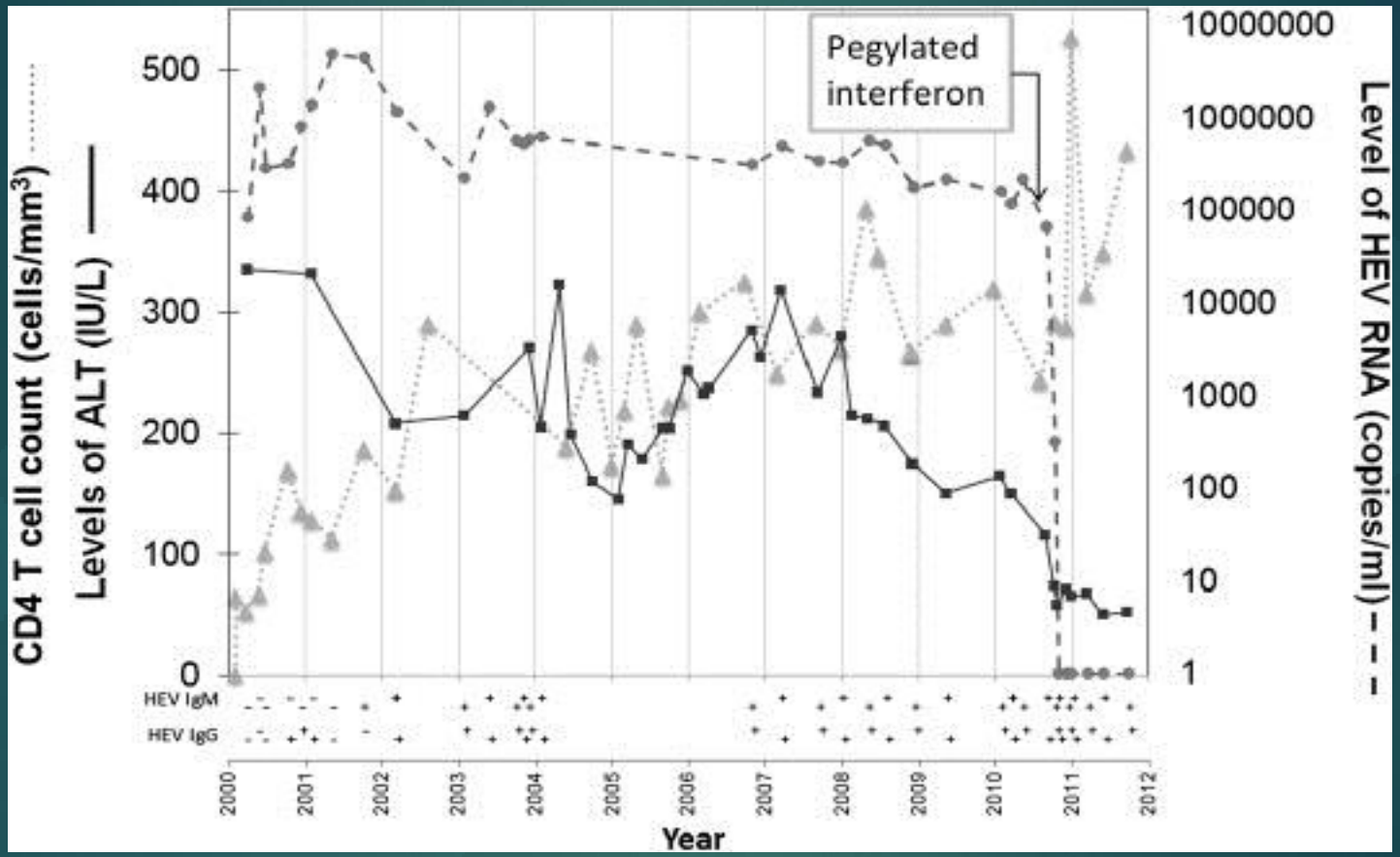


Figure 1. Laboratory Data for a Patient with Coinfection with Human Immunodeficiency Virus (HIV) and Hepatitis E Virus (HEV).

Dalton et al., NEJM, 2009

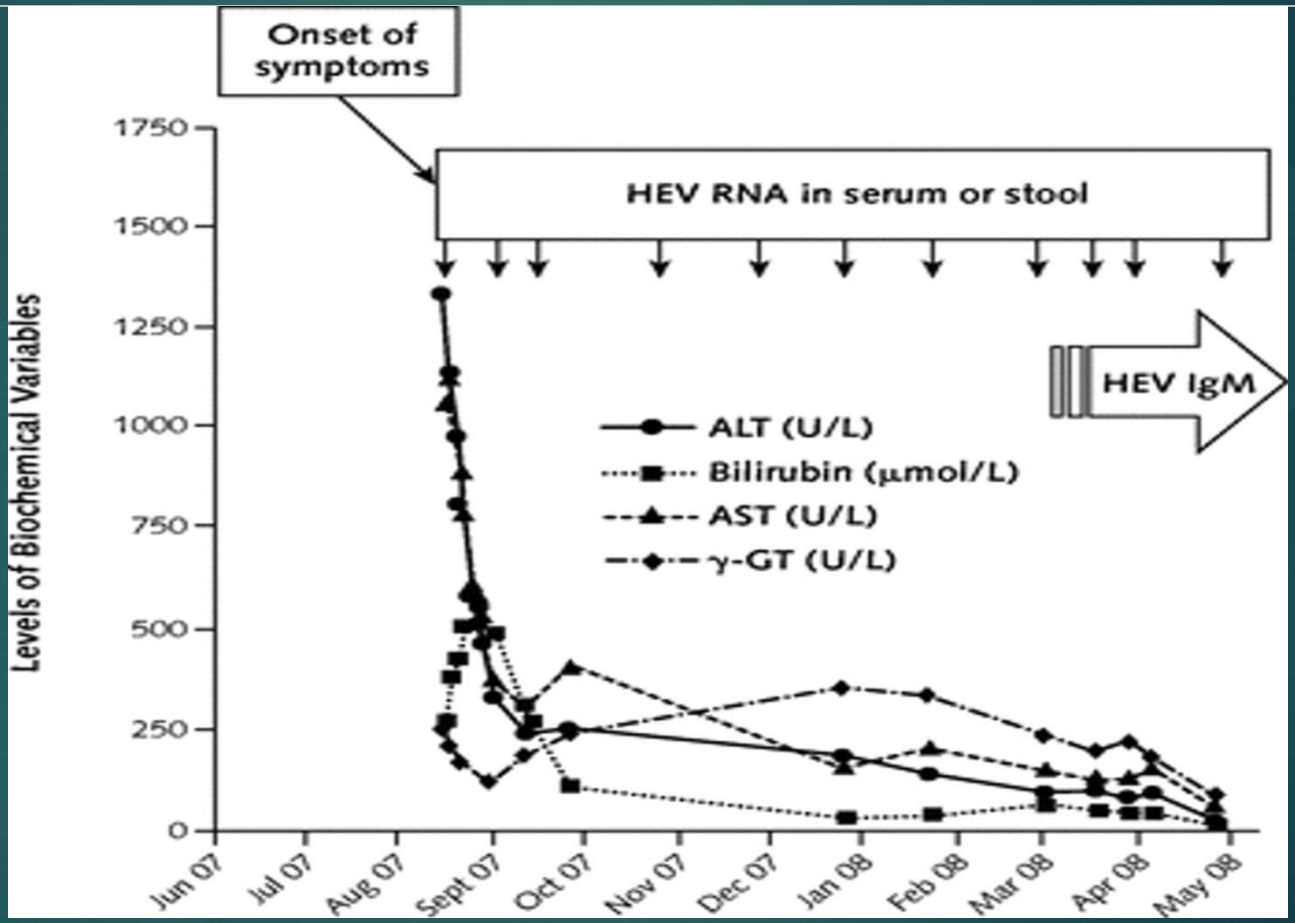
CHRONIC HEV IN HIV
Progression to "Cryptogenic" Cirrhosis



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

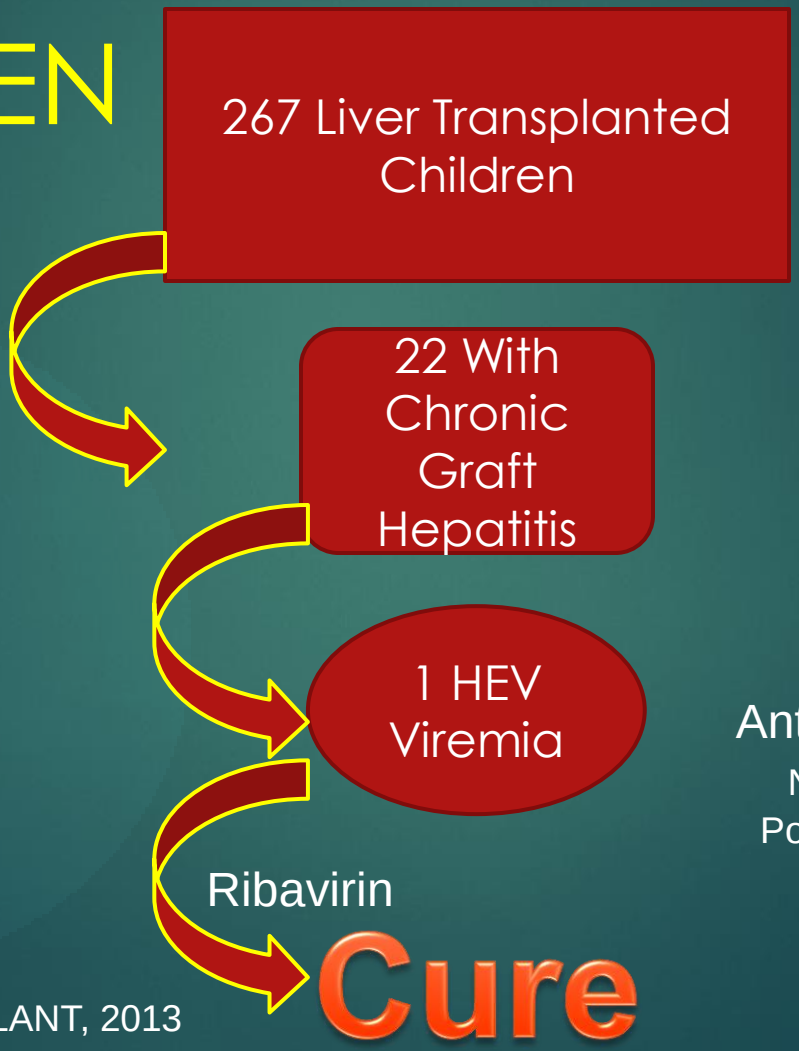


Ann Intern Med. 2009;150(6):430-431.

