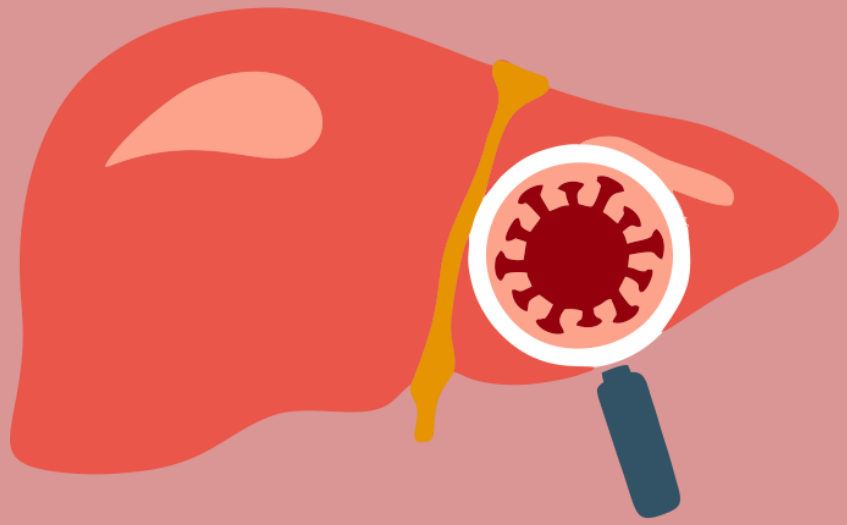
PRAKASH

PRogrammed Approach to Knowledge And Sensitization on Hepatitis



HEPATITIS INDUCTION PROGRAM

Viral Hepatitis

A & E

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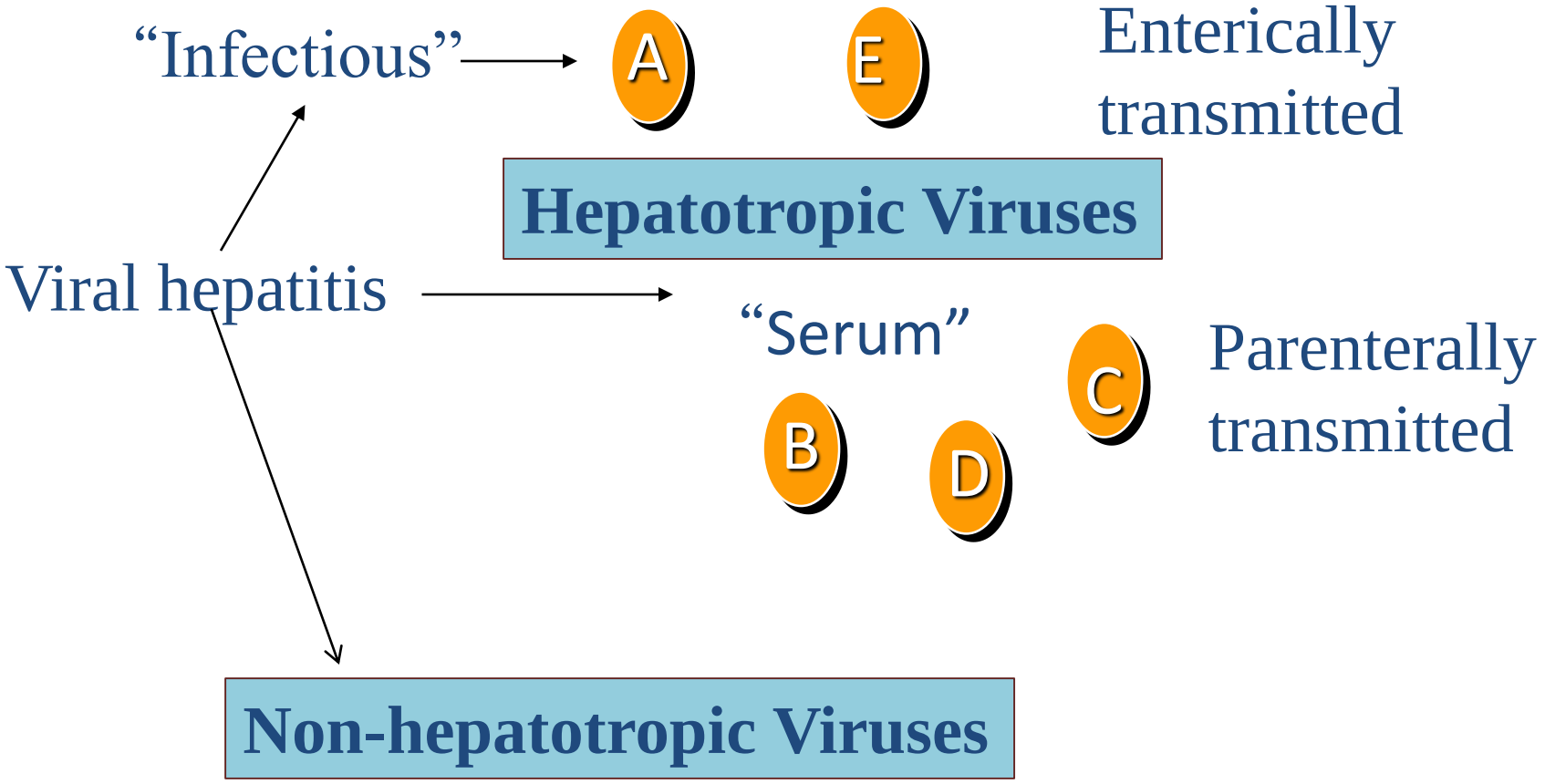
Clinical Virology, ILBS

New Delhi

Viral Hepatitis



: CMV/EBV/HSV/Dengue/influenza

India : Viral Hepatitis

Hyperendemic

Hyperendemic

Viruses causing hepatitis - an overview

	HAV	HBV	HCV	HDV	HEV
Family	Picornaviridae	Hepadnaviridae	Flaviviridae	Delta agent (satellite virus)	Hepeviridae
Nucleic acid	ssRNA	dsDNA	ssRNA	ssRNA	ssRNA
Routes of transmission	Fecal-oral	Parenteral	Parenteral	Parenteral	Fecal-oral
Chronic hepatitis	No	Yes	Yes	Yes	No (Yes- HEV genotype 3)
Oncogenic potential (HCC)	No	Yes	Yes	-	No
Vaccine available	Yes	Yes	No	HBV vaccine is protective	Yes (China)

Viral hepatitis - various clinical presentations

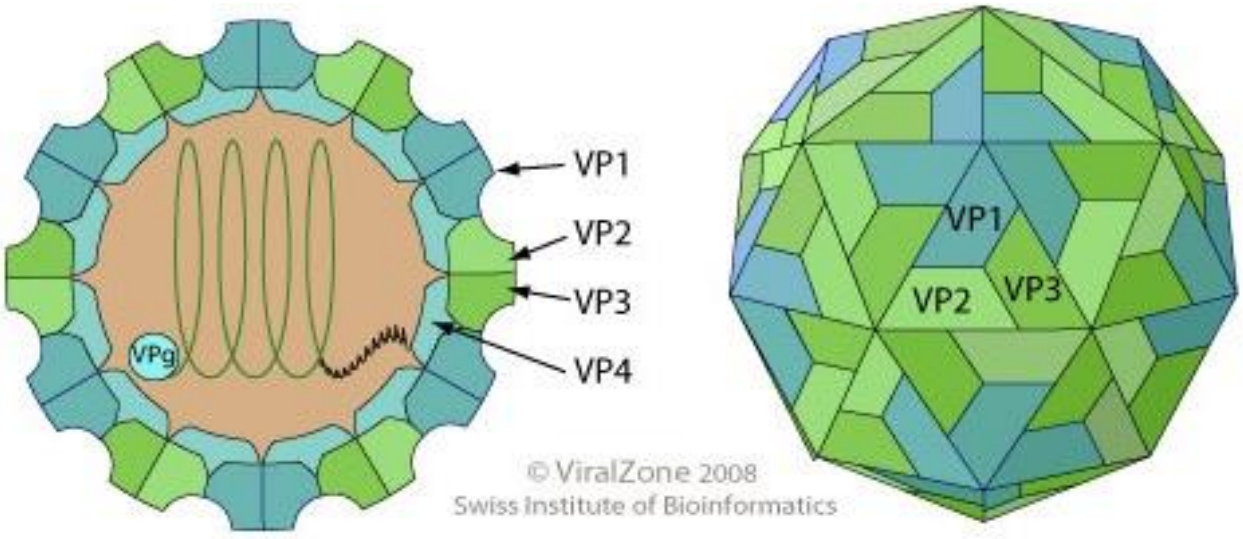
- Acute viral hepatitis (AVH)
- Chronic viral hepatitis - Chronic hepatitis B (CHB)
  - Chronic hepatitis C (CHC)
- Transaminitis
- Acute liver failure (ALF), Fulminant hepatic failure (FHF)
- Acute-on-chronic liver failure (ACLF)
  - acute (precipitating) event on an underlying chronic liver disease (CLD)

↓

- Acute viral hepatitis by HAV
- Acute viral hepatitis by HEV
- Acute viral hepatitis/reactivation (HBV)
- Super-infection with HDV
- Drug induced liver injury (DILI)

