Hepatitis

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Medical Virology,
25 Dec 2015.

Contents of Teaching in Medical Virology Lecture:

Hepatitis

Viral hepatitis

- Acute hepatitis may occur as part of the clinical course of a number of viral infections, including Human Cytomegalovirus, Epstein-Barr virus, Herpes Simplex Virus, Yellow Fever Virus and Rubella.
- But the term **"hepatitis virus"** is usually used to describe infections caused by agents whose primary tissue tropism is the liver.
- To date, at least five hepatitis viruses have been recognised and these have been named, **hepatitis A, B, C, D** and **E.**

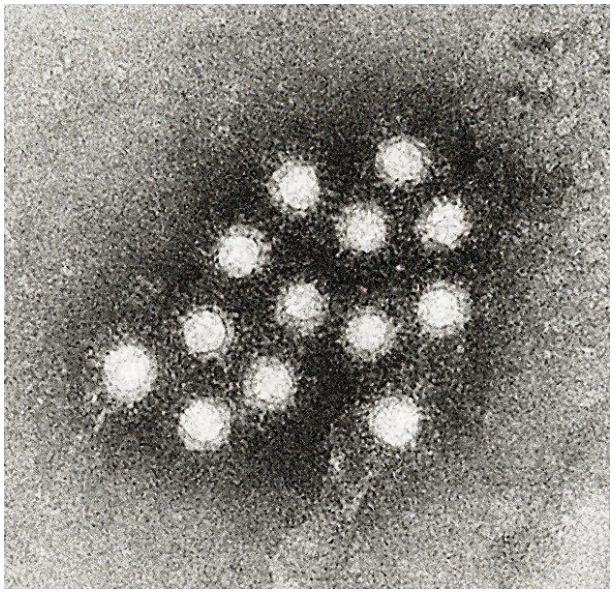
Clinical Features

- Hepatitis due to all these viruses presents clinically in a very similar fashion, especially during the acute phase.
- Thus, a specific diagnosis can only be made in the laboratory.
- The majority of infections are often asymptomatic or produce only mild non-specific symptoms.
- But, common clinical features include: anorexia, nausea, vomiting, right upper quadrant pain and raised liver enzymes AST and ALT.
- Jaundice is the hall mark of infection, but tends to develop late.
- Anicteric cases are also very common.

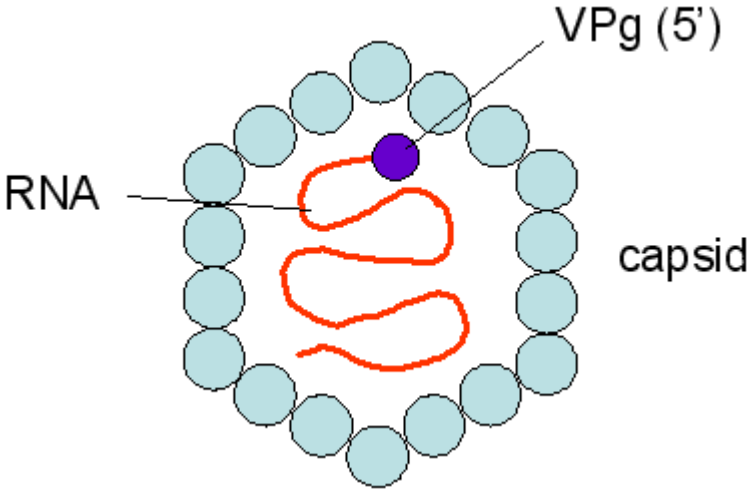
Enterically transmitted hepatitis: A and E

Hepatitis A virus (HAV)

- Order: Picornavirales
  Family: Picornaviridae
  Genus: Hepatovirus
  Species: Hepatitis A virus
  Structure: small; 27 nm in diameter, non-enveloped spherical particle
  Genome: +ssRNA (positive sense, single stranded RNA)