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



Anti-HEV IgG

Negative- MP

Positive- Wantai

Junge et al, PED TRANSPLANT, 2013

HEV Infection in Immunocompetent and Immunosuppressed Patients

	Immunocompetent	Immunosuppressed
Presentation	Often symptomatic	Rarely symptomatic
ALT at Diagnosis	1000-3000 IU/L	100-300 IU/L
HEV Genotype	Genotype 1,2,3, or 4	Only Genotype 3 has been reported
HEV Diagnostics	Increase in IgM and IgG PCR (+) in 75%	Requires PCR Serologic testing unreliable seroconversion may not occur
Outcome	Resolving Hepatitis	Chronic infection in 60% (higher liver) and 10-15% develop cirrhosis

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

Hoofnagle NEJM 2012;367:1237-44

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)
  - High HEV seroprevalence in nonendemic countries (Thomas et al., 1997)
- Different prevalence rates using different assays

Khudyakov Virus Research 2011;161:84-92

Variability of anti HEV IgM assays

Assay	Sensitivity	Specificity
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CDC*	98%	93.4%
International Immuno-Diagnostics (Foster City CA)	82.4%	91.7%
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Diagnostic Systems (Russia)	98%	96.6%
Mikrogen GmbH (Germany)	92.2%	96.1%

*Not commercially available

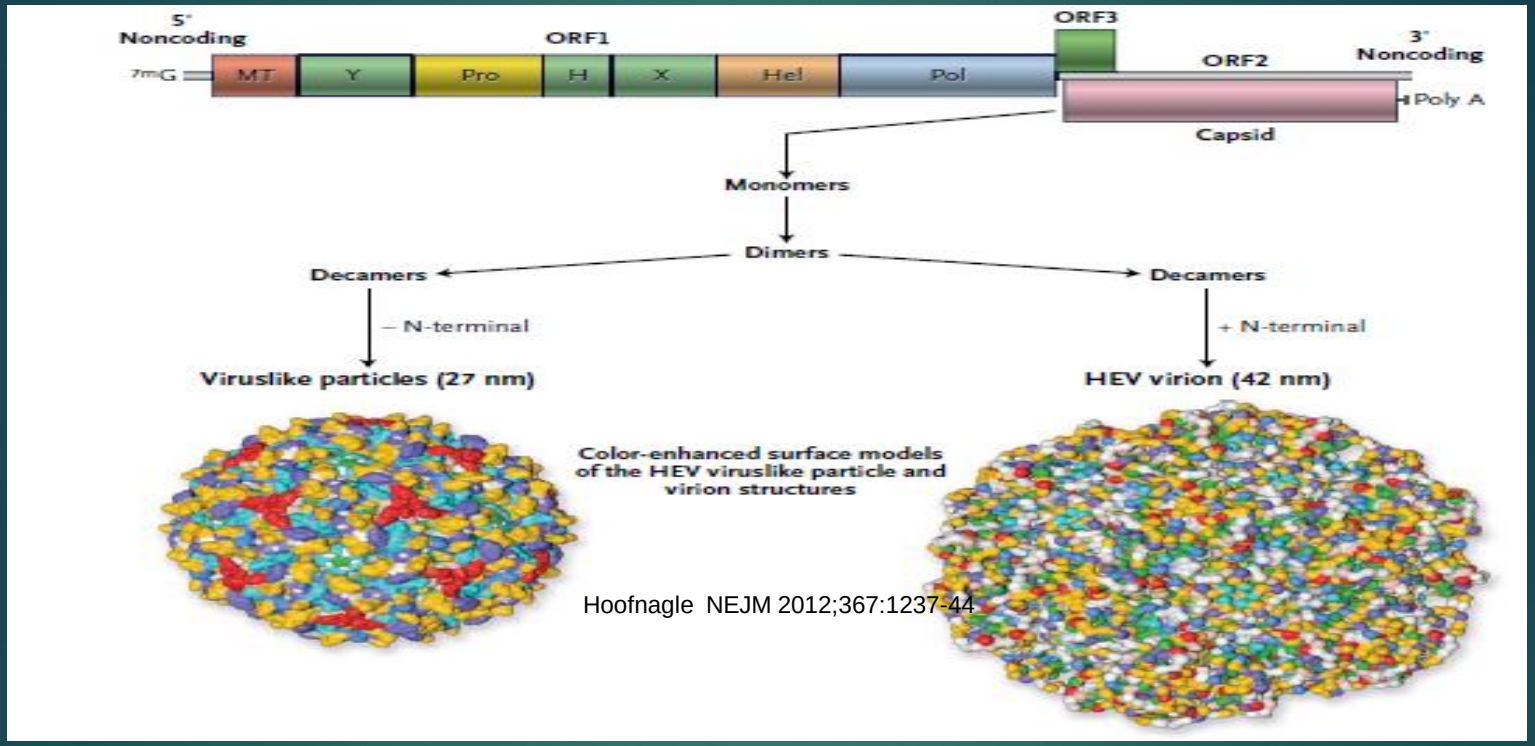
All samples in sensitivy panel wth acute jaundice, (-) ABC and HEV RNA+

All HEV RNA (+) with well defined genotypes

Drobenuic Clin Infect Dz 2010 ;51:e24-27

HEV: Antibody Testing

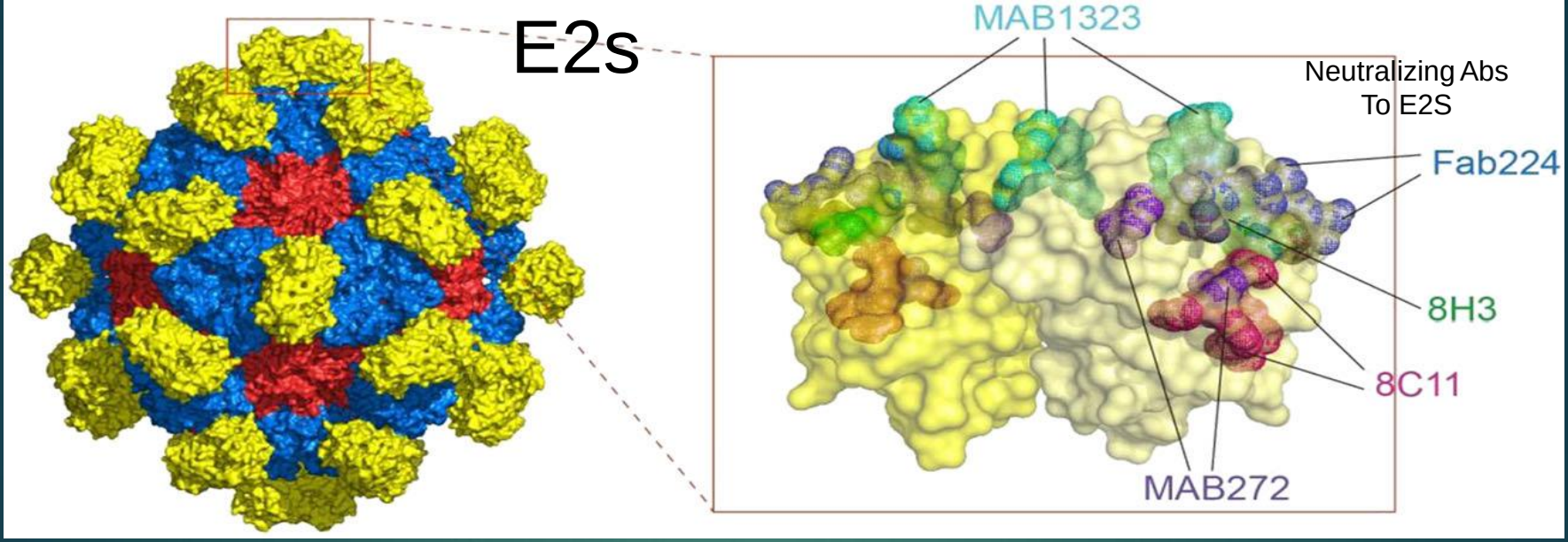
- Synthetic peptides derived from ORF2 and ORF3 proteins are major targets used for HEV diagnostic assays



- Differences in peptides used accounts for differences in sensitivity

Khudyakov Virus Research 2011;161:84-92

Crystal structure of HEV capsid protein and neutralizing sites



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  - Shell (red above) AA 118-313
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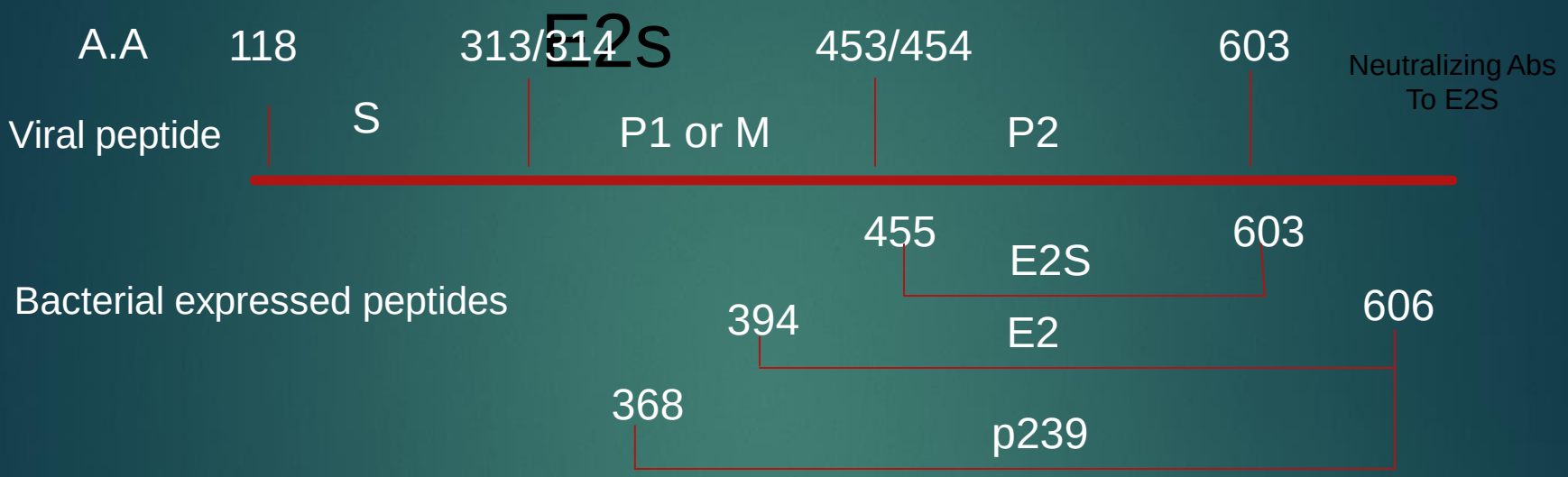
  - Protruding domain (P2 yellow) AA 454-606
- E2s domain is bacterially expressed Peptide (= to P2 domain) contains all identified neutralizing epitopes