↓

- Alcoholic liver disease (ALD)
- Chronic hepatitis B (CHB)
- Chronic hepatitis C (CHC)
- Non-alcoholic fatty liver disease (NAFLD)
- Drug induced liver injury (DILI)
- Autoimmune hepatitis (AIH)
- Cryptogenic liver disease



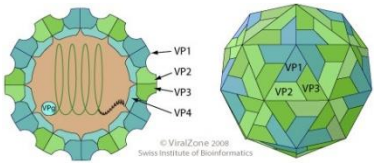
Hepatitis A Virus (HAV)

HAV - taxonomy

Family	Genus	Viruses	Example(s)
Picornaviridae	*Enterovirus*	Enteroviruses	Poliovirus 1-3, Coxsackieviruses A(1-24) & B(1-6), Echoviruses 1-33, Enteroviruses 68-116
		Rhinoviruses	HRV A,B,C - > 149 types
	Parechovirus	Parechoviruses	Parechoviruses 1-14
	Hepatovirus	HAV	Hepatitis A virus (HAV)
	Aphthousvirus	-	Foot-and-mouth disease virus (cattle)
	Cardiovirus	-	Encephalomyocarditis virus (mice)

Hepatitis A virus (HAV)

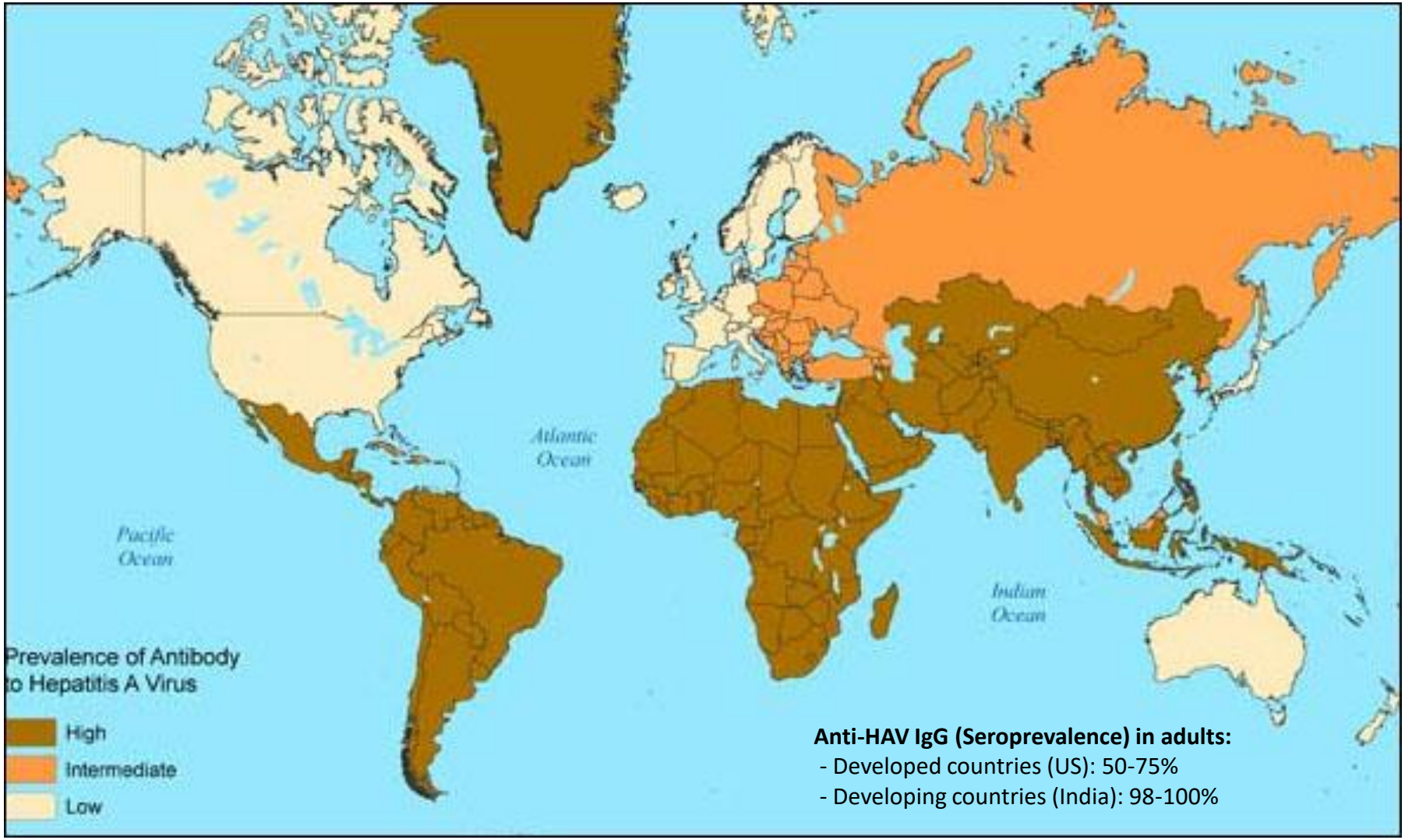
- Family- *Picornaviride*; Genus- *Hepatovirus*



- non-enveloped
- transmission - Feco-oral
  - contaminated water
  - food-borne illness- shellfish (molluscs- oysters, etc.)
- common cause of Acute Viral Hepatitis in the pediatric age group
- Outbreak

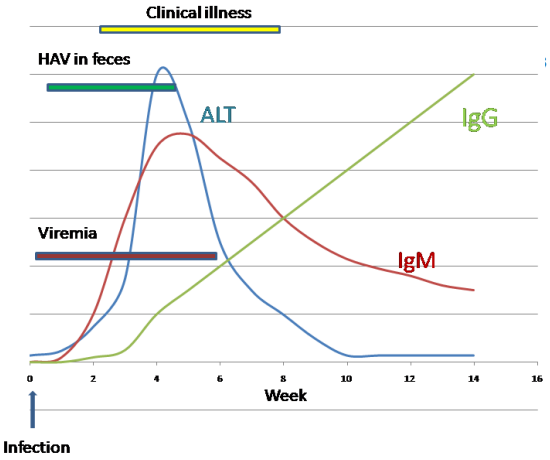
HAV - Sources of Infection

HAV - Global seroprevalence (based on Anti-HAV IgG positivity)



Anti-HAV IgG (Seroprevalence) in adults:
- Developed countries (US): 50-75%
- Developing countries (India): 98-100%

HAV - Clinical spectrum



Incubation period - 15 to 45 days (≈30 days)

- subclinical (asymptomatic) infection
  - in children below 5yrs.- most infections (≈87%) are subclinical
  - in adults- only ≈ 18% are subclinical
- **Acute viral hepatitis (AVH)**
- Fulminant hepatitis (rare)
- ACLF (HAV is the acute precipitating event)

Age of patient with HAV infection	Fulminant HAV infection and mortality rate (%)
< 14 years	0.1
15-39 years	0.3
> 40 years	2.1

HAV does not lead to chronic hepatitis.

HAV vs. HEV

	HAV	HEV
Transmission	Fecal-oral	Fecal-oral
Age at presentation of AVH	First decade of life; ≤ 10 - 15 yrs.	Older adolescents/adults; ≥ 20 - 50 yrs.
Sero-prevalence (IgG)	Anti-HAV IgG: - ubiquitous (≈ 100%) by age 10-15 yrs. in developing countries - **protective into later life**	Anti-HEV IgG: - 20-40% in adults - low (5-10%) before age 15 yrs. - **Non protective**

- Incubation period 10-45 days
- Prodromal stage
- Icteric phase
- Convalescent phase

INCUBATION OR PRECLINICAL PERIOD

10 - 50 days

Asymptomatic

Active replication of virus

PRODROMAL OR PRE-ICTERIC PHASE

several days to more than a week

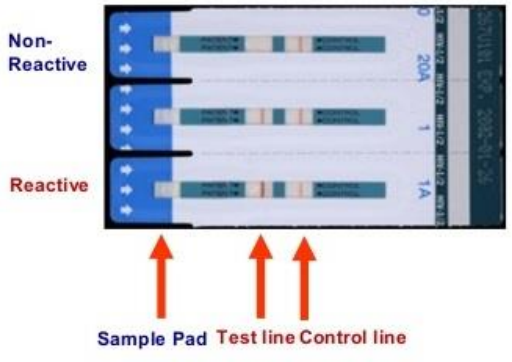
Characterized by loss of appetite, fatigue, abdominal pain, nausea and vomiting, fever, diarrhoea

ICTERIC PHASE

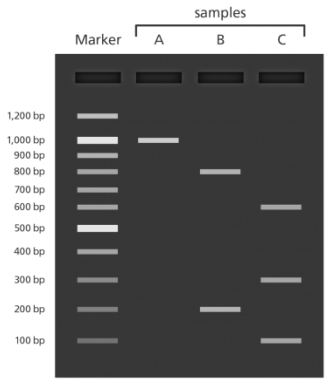
- Jaundice develops at total bilirubin levels exceeding 20 - 40 mg/l.
- Begins within 10 days of the initial symptoms.
- Characterized by golden-brown urine, pale stools, yellowish discoloration of the mucous membranes, conjunctivae, sclerae, and skin.
- Fever usually improves after the first few days of jaundice.
- Viremia terminates shortly after hepatitis develops
- Faeces remain infectious for another 1 - 2 weeks

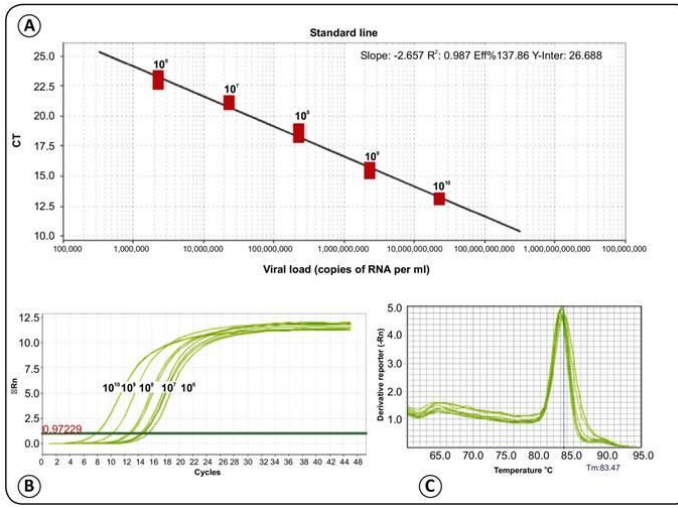
CONVALESCENT PERIOD

- resolution of the disease is slow, but patient recovery complete.
- Relapsing hepatitis occurs in 3 - 20%.