Hepatitis A virus in electron microscope



Hepatitis A virus structure

HAV

Clinical Features

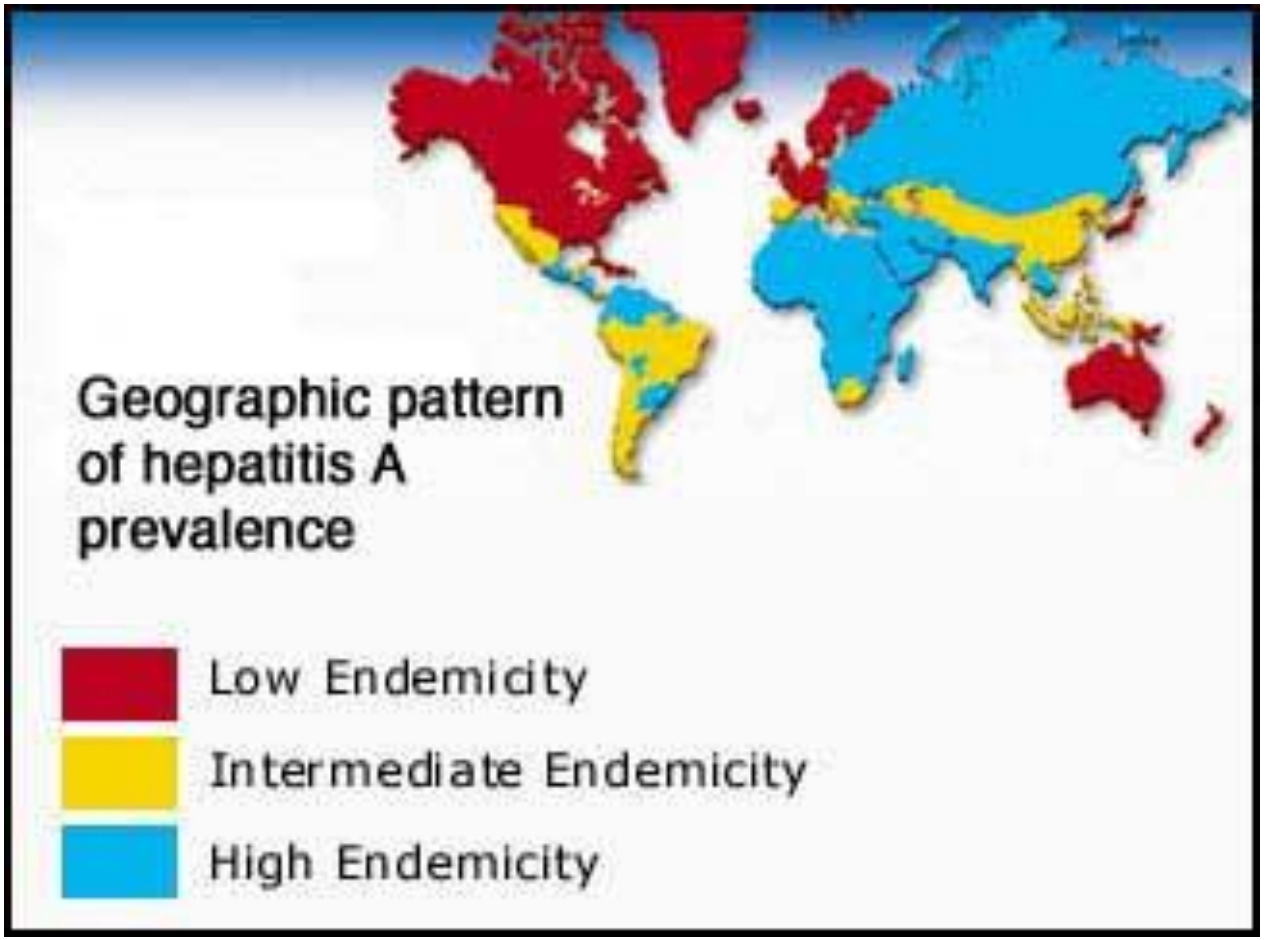
- Incubation period 3-5 weeks (mean 28 days)
- Complications: Fulminant hepatitis: rare; 0.3-1.8 % of cases
- Highest risk: pregnant women, elderly, pre-existing liver disease, other chronic medical conditions

Pathogenesis

- Virus enters via the gut; replicates in the alimentary tract and spreads to infect the liver, where it multiplies in hepatocytes.
- Viraemia is transient. Virus is excreted in the stools for two weeks preceding the onset of symptoms.

Epidemiology

- World-wide distribution; endemic in most countries. The incidence in first world countries is declining. There is an especially high incidence in developing countries and rural areas, where 80-90% of people are infected by the age of 5 years.
- The implication for South Africa is that most people, especially from rural areas, are seropositive, and donated blood/plasma contains sufficient levels of antibodies for use as passive immunity.