Zang Rev Med Virol 2012;22:339-349, Kamar Lancet 2012;379:2477-2488

Bacterial Expressed Peptides from Viral P2 Domain used for Anti-HEV Testing



- Peptide pE2 contains 66 additional AA with extension into P1
  - appears to stabilize dimeric structure, making it a useful dx agent
- p239 adds 26 more AA in P1 domain.
  - Additional of 26 AA results in formation of a Virus like particle, enhancing immunogenicity
- The antigenicity of these peptides is virtually similar

Zang Rev Med Virol 2012;22:339-349,

HEV: Differences Main anti-IgG assays

- The GL assay (IgG kit by Genlabs [Singapore])
  - Used to be most commonly used assay world wide
  - Serum Ab directed to recombinant peptides ORF2(negative terminal) and ORF3 (complete protein)
  - Lack of E2s domain (On ORF2)

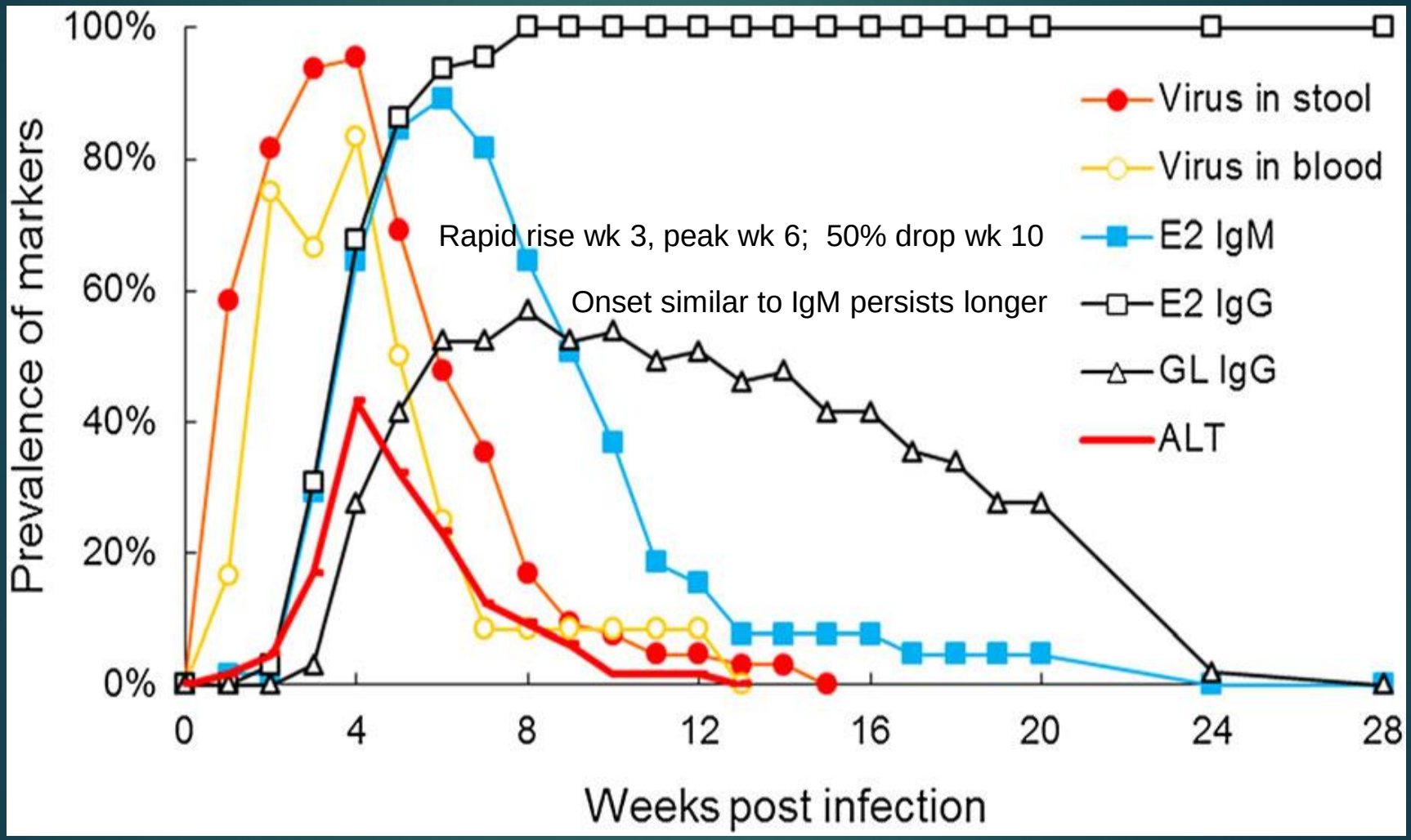
Bendall J Med Virol 2010;82:799-805
Zang Rev Med Virol 2012;22:339-349Park BMC Inf Disease 2012;12:1-6

HEV: Difference 2 anti-IgG assays

- Wantai (PEG2) E2 assay
  - 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

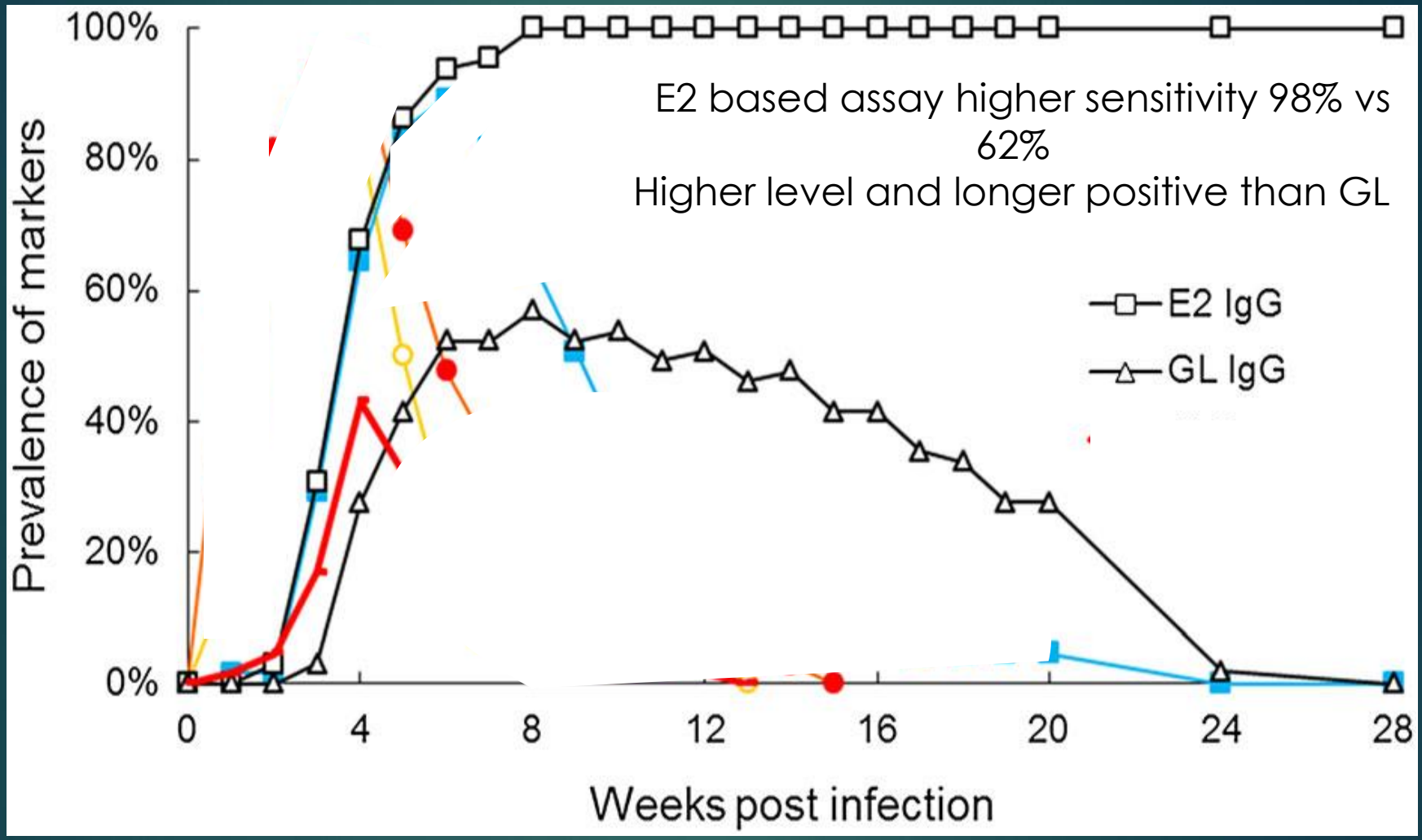
Bendall J Med Virol 2010;82:799-805
Zang Rev Med Virol 2012;22:339-349Park BMC Inf Disease 2012;12:1-6

HEV infection 86 Rheus monkeys



Zang Rev Med Virol 2012;22:339-349

Hepatitis E 86 Rheus Monkeys



Zang Rev Med Virol 2012;22:339-349

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 versus2.5Wu/mL
  - (+) in more sera from PCR-confirmed cases (98% vs. 56%)
  - Remained (+) longer post infection;