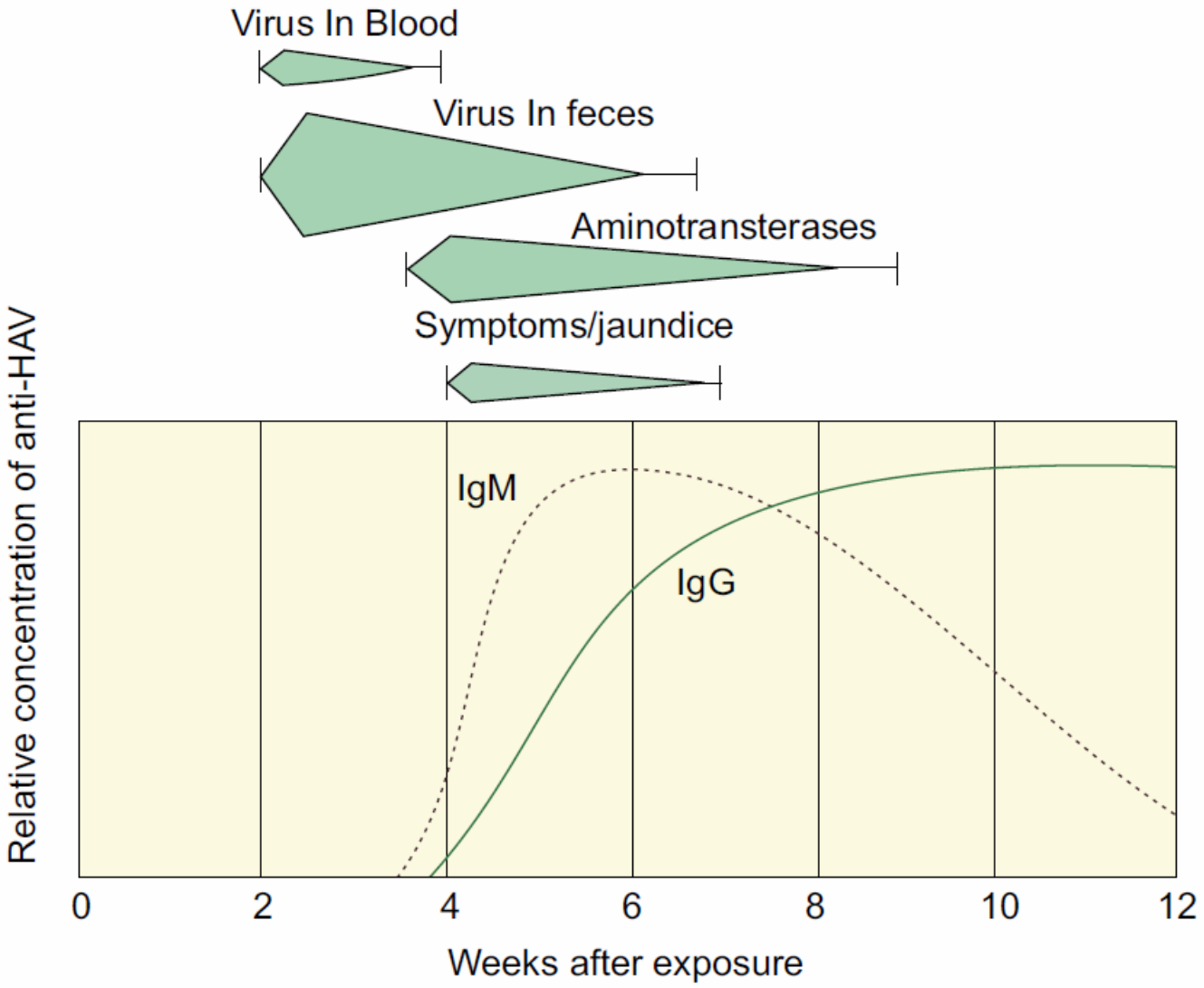

Non-Reactive
Reactive
Sample Pad
Test line
Control line


ARCHITECT plus
Abbott


samples
Marker
A
B
C
1,200 bp
1,000 bp
900 bp
800 bp
700 bp
600 bp
500 bp
400 bp
300 bp
200 bp
100 bp


A
Standard line
Slope: -2.657 R²: 0.987 Eff%137.86 Y-Inter: 26.688
CT
Viral load (copies of RNA per ml)
B
Cycles
0.97229
C
Derivative reporter (-Rn)
Temperature °C
Tm:83.47

Laboratory diagnosis of HAV

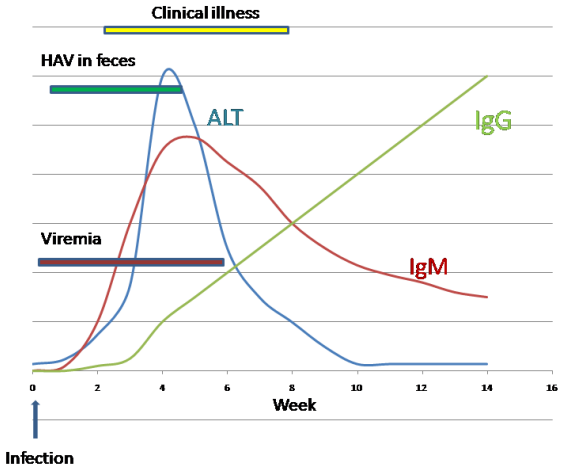


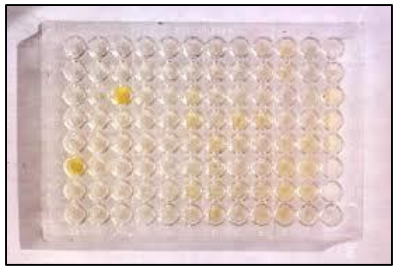
HAV - Laboratory diagnosis

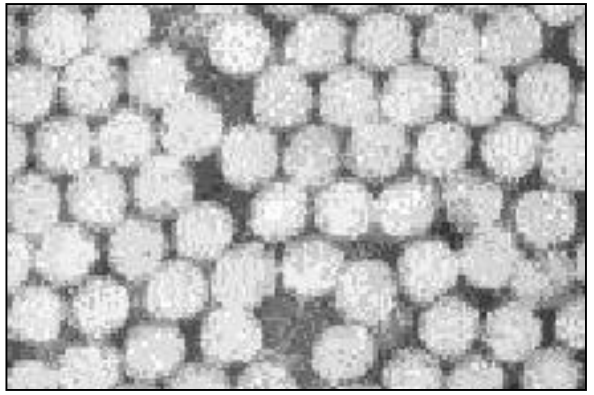
1. **Serology (Antibody detection):**
   - **Anti-HAV IgM**
   - **Anti-HAV IgG**

2. **Molecular assays (NAT):**
   - **RT-PCR for HAV RNA** (from stool, blood)

3. **Electron Microscopy** (stool):
   - virions ≈27 nm







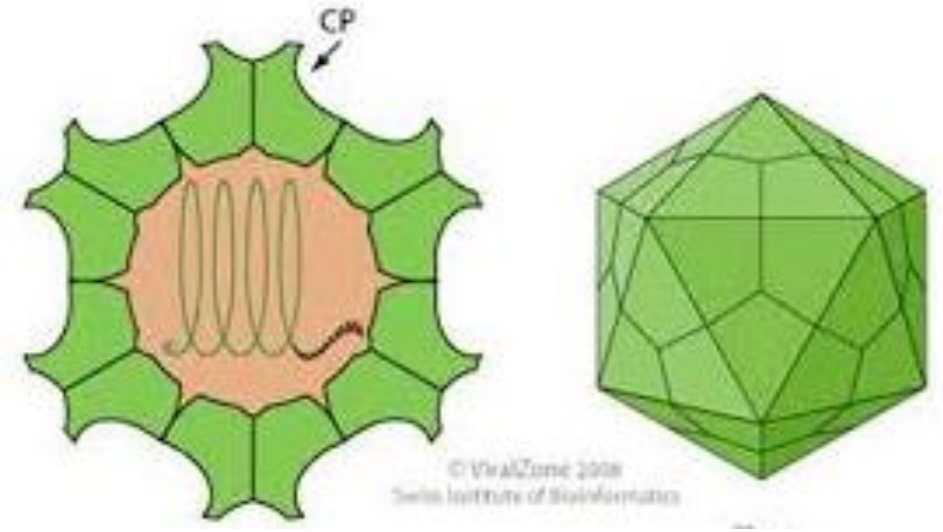
Laboratory diagnosis of HAV

TESTS	IMPORTANCE
Anti-HAV IgM	▪ Confirms diagnosis of acute Hepatitis A ▪ Rapidly increases in titer over 4-6 weeks ▪ Declines to non-detectable within 3-6mths
Total Anti-HAV	▪ In the absence of either IgM anti-HAV or an abnormal ALT level, it is suggestive of previous infection with HAV or successful vaccination and protection against future infection
HAV RNA	▪ HAV or viral antigen can be detected in the stools of patients 1 to 2 weeks before symptoms develop ▪ Done mainly in blood if patients are immunosuppressed
Liver function tests	▪ LFTs especially ALT and AST are sensitive measures of parenchymal liver damage but are not specific for hepatitis A.