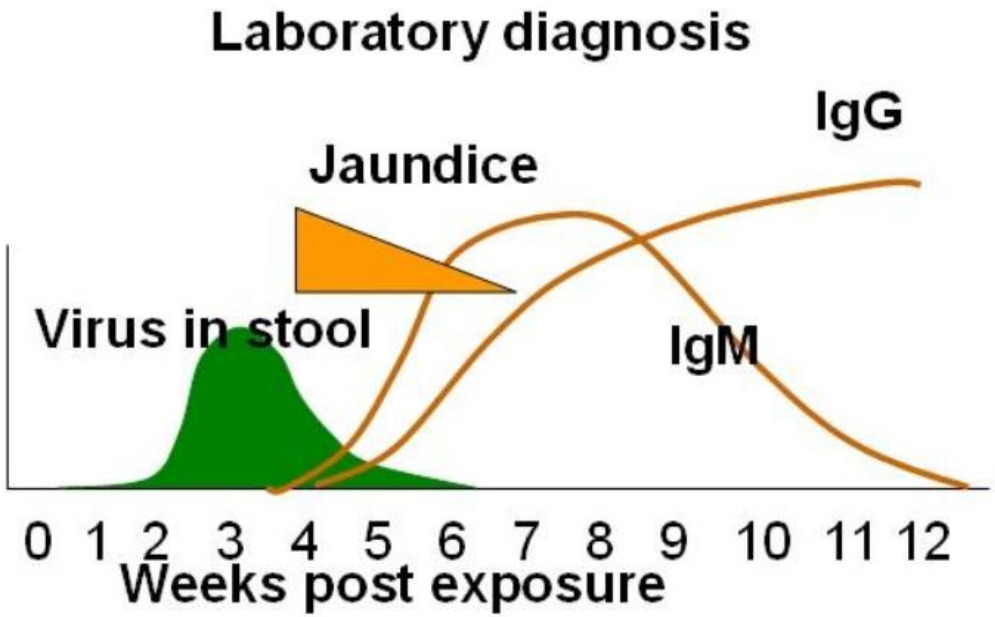


Hepatitis A Virus Transmission

- Close personal contact
  (e.g., household contact, sex contact, child day care centers)
- Contaminated food, water
  (e.g., infected food handlers, raw shellfish)
- Blood exposure (rare)
  (e.g., injecting drug use, transfusion)

Laboratory Diagnosis

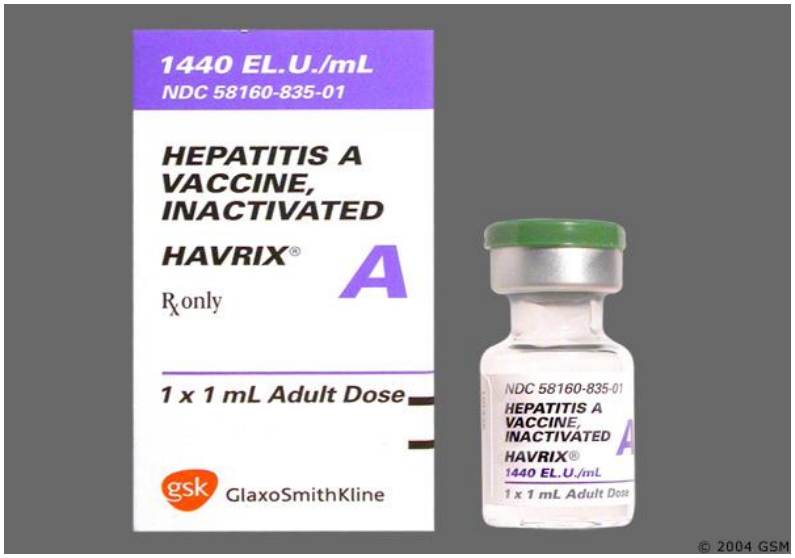
- **Acute infection** : HAV-IgM in serum by EIA.
- **Past Infection i.e. immunity**: HAV-IgG by EIA.
- **Cell culture** – difficult and take up to 4 weeks, not routinely performed
- **Direct Detection** – EM, RT-PCR of faeces. Can detect illness earlier than serology but rarely performed.

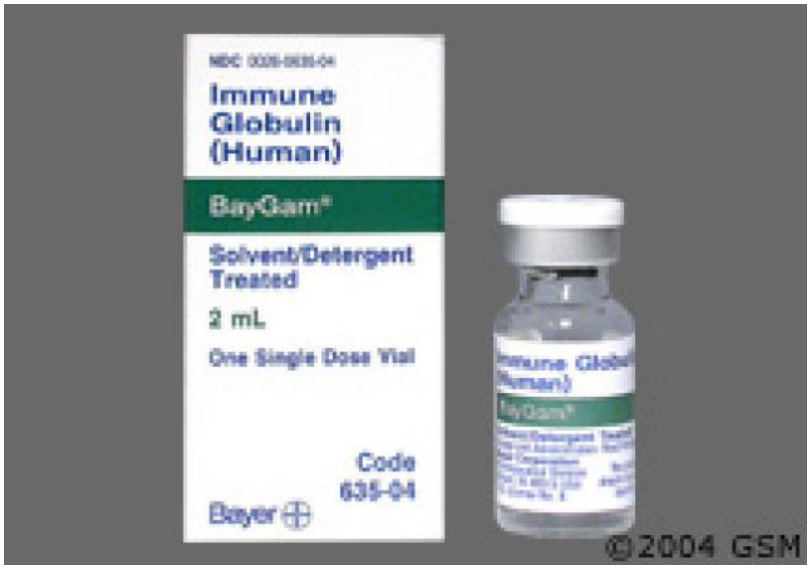


HAV

Prevention

- **Active Immunization:** Inactivated cell culture derived vaccine is available; it is recommended for travellers to third world countries and, indeed, all adults who are not immune. It is the recommended form of post-exposure prophylaxis if the exposure is identified early, and if there are no predisposing risk factors for severe disease. If there are such risk factors, or if prophylaxis is delayed, passive immunization in addition to vaccination is recommended.
- **Passive immunisation:** Normal immunoglobulin (antibody prepared from pooled human serum) given to close contacts of acute cases. Protection is short lived: three months.