- E2 assay with higher estimate of prevalence in
  - 500 UK blood donors (16.2% vs. 3.6%)
  - *147 health check pts Korea 23.1% vs 14.3%

Bendall J Med Virol 2010;82:799-805
*Park BMC Inf Disease 2012;12:1-6

Conclusion Anti-HEV Ab Testing in Immunocompetent Patients

- Acute HEV can be accurately diagnosed using Anti-HEV IgM Ab testing
- 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

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-**P**CR targeting ORF2 or ORF3 should be accurate for diagnosis of chronic HEV
- Need standardization to more accurately characterize viral levels

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

Baylis J Clinical Microbiology;2011;1234-1239

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Baylis J Clinical Microbiology;2011;1234-1239

Treatment

- Supportive care
- Consider ribavirin

Table 1. Characteristics of solid organ transplant recipients with HEV infection

	Age	TX-organ	Sex	Immunosuppression	Peak ALT	Peak INR	Therapy	Clearance of HEV within less than	Outcome
OLT 1	34	Liver	m	tac, mmf, decortin	239	1.3	Reduction of IS	3 months	SVR (follow-up >2 years)
OLT 2	40	Liver	m	ciclo, mmf, decortin	555	1.0	Reduction of IS	30 months	SVR (follow-up >2 years)
KTR 1	43	Kidney	m	tac, mmf, decortin	359	1.0	Reduction of IS	6 months	SVR (follow-up >2 years)
KTR 2	65	Kidney	m	ciclo, mmf, decortin	1566	1.0	Ribavirin	1 month	SVR (follow-up >2 years)
KTR 3	50	Kidney	m	ciclo, mmf, decortin	160	1.0	Ribavirin	2 months	SVR (follow-up >5 months)
KTR 4	40	Kidney	m	tac, mmf	342	1.1	Ribavirin	2 months	SVR (follow-up >4 months)
KTR 5	54	Kidney	m	ciclo, sirolimus	2053	1.1	Ribavirin	1 month	SVR (follow-up >4 months)
HTR 1	50	Heart	f	ciclo, decortin, everolimus	217	1.0	Ribavirin	2 month	SVR (follow-up >2 years)
HTR 2	66	Heart	m	ciclo, decortin, everolimus	209	1.1	Ribavirin	1 month	SVR (follow-up >2 years)
HTR 3	57	Heart	m	ciclo, decortin, azathioprine	211	1.1	Ribavirin	1 month	SVR (follow-up >2 years)
HTR 4	58	Heart	m	tac, decortin, everolimus	315	1.1	Ribavirin	No clearance	Patient died from liver cirrhosis-associated complications
LuTR 1	48	Lung	f	tac, mmf, decortin	89	1.4	Ribavirin	2 months	SVR (follow-up >2 months)
LuTR 2	56	Lung	f	ciclo, mmf, decortin	254	1.1	Ribavirin	2 months	SVR (follow-up >7 months)
LuTR 3	32	Lung	m	ciclo, mmf, decortin	270	1.0	Ribavirin	No clearance	Patient died because of failure of lung transplant (6 weeks after begin of treatment)
LuTR 4	41	Lung	m	tac, mmf, decortin	215	1.6	No therapy	No clearance	Patient died before diagnosis of HEV infection (retrospectively identified)

Liver International ISSN 1478-3223

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[2,8], Juergen Wenzel[9], Michael P. Manns[1,2] and Heiner Wedemeyer[1,2,†]

Treatment of hepatitis E with ribavirin

Pischke *et al.*

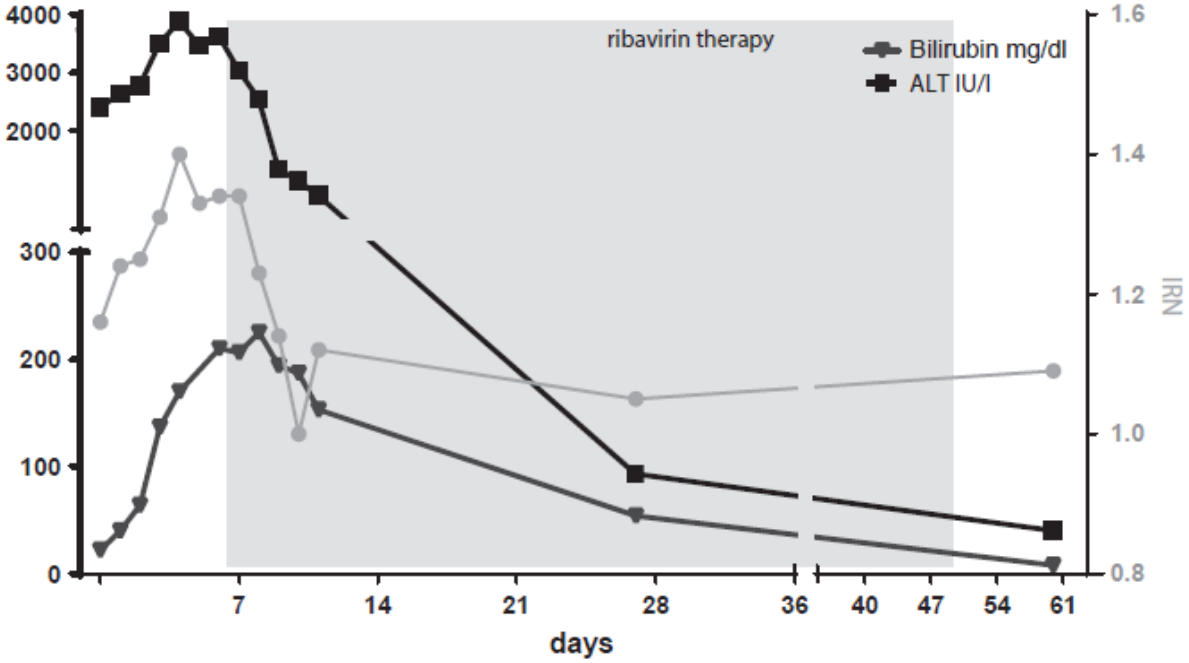

Fig. 1. Course of bilirubin, ALT and INR in a patient with acute hepatitis E, treated with ribavirin for 6 weeks.

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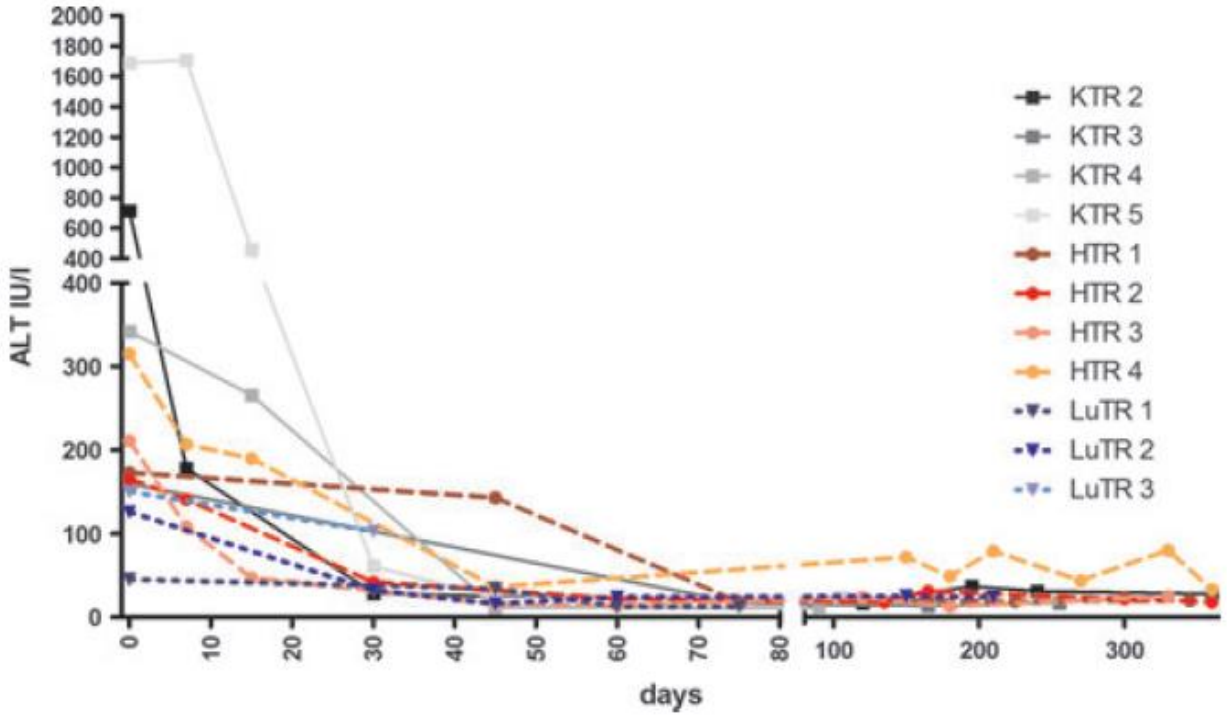

Fig. 2. One-year follow-up (from the beginning of ribavirin treatment) of ALT levels in transplant recipients treated with ribavirin. Patient HTR 4 suffered from viral breakthrough. Patient LuTR 3 died from non liver or therapy-associated death.