HAV prophylaxis

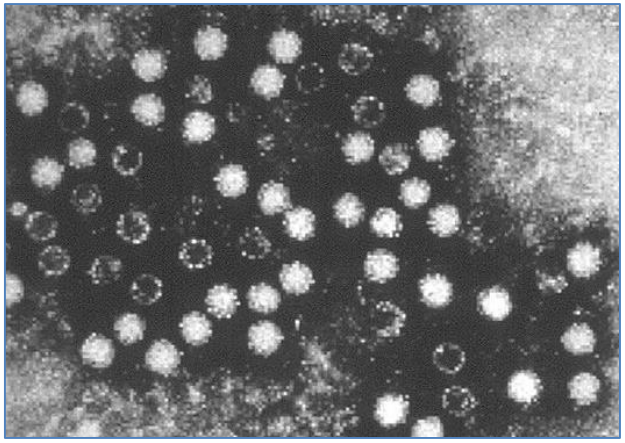
- HAV vaccine
  - formalin inactivated, human diploid cell culture based
  - two doses, 6-12 months apart
  - indications - non-immune residents in an endemic region
    - travel from developed country to an endemic area
    - men who have sex with men (MSM) etc.

- HAIG - passive immunization with immunoglobulin
  - given before or within 14 days of exposure

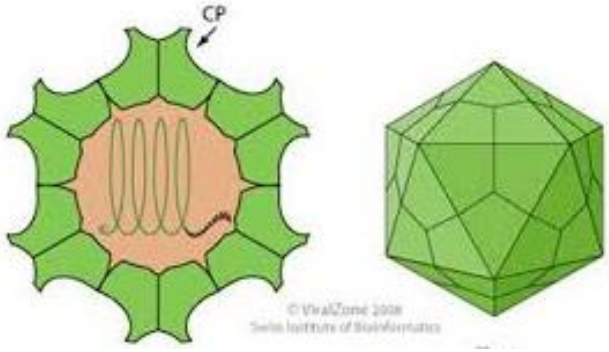


Hepatitis E Virus (HEV)

HEV - Introduction



- Family- *Hepeviridae;* Genus- *Hepevirus*
- Virion- 27-34 nm, non-enveloped
- ssRNA, positive sense
- 7.2-kb genome
- 4 genotypes- HEV1,2,3 and 4
- one serotype



- Clinical spectrum - **Acute viral hepatitis (AVH)**
  - **Fulminant hepatic failure (FHF)- pregnancy**
  - **Chronic hepatitis E - in the immunocompromised**

Discovery of HEV

Hepatitis E

Seminar

Nassim Kamar, Richard Bendall, Florence Legrand-Abravanel, Ning-Shao Xia , Samreen Ijaz, Jacques Izopet, Harry R Dalton

Hepatitis E virus (HEV) was discovered during the Soviet occupation of Afghanistan in the 1980s, after an outbreak of unexplained hepatitis at a military camp. A pooled faecal extract from affected soldiers was ingested by a member of the research team. He became sick, and the new virus (named HEV), was detected in his stool by electron microscopy. Subsequently, endemic HEV has been identified in many resource-poor countries. Globally, HEV is the most common cause of acute viral hepatitis. The virus was not initially thought to occur in developed countries, but recent reports have shown this notion to be mistaken. The aim of this Seminar is to describe recent discoveries regarding HEV, and how they have changed our understanding of its effect on human health worldwide.

- 1983- Balayan MS successfully transmitted the disease into himself
- oral administered pooled stool extracts of 9 patients
- developed acute viral hepatitis
- demonstrated virus particles in his own stool by IEM

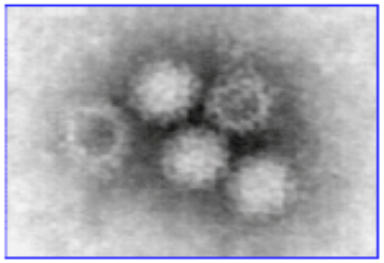
Lancet 2012; 379: 2477–88

Hepatitis E virus (HEV)

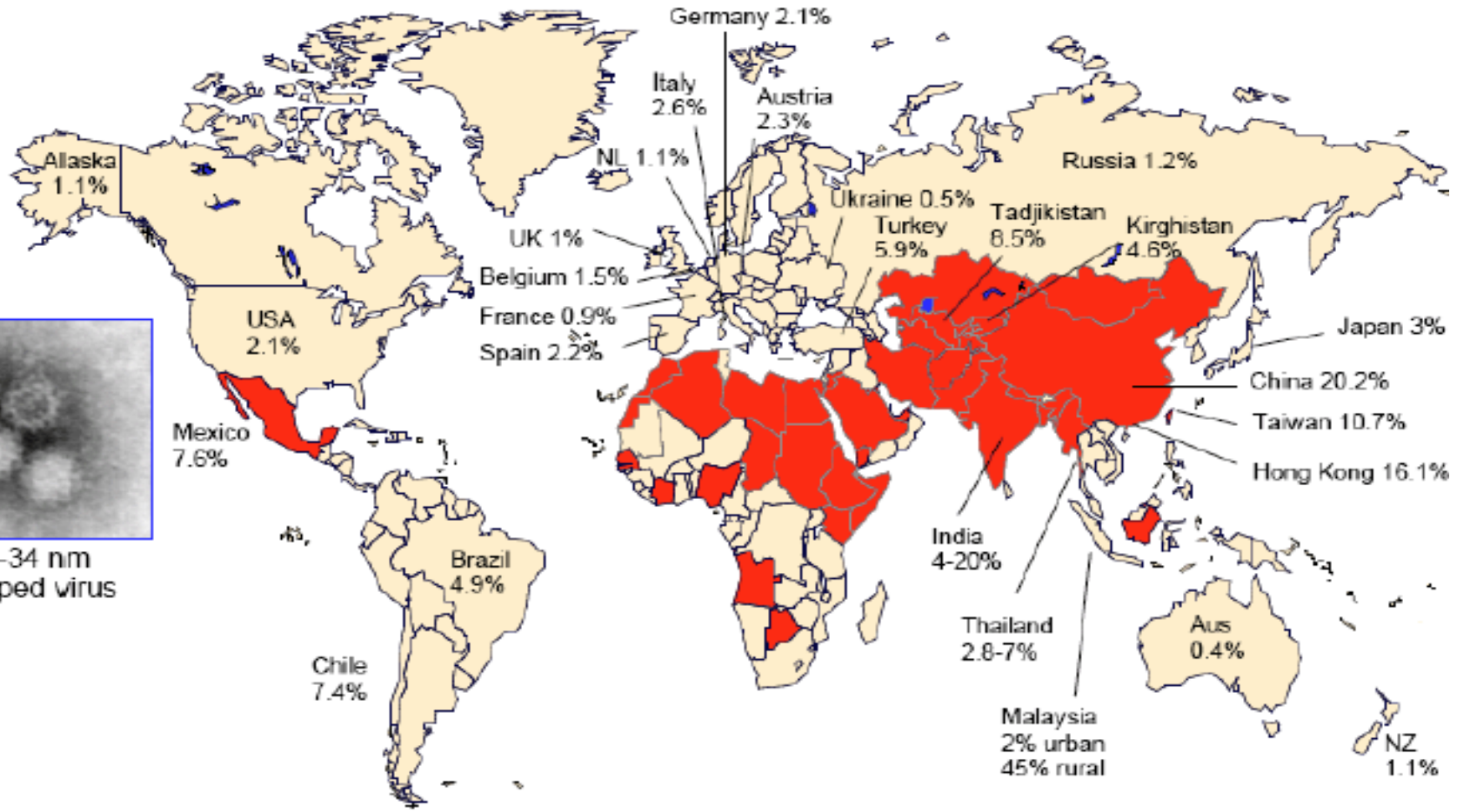
- HEV infection is the most frequent cause of acute sporadic and epidemic hepatitis in India (30-70% cases are due to HEV)
- Dual infection with HAV and HEV have been more frequently reported among children with ALF
- HEV is also the major cause of Acute liver failure in India
- high mortality rate in pregnant women (25% in the third trimester)