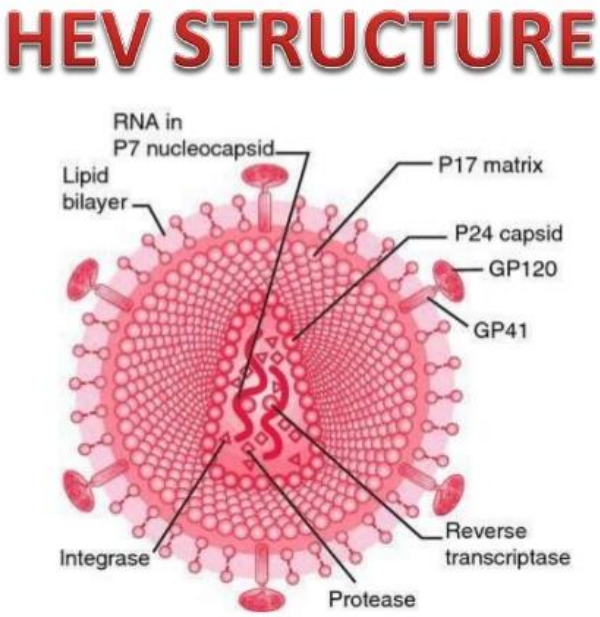
Hepatitis E

Hepatitis E virus (HEV)
Virology

- Order: none
- Family: Hepeviridae
- Genus: Hepevirus Species: Hepatitis E virus
- Structure: 27-34 nm in diameter, non-enveloped spherical particle
- Genome: +ssRNA (positive sense, single stranded RNA)

Clinical Features

- Incubation period: 45 days [2-9 weeks]
- Acute, self limiting hepatitis
- Most cases occur in young adults, 15-40 years

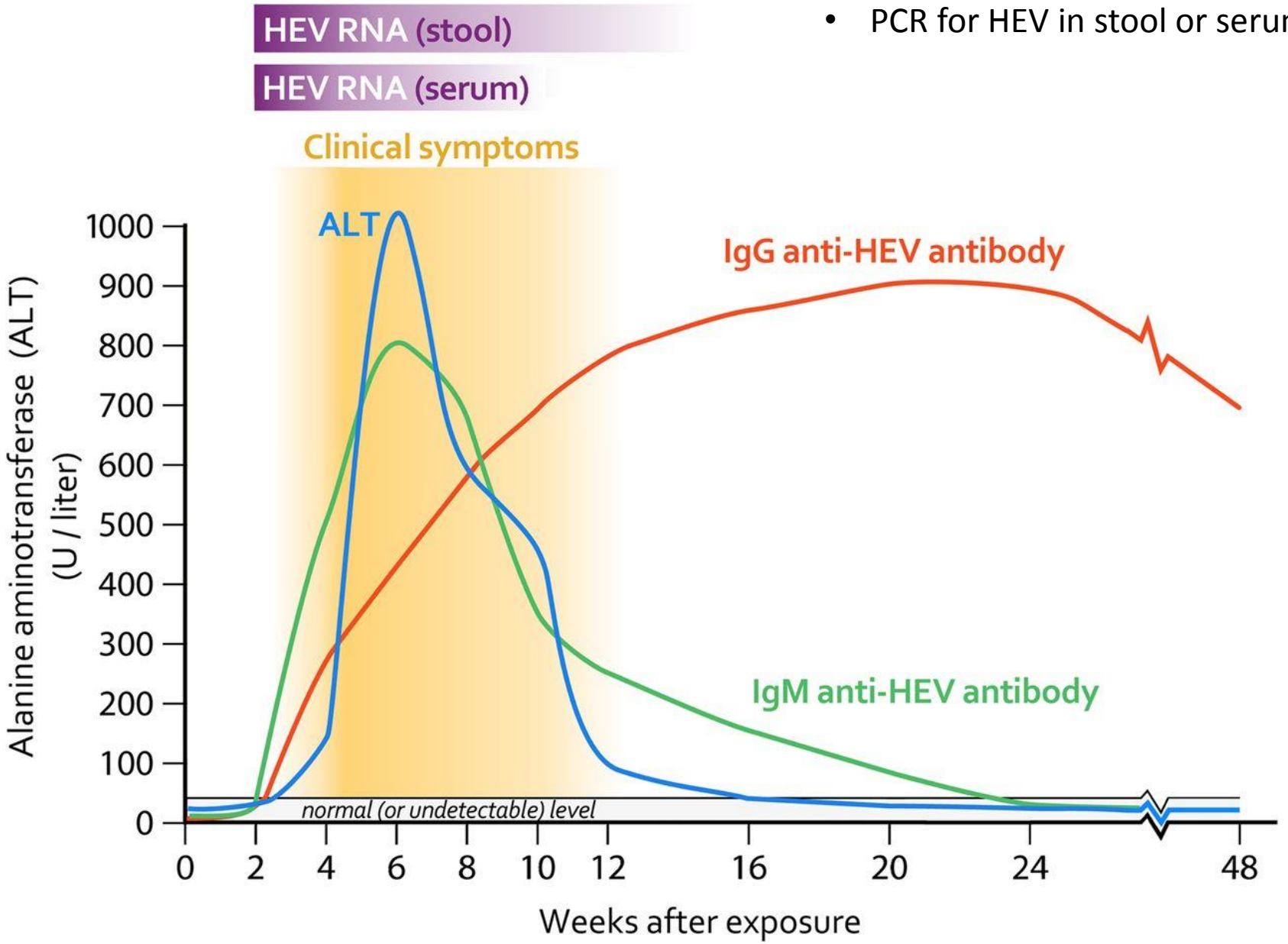


How serious is Hepatitis E?