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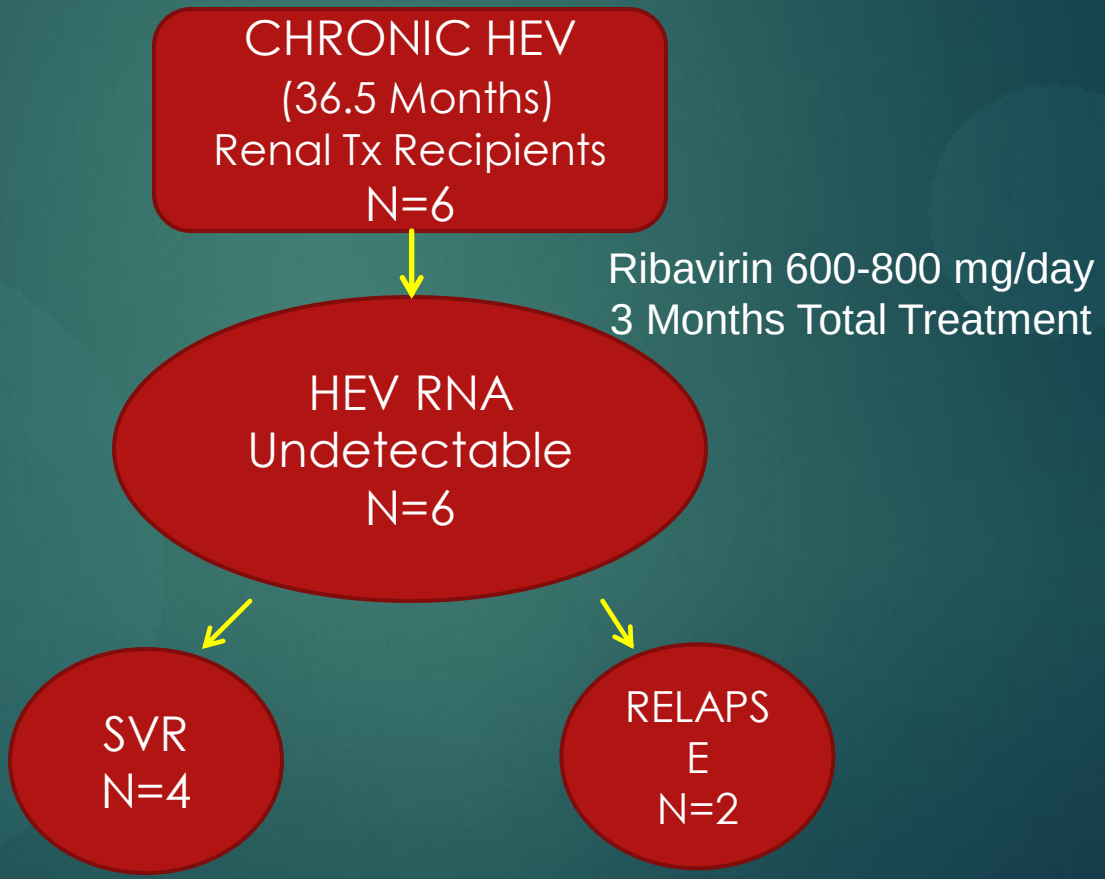
Table 1. Treatment of Patients With Chronic HEV Infection

Group of patients	Treatment	Outcomes	First author, year
16 Liver and kidney transplantation patients with chronic HEV infection	Reduction of immunosuppression	4 of 16 patients HEV RNA–negative	Kamar, 2011[78]
56 Liver and kidney transplant recipients with chronic HEV infection	Reduction of immunosuppression	18 of 56 patients HEV RNA–negative	Kamar, 2011[63]
3 Liver transplant recipients with chronic HEV infection	3-month course with pegylated interferon-alfa-2a	2 of 3 patients cleared HEV RNA, 1 relapsed after treatment	Kamar, 2010[79]
2 Liver transplant recipients with chronic HEV infection	16 weeks or 1 year of treatment with pegylated interferon-alfa-2b	2 of 2 patients cleared HEV RNA	Haagsma, 2010[80]
1 HIV-infected patient with chronic HEV infection	6 months pegylated interferon monotherapy, followed by 12 weeks of therapy with the combination of interferon and ribavirin	Patient tested negative for HEV RNA	Dalton, 2011, Ann Intern Med[88]
7 Recipients of solid organ transplants	Treatment with ribavirin monotherapy for 5 months	6 of 7 patients cleared the virus, and 1 is still a carrier of HEV	Unpublished data from our group
6 Recipients of solid organ transplants	Treatment with ribavirin monotherapy for 3 months	4 of 6 patients achieved sustained virologic response, 2 relapsed	Kamar, 2010[82]
9 Patients with various conditions of immunosuppression	Treatment with ribavirin monotherapy for 3 months	9 of 9 patients cleared the virus, no relapse	Mallet, 2010, AASLD Annual Meeting[56]

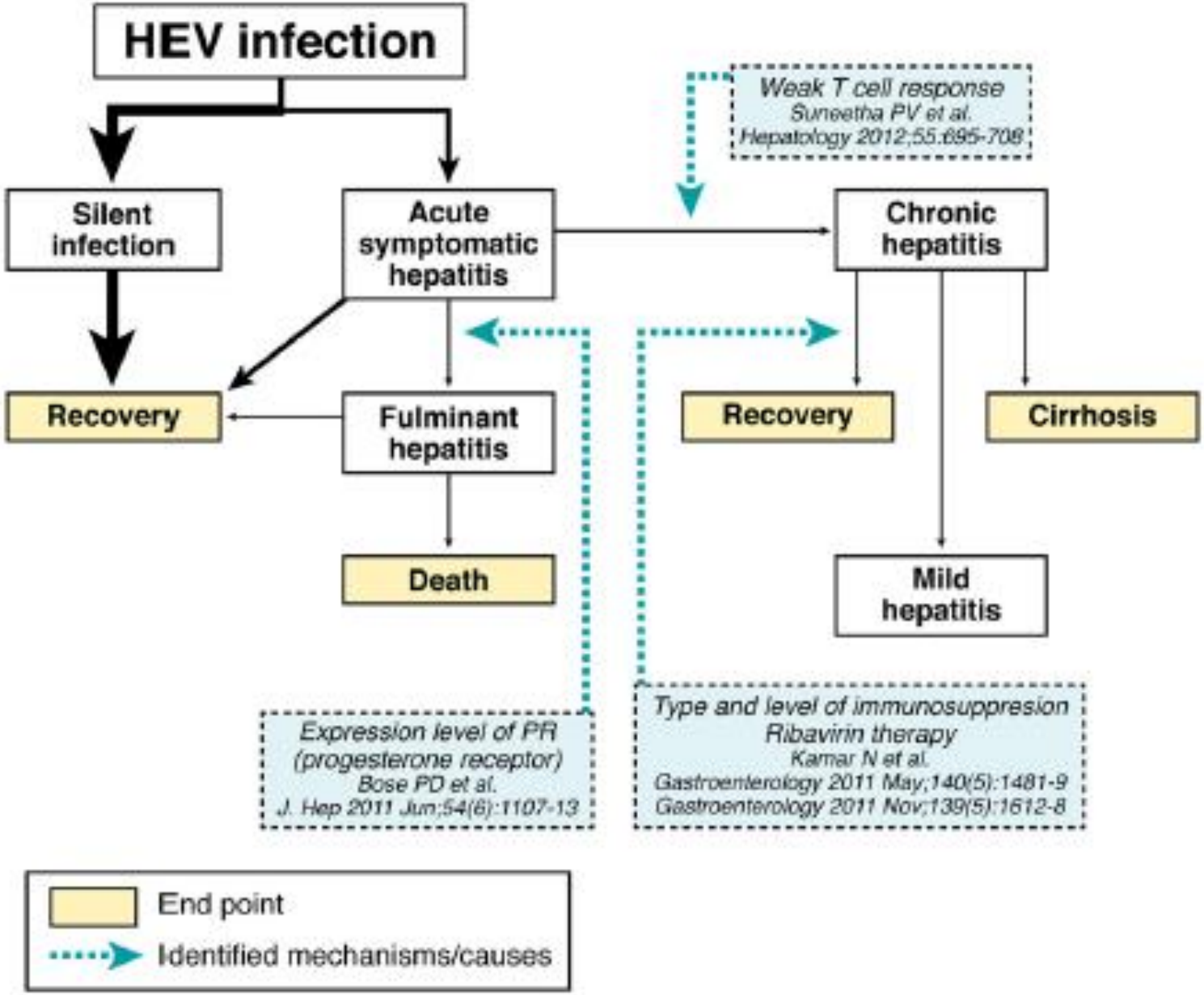
AASLD, American Association for the Study of Liver Disease.

GASTROENTEROLOGY 2012;142:1388–1397

RIBAVIRIN THERAPY FOR HEV in Renal Tx recipients



Kamar et al, GASTROENTEROLOGY, 2010



GASTROENTEROLOGY 2012;142:1388–1397

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 immunosupressed 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 solders 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*