HEV - Global seroprevalence

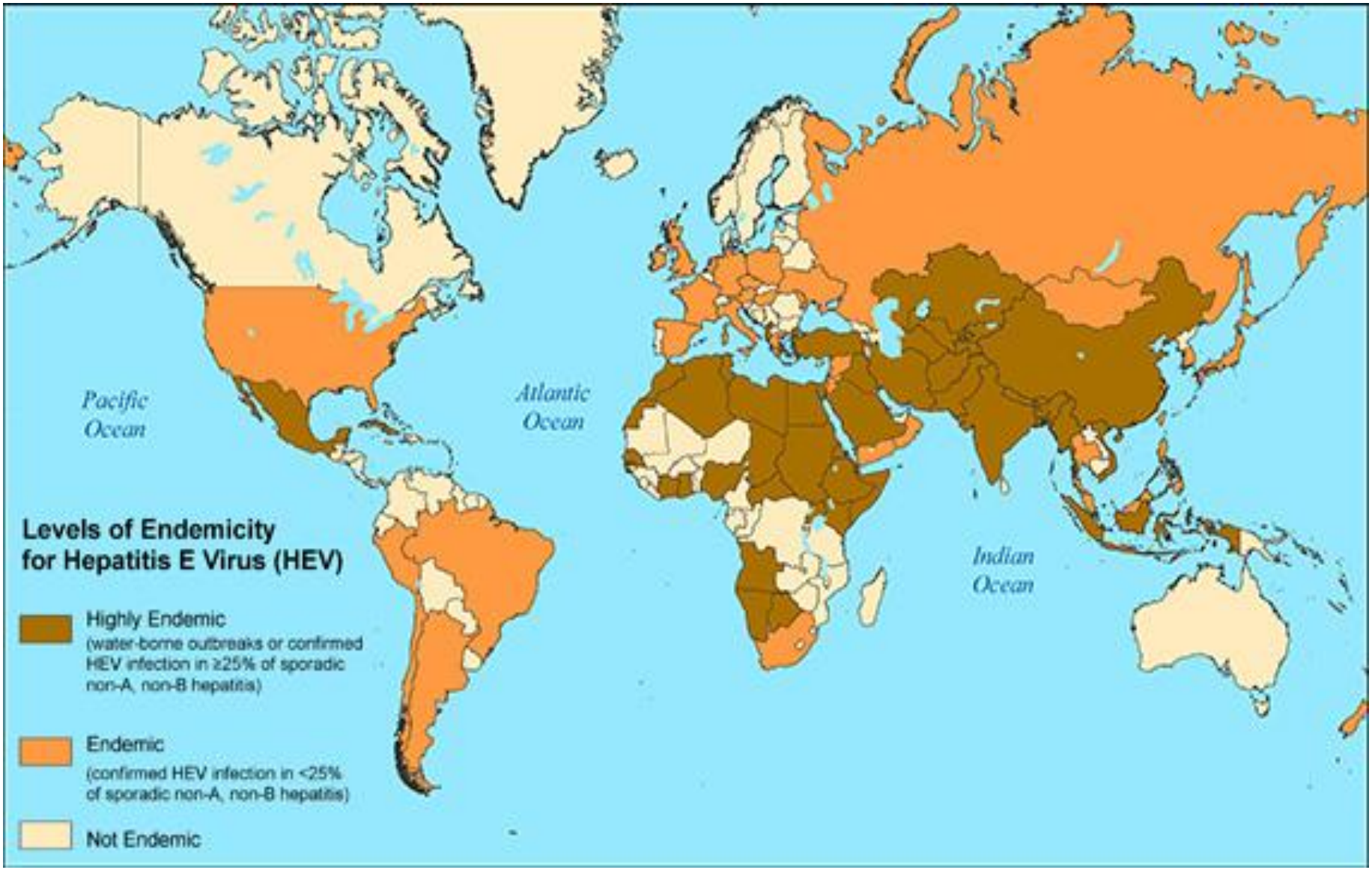
- commonest cause of enterically transmitted AVH
- occurs worldwide
- Seroprevalence (as determined by Anti-HEV IgG)



HEV: 27-34 nm
nonenveloped virus

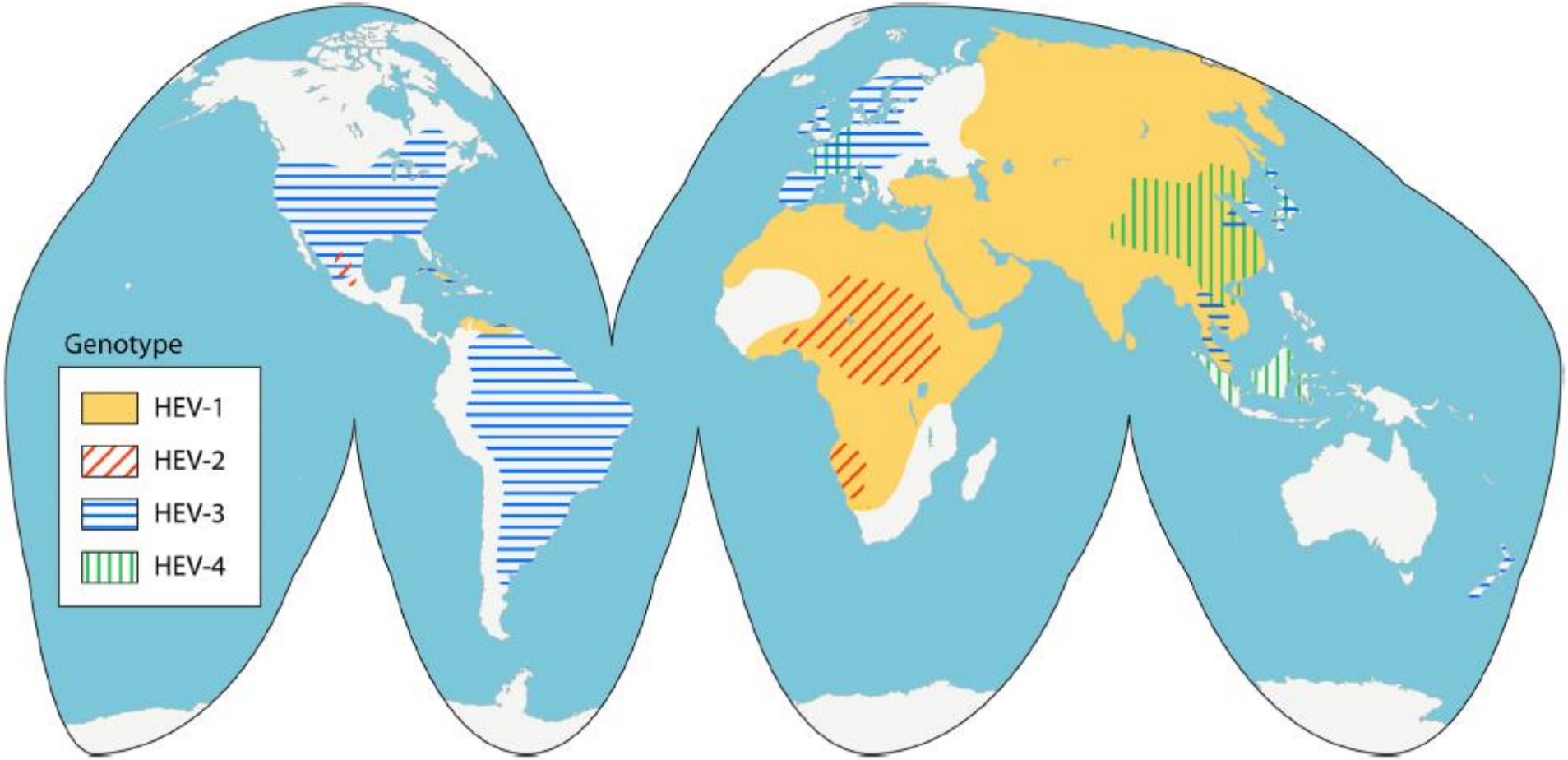


HEV - Global prevalence (endemicity)