- Most people with hepatitis E recover completely.
- During HEV outbreaks, the overall case-fatality rate is about 1%.
- However, for pregnant women, hepatitis E can be a serious illness with mortality reaching 10%–30% in their third trimester of pregnancy.

HEV infection and diagnosis

- Virus cannot be cultured in vitro
- Specific IgM detection by ELISA
- PCR for HEV in stool or serum

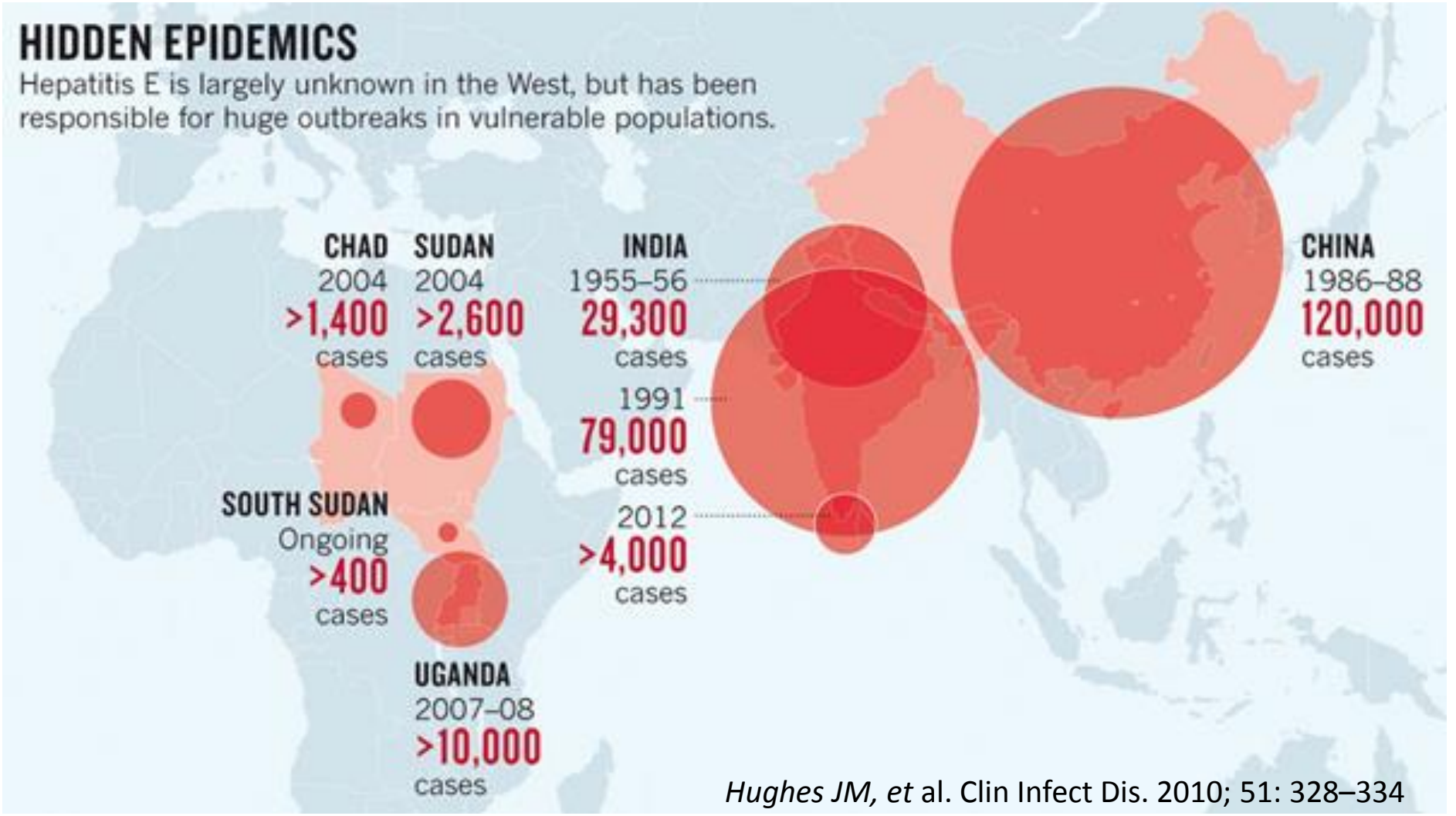


Transmission and Exposure of HEV

How is the Hepatitis E virus spread?