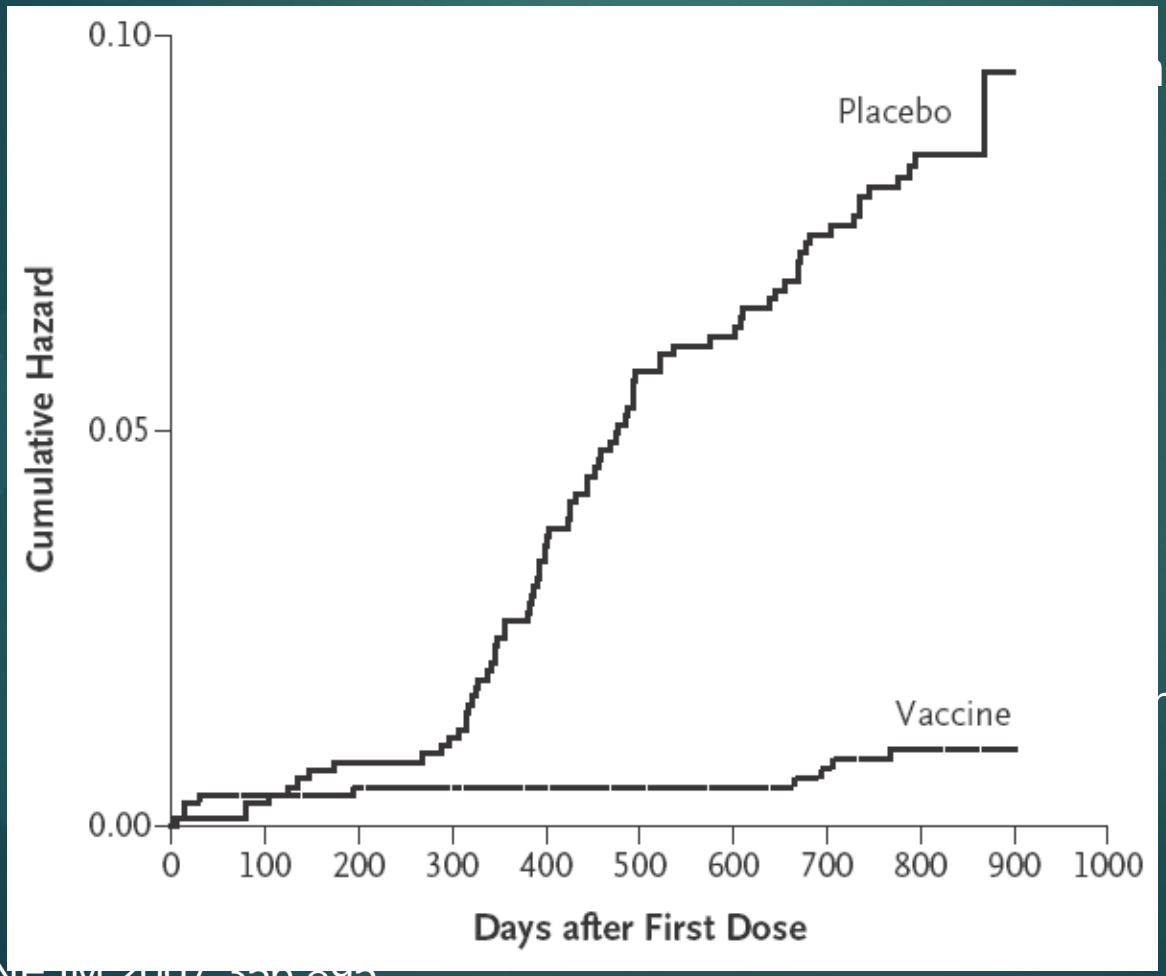
Hepatitis E Vaccine Application*

- 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



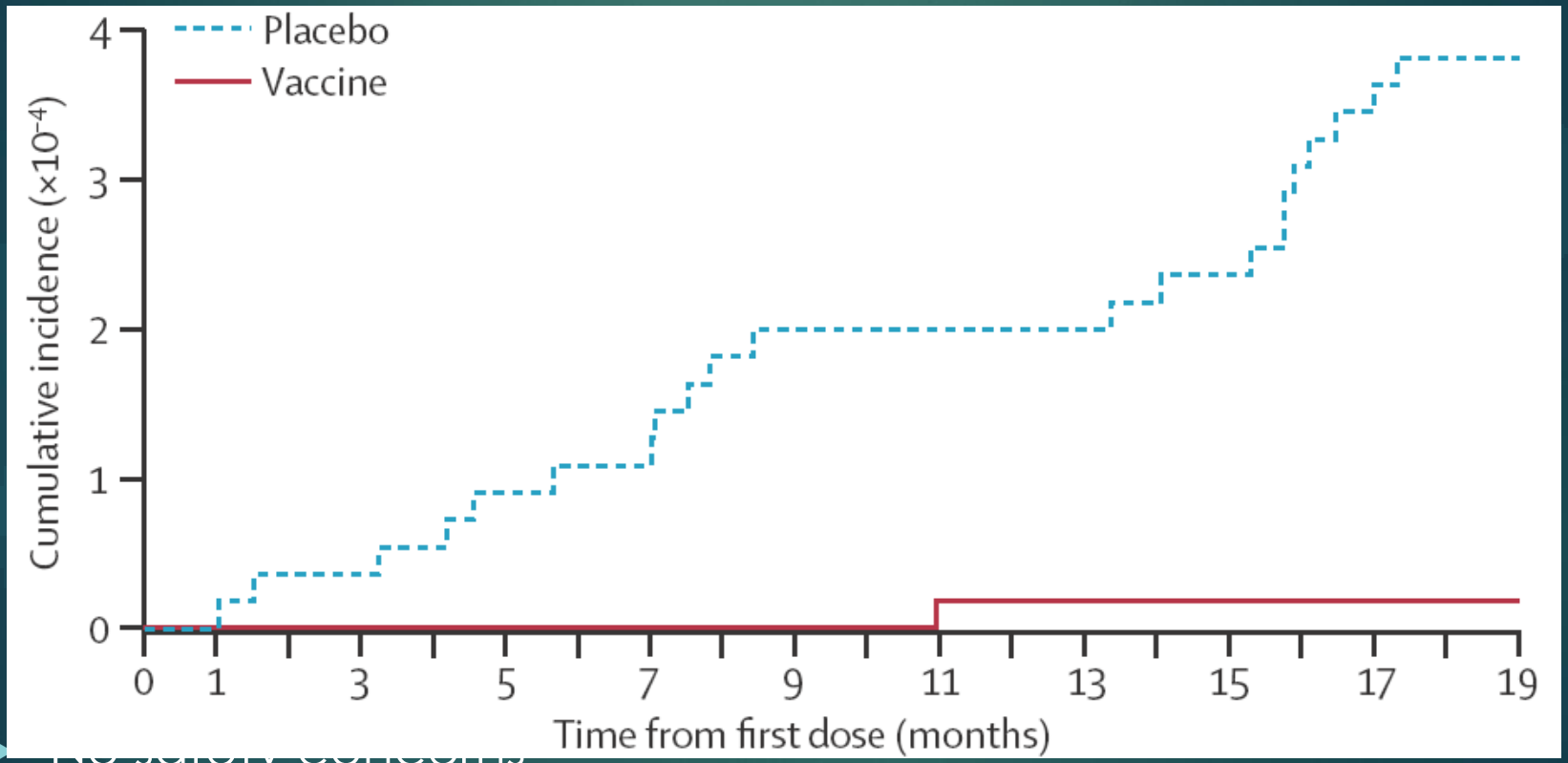
=66 (7.4%)

=3 (0.3%)

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



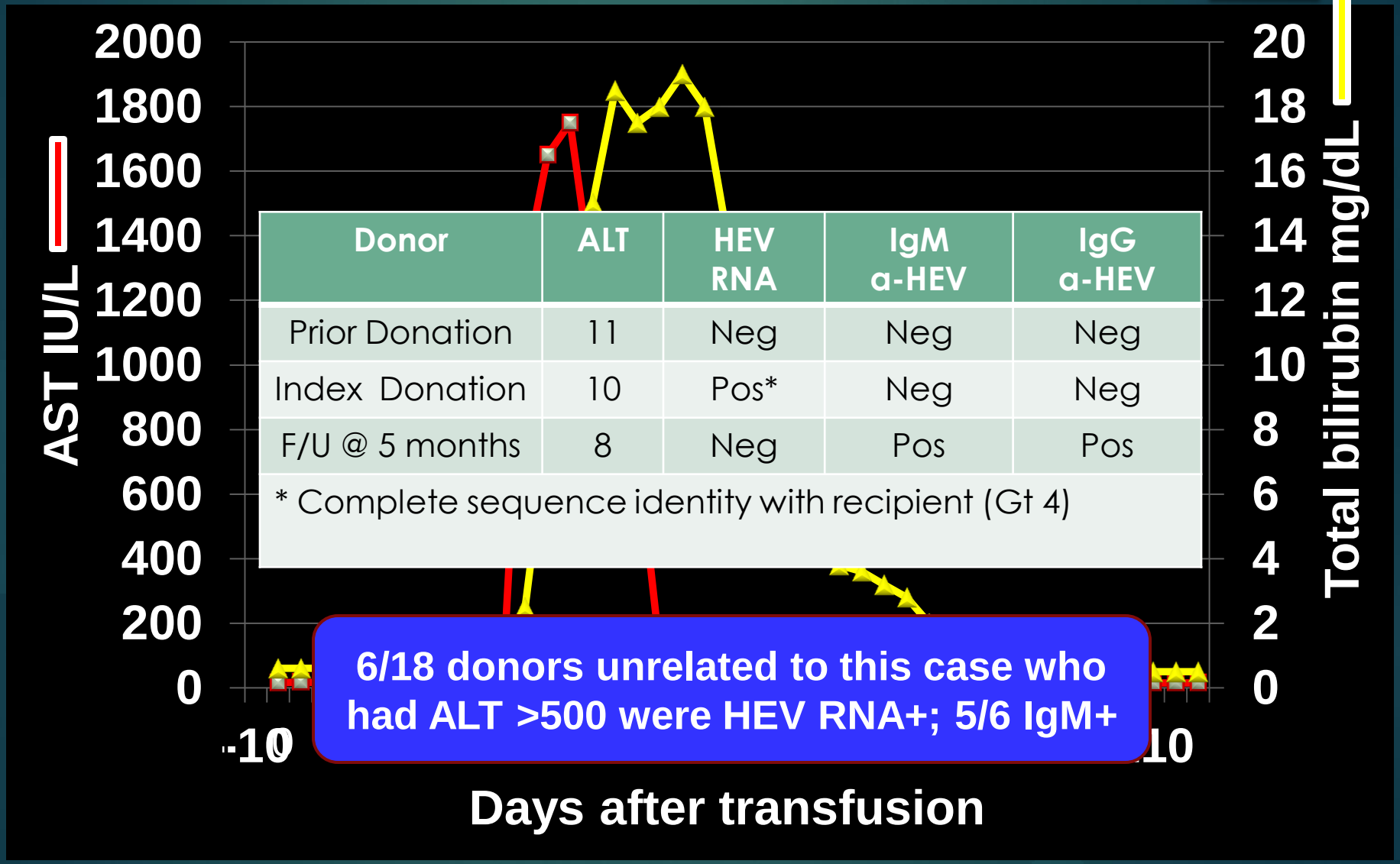
- No safety concerns

Zhu Lancet 2010;376:895

Blood Donor Testing

- The next phase?