Eyasu H, *Clin Infect Dis*, 2010

HEV - Global distribution of genotypes

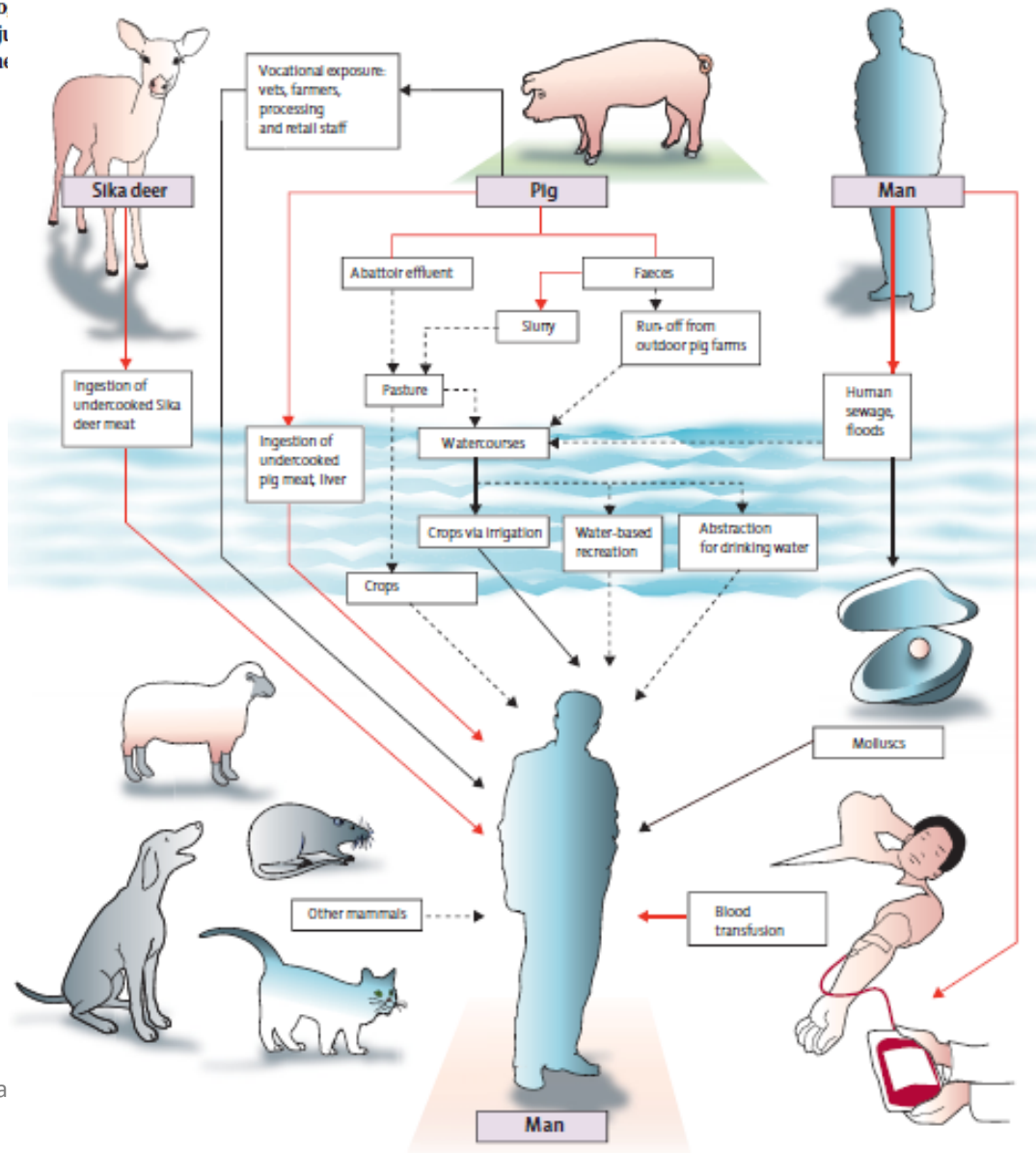


Hepatitis E: an emerging infection in developed countries

Harry R Dalton, Richard Bendall, Samreen Ijaz, Malcolm Banks

Hepatitis E is endemic in many developing countries where it causes substantial morbidity. In industrialised countries, it is considered rare, and largely confined to travellers returning from endemic areas. However, there is now a growing body of evidence that challenges this notion. Autochthonous hepatitis E in developed countries is far more common than previously recognised, and might be more common than hepatitis A. Hepatitis E has a predilection for older men in whom it causes substantial morbidity and mortality. The disease has a poor pro existing chronic liver disease, and is frequently misdiagnosed as drug-induced liver inj infection remain uncertain, but it might be a porcine zoonosis. Patients with unexplain for hepatitis E, whatever their age or travel history.

Lancet Infect Dis 2008;
8: 698–709



HEV transmission routes in developed countries

HEV - Epidemiology

	HEV in developing countries	HEV in developed countries
Genotypes	1 and 2	3 and 4
Source of infection	Humans	Zoonotic (**pigs** are primary host)
Route of infection	Fecal-oral via infected water	Fecal-oral via infected pig meat, infected water
Outbreaks	Yes, can involve thousands of cases	No (occasional small case clusters from point-source food outbreaks)
Seasonality	Yes e.g. outbreaks during monsoons	No

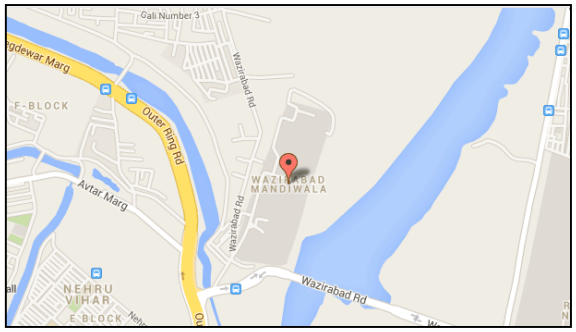
Most cases of AVH-E in developed countries are autochthonous

HEV Epidemiology - India

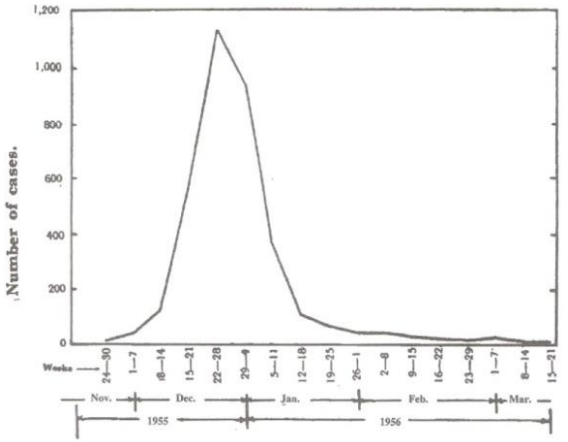
- globally commonest cause of AVH in those ≥ 15 yrs, adults
- endemic , >22,00,000 cases/year
- 30-60% of all cases of sporadic hepatitis are due to HEV
- occasional epidemics
- HEV genotype 1 (HEV1)

Kamar N. *Clin Microbiol Rev*, 2014

Delhi, 1955-1956



- 1 Dec.1955 to 20 Feb.1956
- first reported large outbreak of hepatitis later serologically attributed to HEV
- Jamuna river flooded its banks following monsoons
- Wazirabad water treatment plant got contaminated by nearby sewage drains

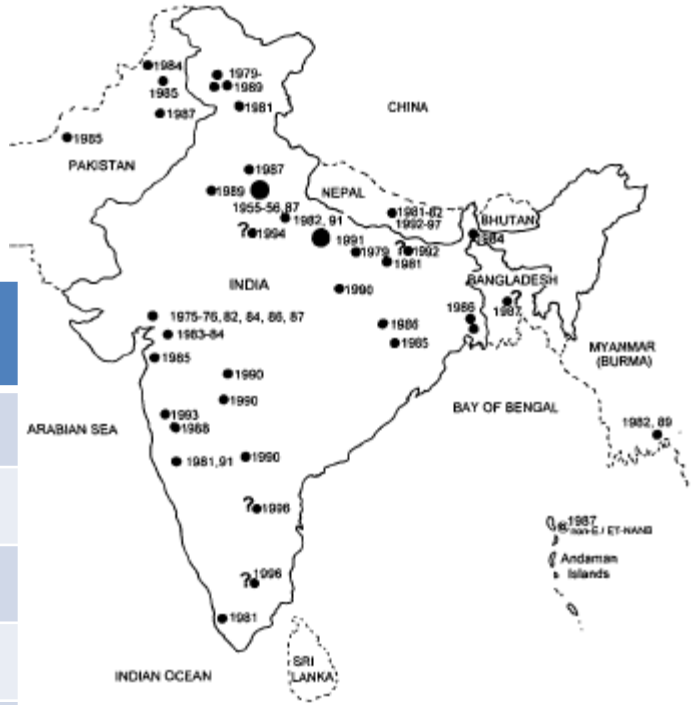


- 29,300 cases reported
- single peak, rapid dissipation; no secondary or tertiary waves
- mortality in pregnancy- 10%

Viswanathan R. *Hepatitis Frontiers*, 1957

HEV epidemics - India

Year	Region / City	Population affected (N)	Mortality, Overall (%)	Mortality, Pregnancy (%)
1955-56	Delhi	29,300	0.2	5/48 (11)
1960	Kharagpur	65	-	-
1961	Aurangabad	865	0.3	-
1966	Siliguri	4,287	0.1	-
1975-76	Ahmedabad	2,572	2.4	-
1978	Kashmir	275	3.6	6/8 (75)
1990-91	Kanpur	79,091	0.1	13/48 (27)
2008	Shahbad (Har)	160	-	-
2010	Nellore	23,915	1.3	-



Acharya SK , *Natl Med J India,* 2006
Vivek R, *Trop Med Int Health*, 2010

HEV - Clinical spectrum

- Acute Viral Hepatitis (AVH)
- Acute-on-chronic liver failure (ACLF)
- Fulminant Hepatic failure (FHF) in pregnancy
- Chronic Viral Hepatitis E - in the immunocompromised
- Extrahepatic manifestations

HEV and DILI

HEV infection may be misdiagnosed as DILI. Drug-induced liver injury (DILI) occurs more frequently in the elderly (in whom polypharmacy is common), as does autochthonous hepatitis E, and the clinical features may be indistinguishable. The diagnosis of DILI is based on a number of criteria. These include a temporal relationship between starting a drug and developing hepatitis (usually 5 to 30 days), a temporal relationship between stopping the offending drug and the resolution of hepatitis, and the exclusion of all other causes of hepatocellular injury. In a study of a group of patients with criterion-referenced DILI from Southwest England, it was found that the diagnosis of DILI had been made in error in some patients, as 13% of patients with "DILI" who were tested retrospectively had HEV genotype 3 infection (24). In a more recent study from the United States, 3% of patients with "DILI" had been diagnosed erroneously, as they had hepatitis E on subsequent testing (25). These studies illustrate the importance of excluding other causes of hepatocellular injury before making a diagnosis of DILI and suggest that the diagnosis of DILI should not be made without excluding HEV infection, particularly in individuals with high serum alanine aminotransferase levels.