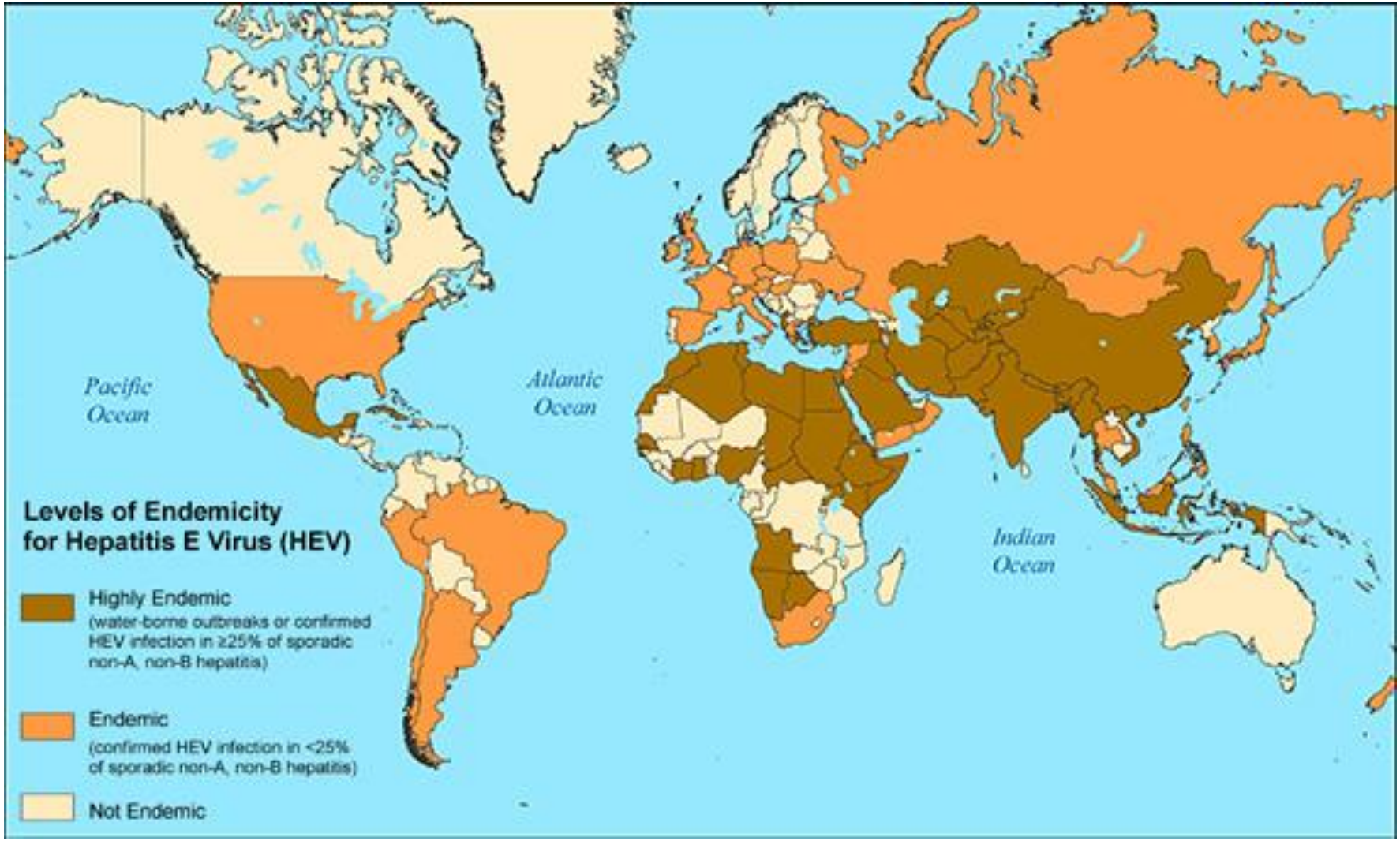
- Hepatitis E virus is usually spread by the fecal-oral route.
- The most common source of HEV infection is fecally contaminated drinking water.
- In developing countries, HEV genotypes 1 and 2 are spread by fecally contaminated drinking water.
- In developed countries sporadic cases of HEV genotype 3 have occurred following consumption of uncooked/undercooked pork or deer meat.
- Consumption of shellfish was a risk factor in a recently described outbreak in a cruise ship.
- HEV genotype 4, detected in China, Taiwan, and Japan, has also been associated with foodborne transmission

HEV outbreaks



Hughes JM, et al. Clin Infect Dis. 2010; 51: 328–334

HEV epidemiology



HEV genotypes

Characteristics	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Geographic Location	Africa and Asia	Mexico, West Africa	Developed Countries	China, Taiwan, Japan
Transmission route	Water-borne Fecal- oral Person to Person	Water-borne Fecal-oral	Food-borne	Food-borne
Groups at high risk for infection	Young Adults	Young Adults	Older Adults (>40 years) and Males Immuno-compromised persons	Young Adults
Zoonotic transmission	No	No	Yes	Yes
Chronic Infection	No	No	Yes	No
Occurrence of Outbreaks	Common	Smaller scale outbreaks	Uncommon	Uncommon