Clinical Course of Transfusion-Transmitted HEV: First case in Japan



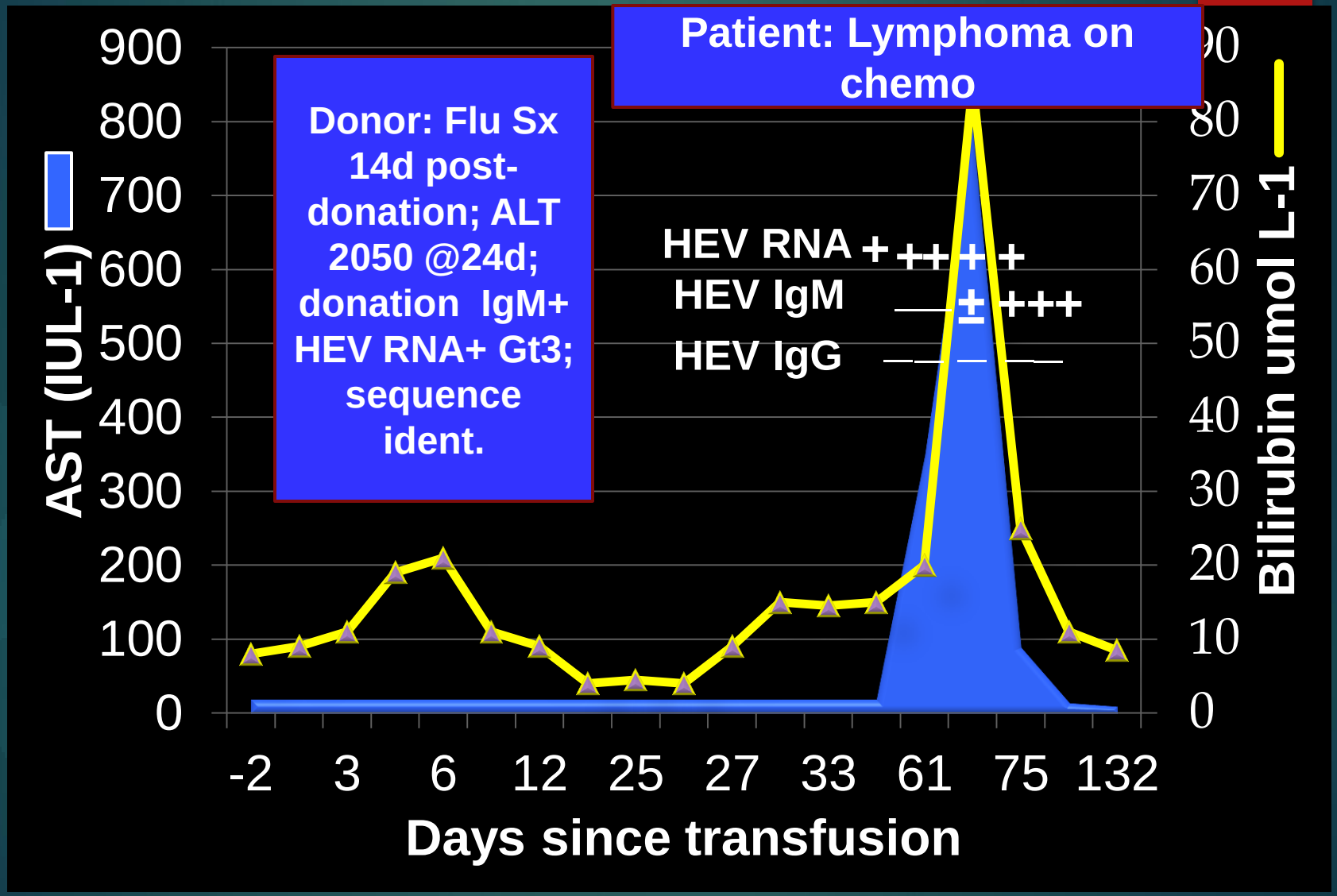
Donor	ALT	HEV RNA	IgM a-HEV	IgG a-HEV
Prior Donation	11	Neg	Neg	Neg
Index Donation	10	Pos*	Neg	Neg
F/U @ 5 months	8	Neg	Pos	Pos

* Complete sequence identity with recipient (Gt 4)

6/18 donors unrelated to this case who had ALT >500 were HEV RNA+; 5/6 IgM+

Matsubayashi K. Transfusion 2004;44:934

Transfusion-Transmitted HEV: First Case in England



Boxall, E. Transfusion Medicine 2006

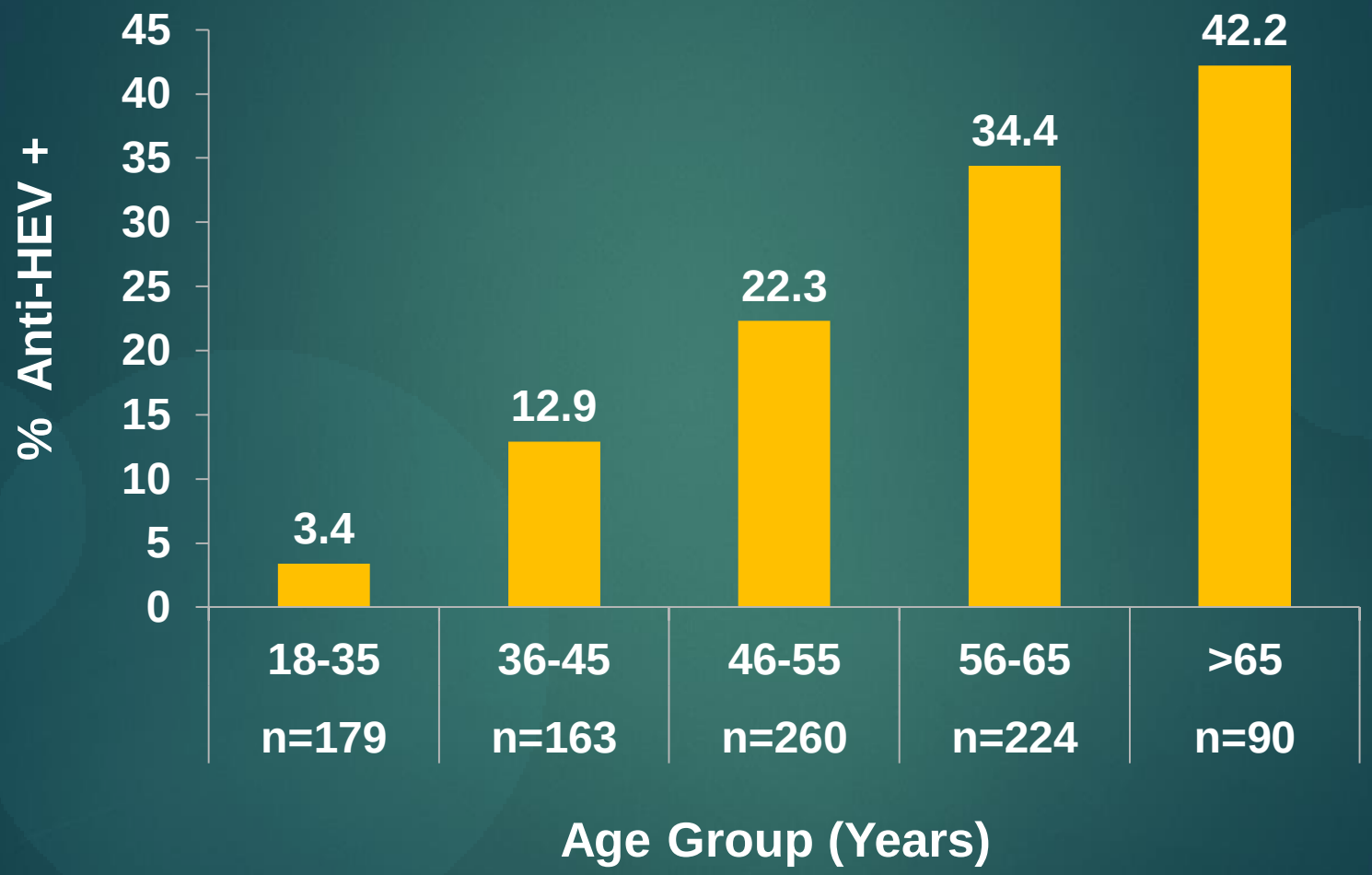
HEV MARKERS IN NIH VOLUNTEER BLOOD DONORS

No. Tested	Anti-HEV IgG+	Anti-HEV IgM+	HEV RNA*
1939	364 (18.8%)*	8 (0.4%)	0 (0%)