Kamar N. *Clin Microbiol Rev*, 2014

HEV in pregnancy
- maternal & neonatal health

- Maternal - ↑ risk of hemorrhage, eclampsia
  - Fulminant hepatic failure (FHF)
  - Hepatic encephalopathy
  - DIC
- Fetal - stillbirths
  - vertical transmission- ↑ neonatal morbidity & mortality

- Case fatality rate (CFR)- 10-42%, usually in the 3rd trimester

- HEV genotypes 1 & 2 only

Adverse outcomes are unique to genotypes 1 & 2 (NOT genotypes 3 & 4)

Unusual genotype 1 in Egypt

D/Ds of liver diseases/jaundice during pregnancy

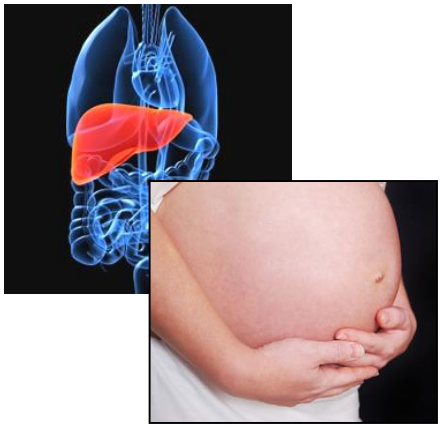
- Acute fatty liver of pregnancy

  - disorder of mitochondrial fatty acid metabolism
  - LCHAD (long-chain 3-hydroxyacyl-coenzyme A dehydrogenase) deficiency

- Fulminant hepatic failure (FHF) following AVH-E

- Pre-eclampsia, HELLP syndrome

- Intrahepatic cholestasis of pregnancy

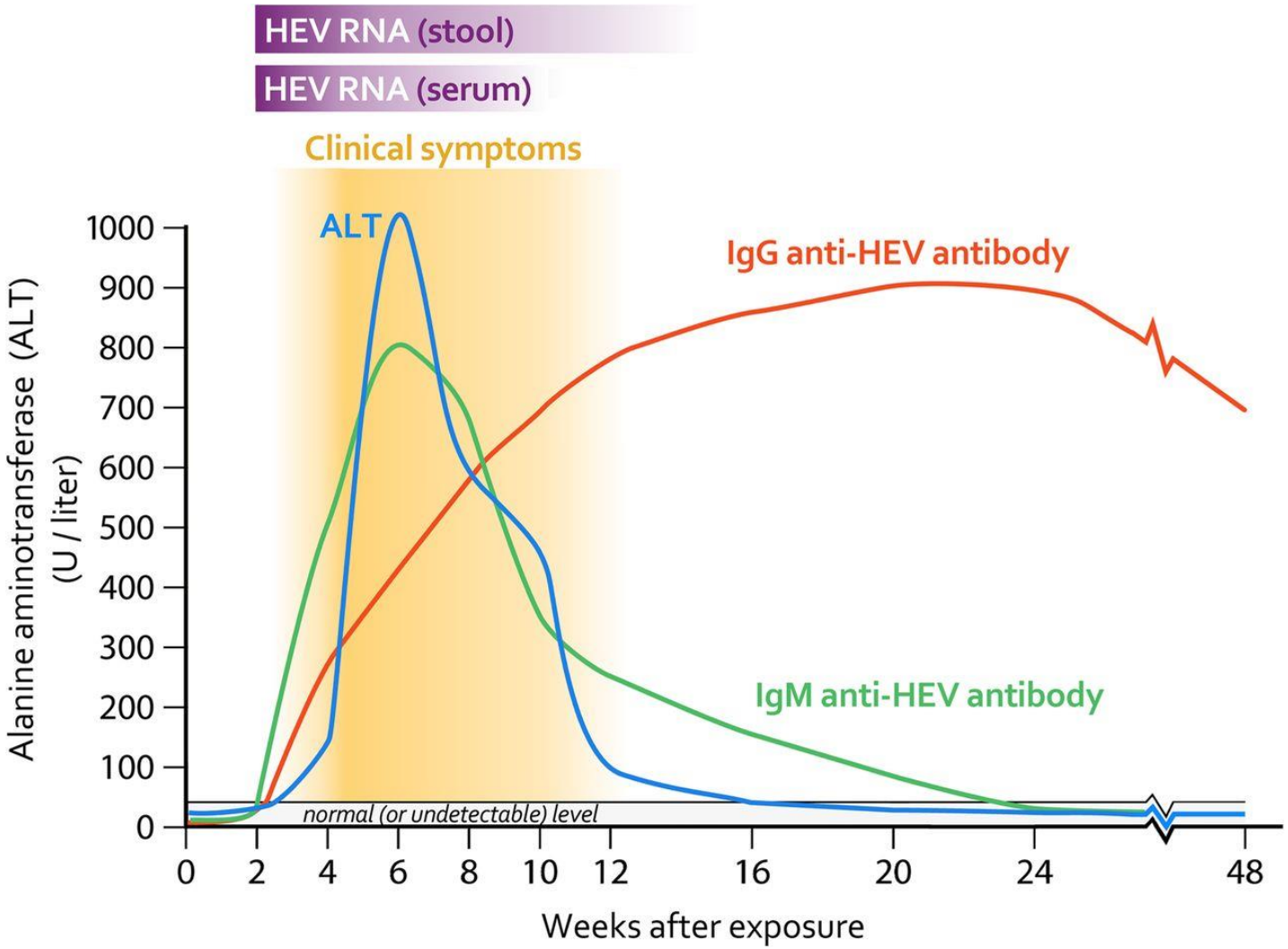


Extrahepatic manifestations of HEV

- Neurological disorders (HEV1, HEV3)
  - Guillain-Barre syndrome- [Anti-ganglioside GM 1,2 positive]
  - Bell's palsy
  - acute transverse myelitis
  - neuralgic amyotrophy
- Renal ds. (HEV1, HEV3)
  - MPGN, MGN
- Acute pancreatitis (HEV1)
- Hematological - thrombocytopenia
  - aplastic anaemia

HEV – Diagnosis

- **AVH-E Incubation period = 4 weeks (2 weeks to 6 weeks)**



Krain LJ. *Clin Microbiol Rev*, 2014

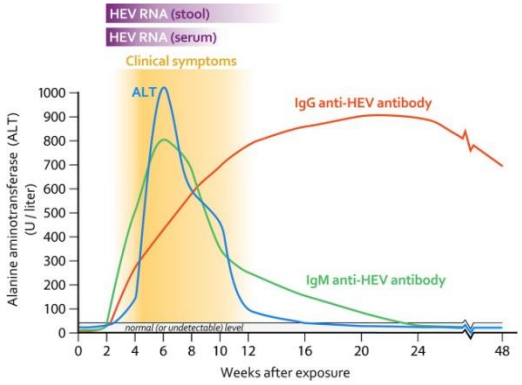
HEV - Lab. diagnosis

1. Antibody detection (ELISA)- ORF2/ORF3 peptides
   - Anti-HEV IgM (persist for 2-3 mo, rarely 6 mo.)
   - Anti-HEV IgG (persist for a few yrs.)