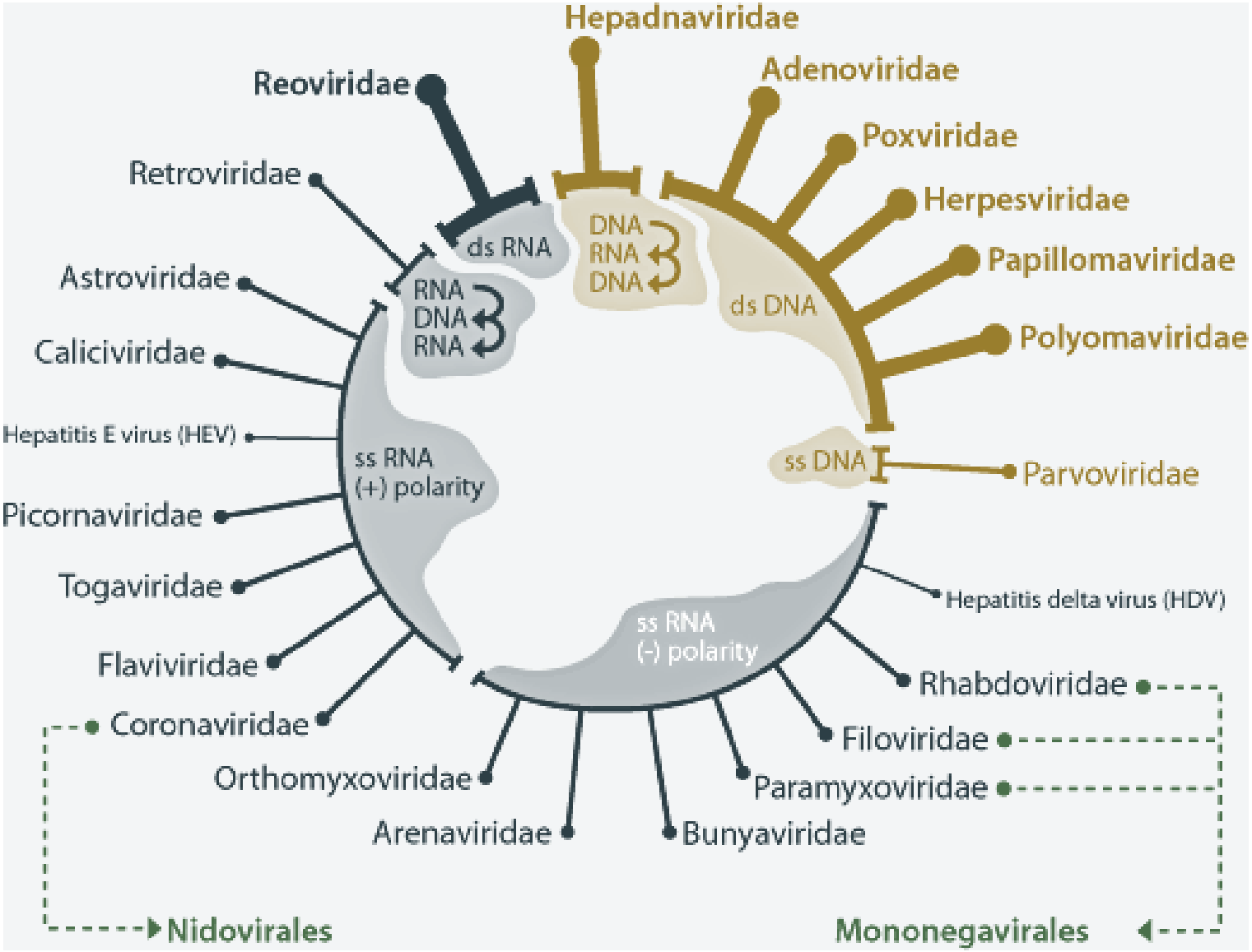
Parenterally transmitted hepatitis: B, C and D

Hepatitis B virus (HBV)