* 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



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+

250 Non-Tx → 2 (0.8%) IgM + HEV RNA+

P<.001

OR= 12

Prospective Study

25 Tx → 3/22 (13.6%) Suscept. HEV Infected

25 Non-Tx → 0/25 Suscept. HEV Infected

Traced to 4 donors HEV RNA+; IgM+

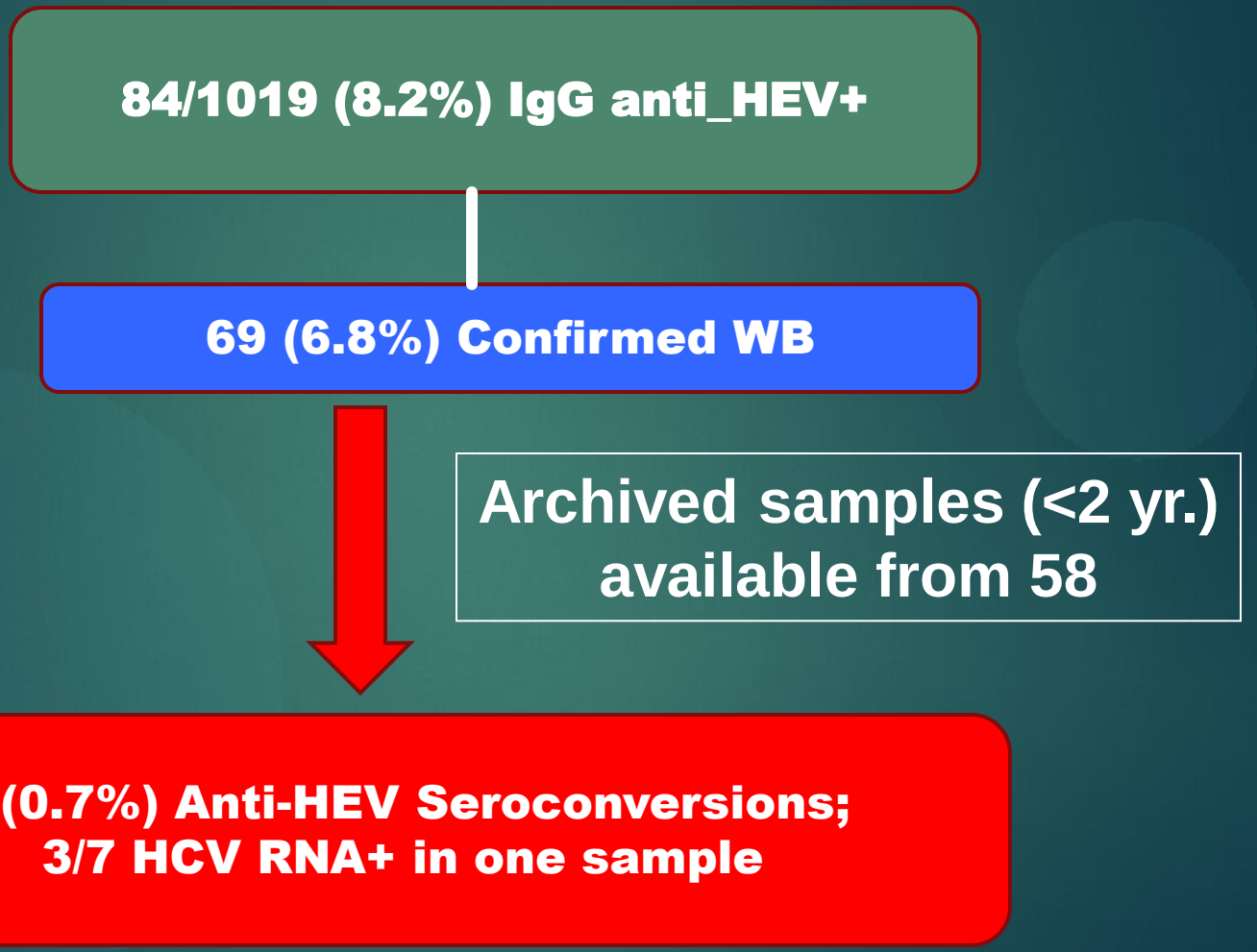
Retro: Tx <3 Mos. Pre-test

13/30 (43%) HEV RNA+ ↔ 1/50 (2%) HEV RNA+

P<.001

OR=37

Seroprevalence and Incidence of HEV Infection in German Blood Donors



Juhl, D: Transfusion 2013

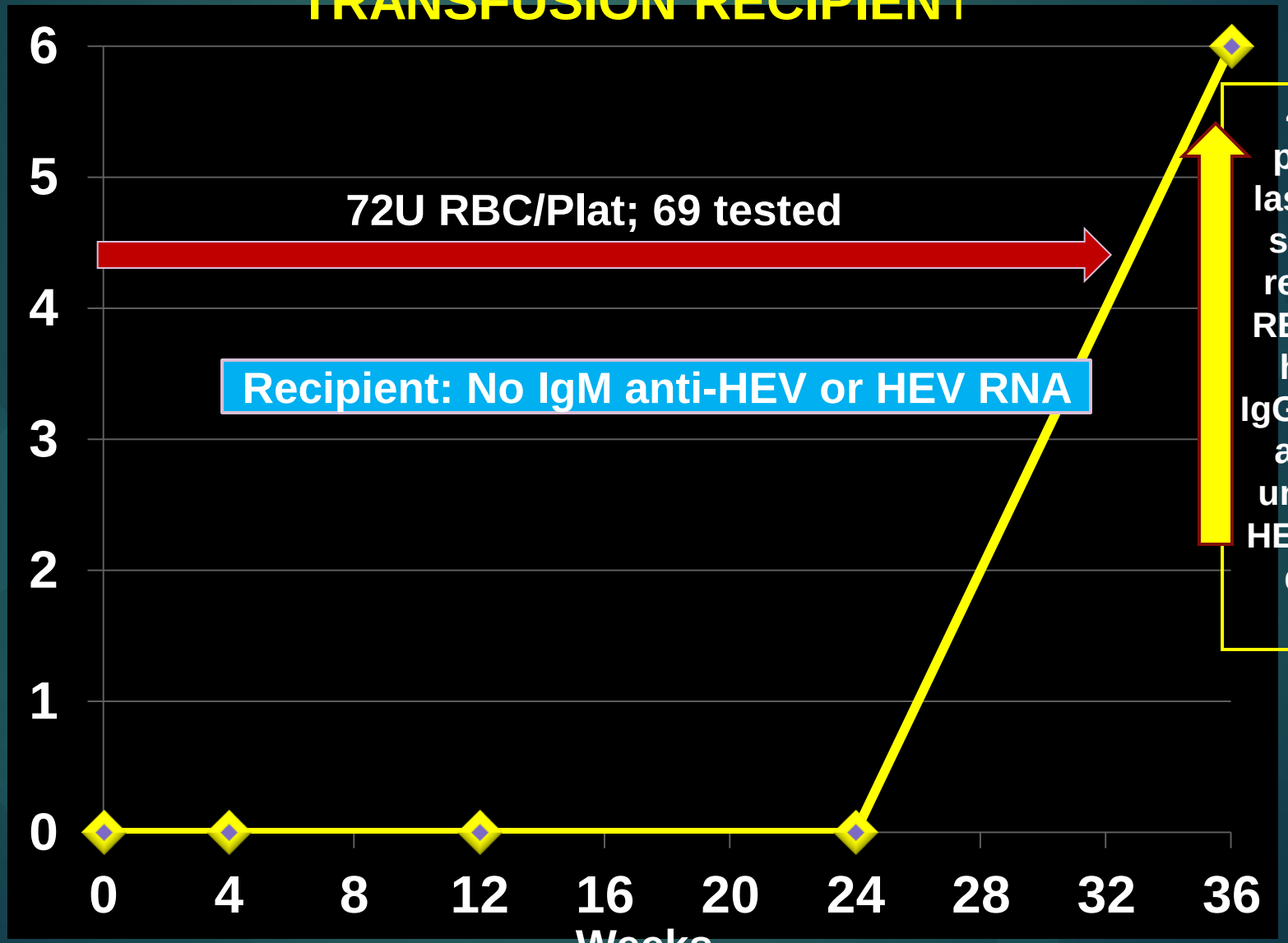
PROSPECTIVE EVALUATION OF HEV TRANSMISSION IN 362 TRANSFUSED PATIENTS

No. Anti-HEV IgG Seroconversions	No. Anti-HEV IgM+ or HEV RNA+	No. New Infections
2 (0.5%)**	0	0**

** 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

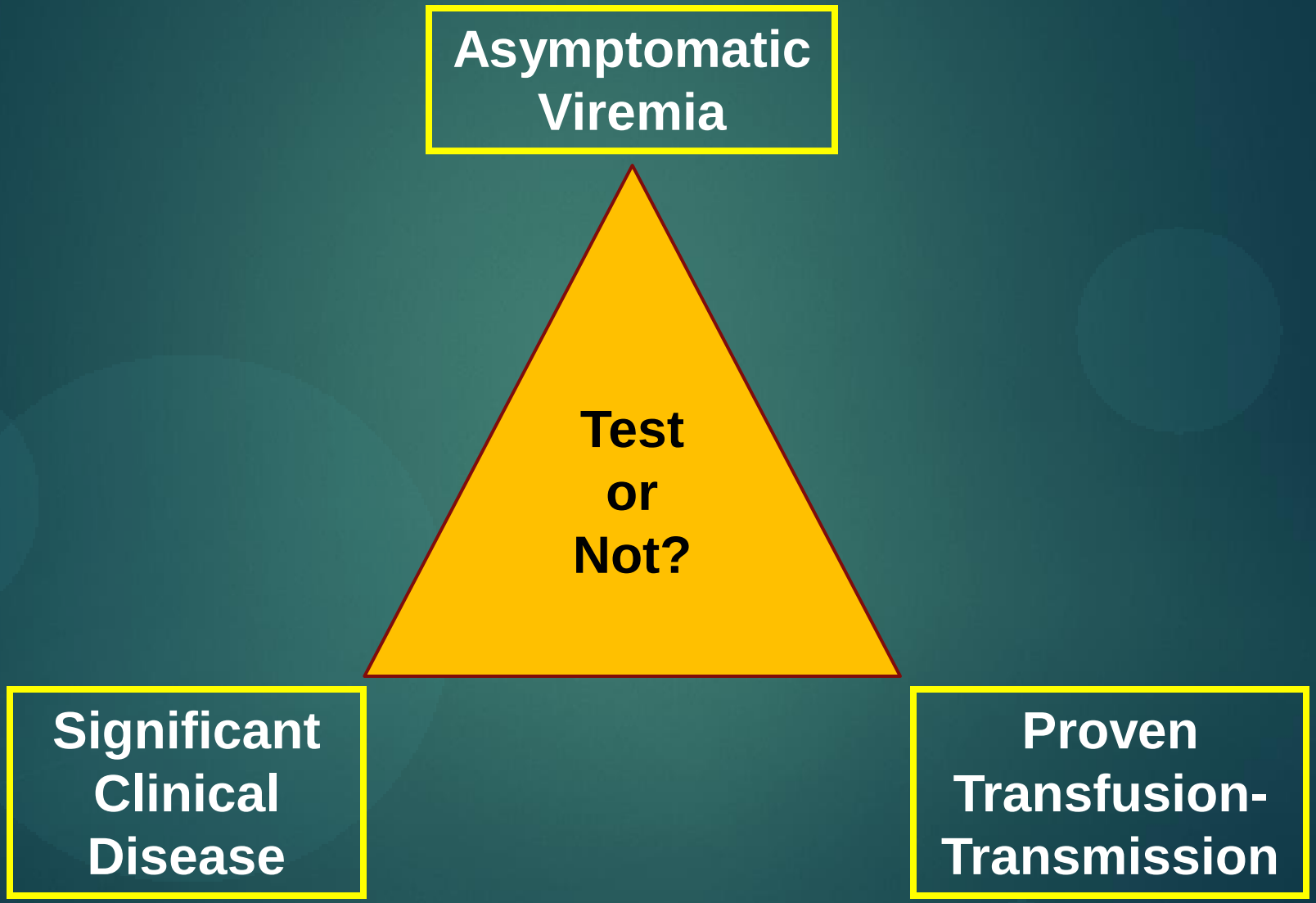
Upper bound of zero observed transmissions is 0.8%

COURSE OF ANTI-HEV IgG EVOLUTION IN A PROSPECTIVELY FOLLOWED SEROCONVERTING TRANSFUSION RECIPIENT



Should blood donors routinely be screened for evidence of HEV infection?

THE TRIANGLE OF TRANSFUSION TESTABILITY



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?