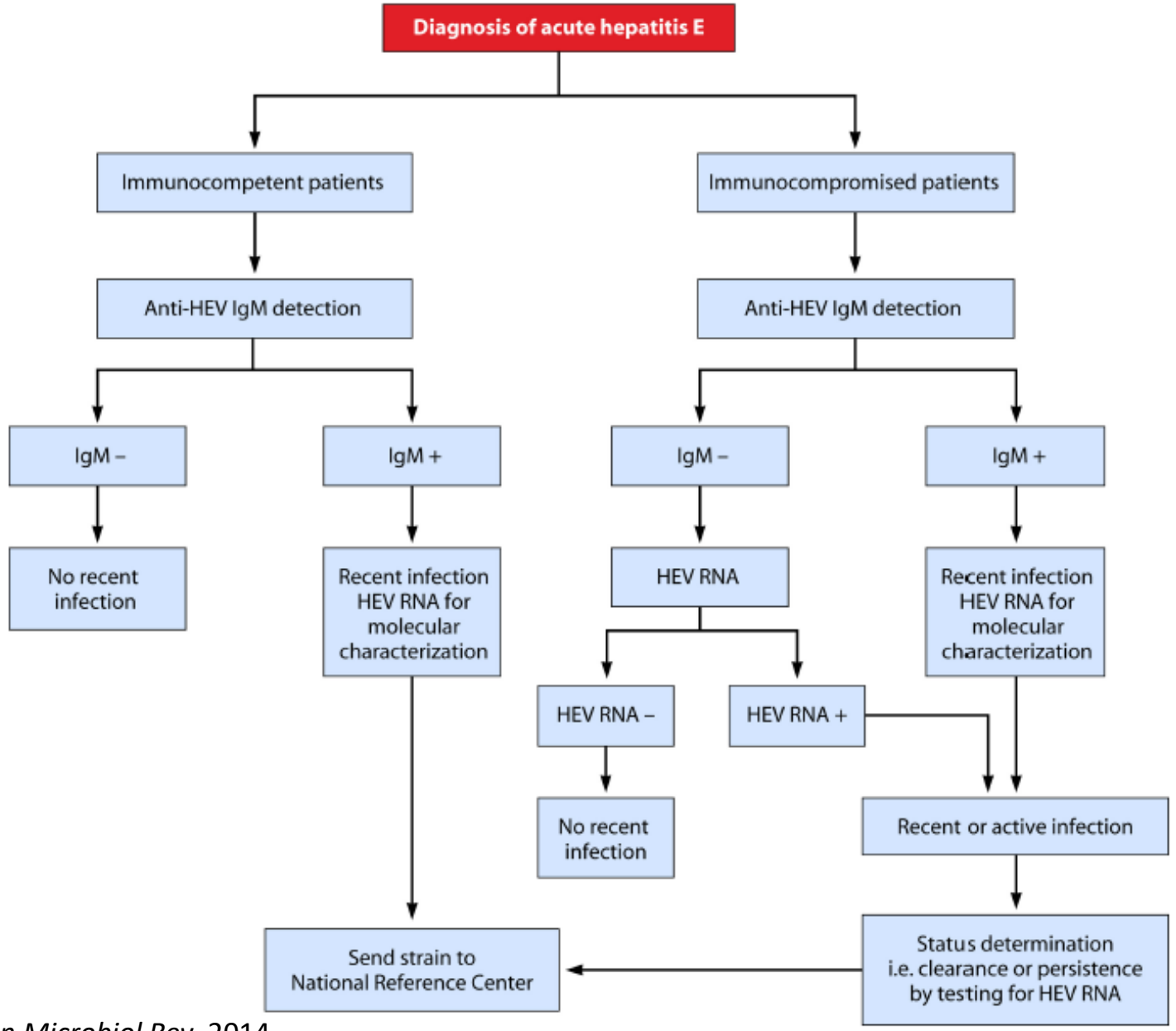
2. Antigen detection (ELISA)- ORF2/ORF3
   - HEV Ag (positive between 3 to 6 wks. approx.)

3. Molecular techniques - RT-PCR

4. Immune Electron Microscopy (IEM)



Lab. diagnosis of AVH-E



Krain LJ. *Clin Microbiol Rev*, 2014

Chronic Hepatitis E

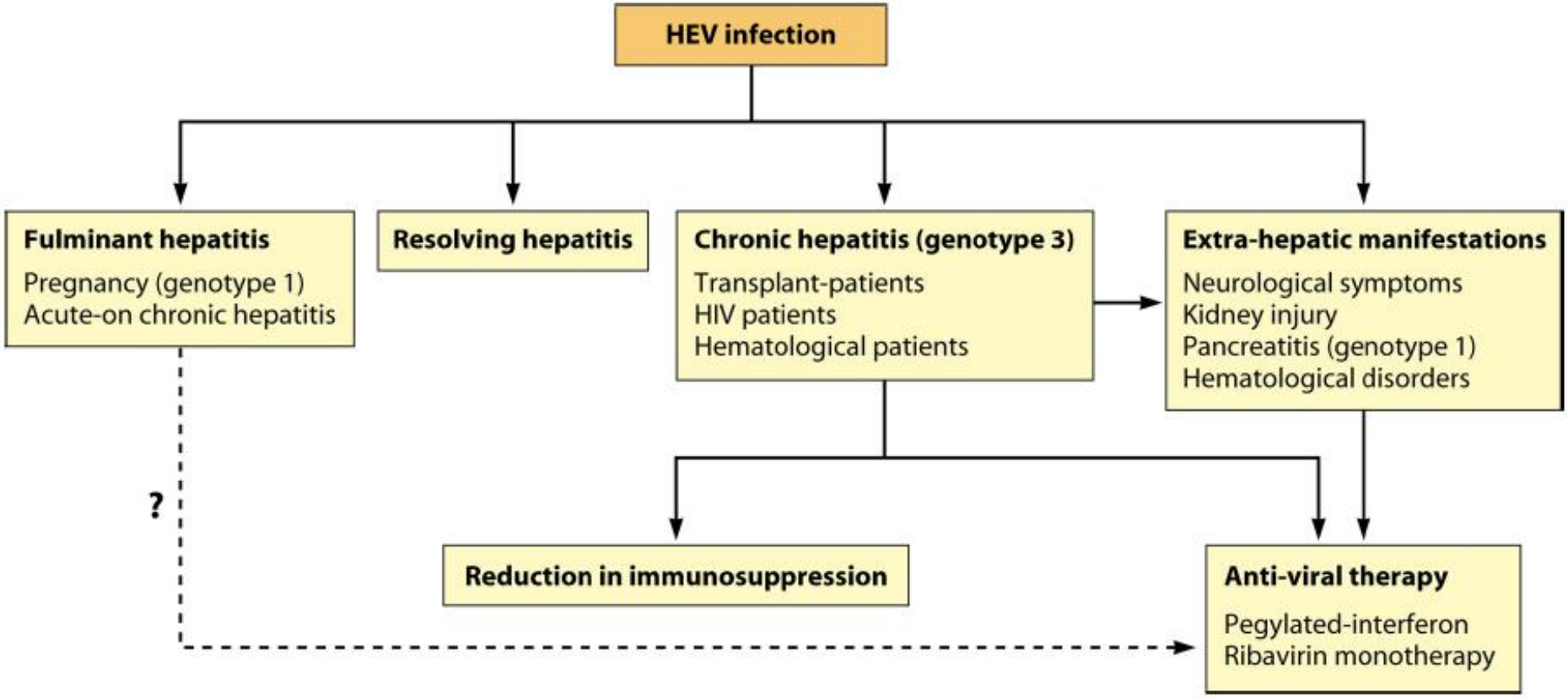
- HEV RNA-positive serum or stools for ≥3 months
- No report till date in developing countries .
- Increasingly reported in developed countries with HEV genotype 3 infection
- Risk population

❑ Solid organ transplant patients on immunosuppressive treatment (60% of HEV patients evolve to CHE).

❑ HIV infected patients

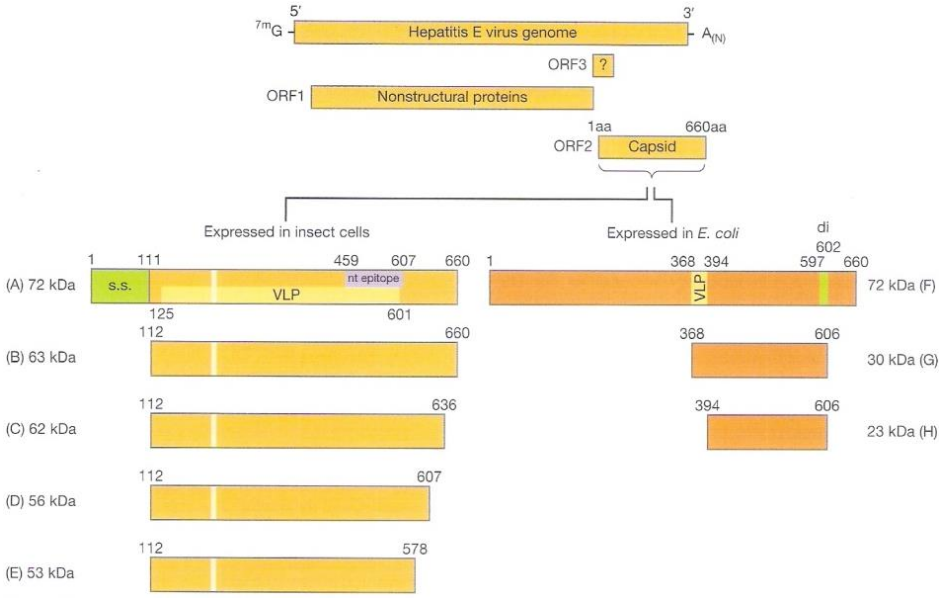
❑ Patients with hematological malignancies

HEV - clinical spectrum and its management



Kamar N. *Clin Microbiol Rev*, 2014

HEV vaccines



Manufacturer	Truncated capsid antigen (ORF2)	HEV genotype used	Expressed from	Vaccinated population	Dose	Regimen (months)	HEV genotype in population tested	Efficacy (95% CI)
GlaxoSmithKline, Belgium	aa 112-607	HEV 1	Baculovirus in insect cells	Military (young Nepalese men)	20 µg, with alum	0, 1, 6	HEV 1	95.5% (89% - 99%)
Xiamen Innovax Biotech, China	aa 368-606	HEV 1	*Escherichia coli*	General (Chinese adults, all ages)	30 µg , with alum	0, 1, 6	HEV 4	100 % (72%-100%)

Plotkins. *Vaccines*, 2013


Thank you!