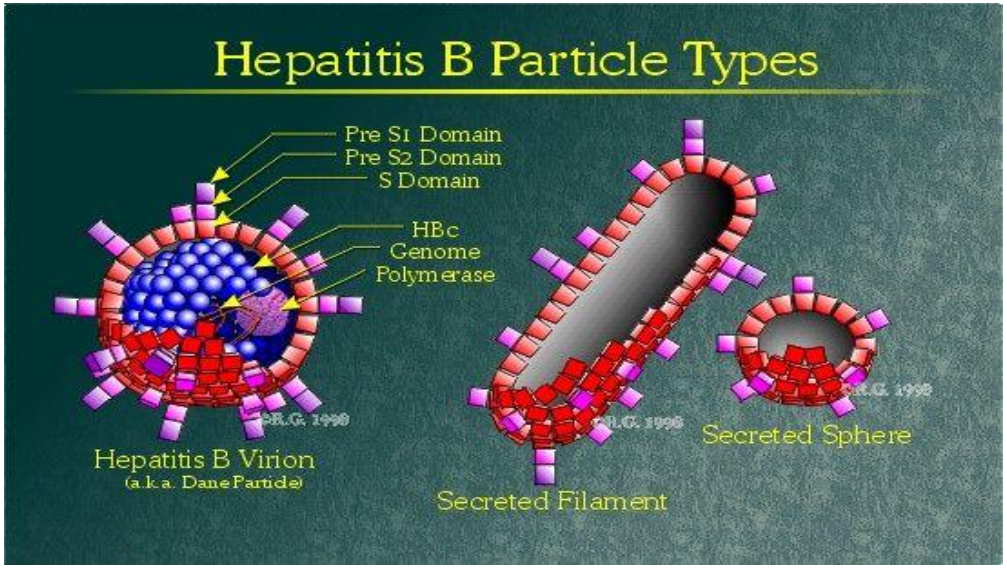
Family	Genus	Species
Hepadnaviridae	*Orthohepadnavirus*	Hepatit B virus
	Avihepadnavirus	Duck hepatit B virus

- Structure: 42 nm in diameter, enveloped spherical particle [also called the Dane particle]
- Genome: circular DNA, incompletely double stranded; 3.2 kilobases in size
  Excess surface antigen is produced, forming spheres and cylinders 22nm in diameter

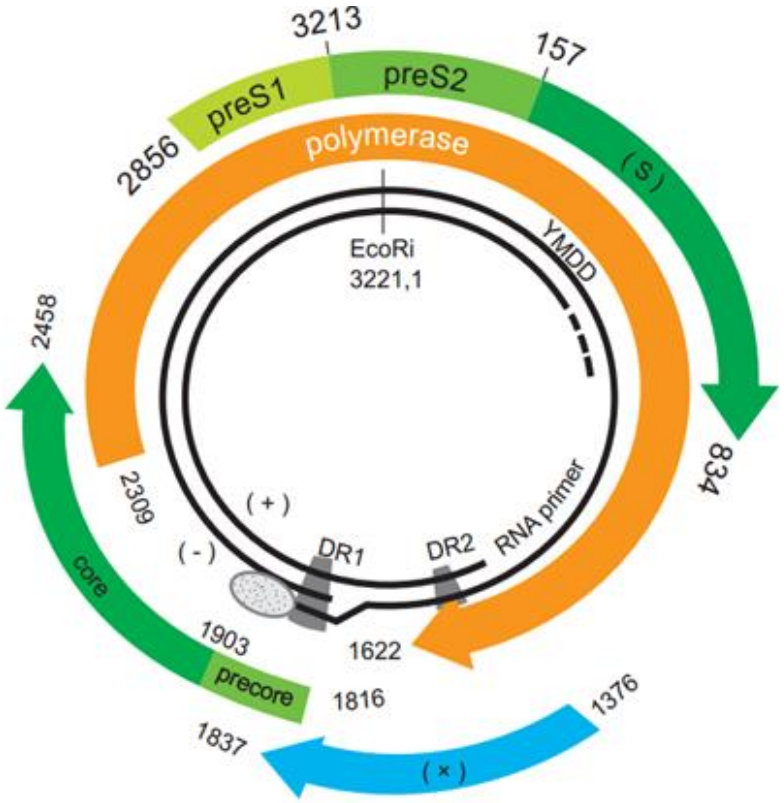
Viral taxonomic diagram



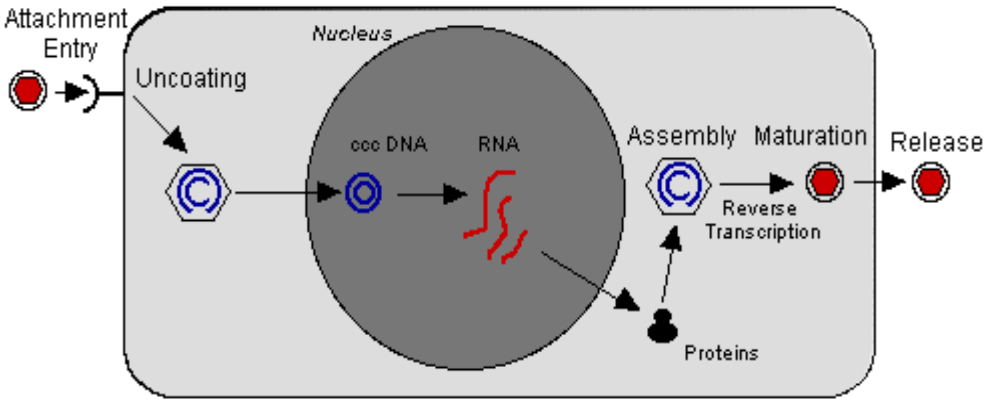
International Committee on Taxonomy of Viruses Database
http://www.antiviralintelistrat.com/1/viral_taxonomy



HBV genome organization and life cycle



Dane particle;
"covalently closed circular "
ccc DNA

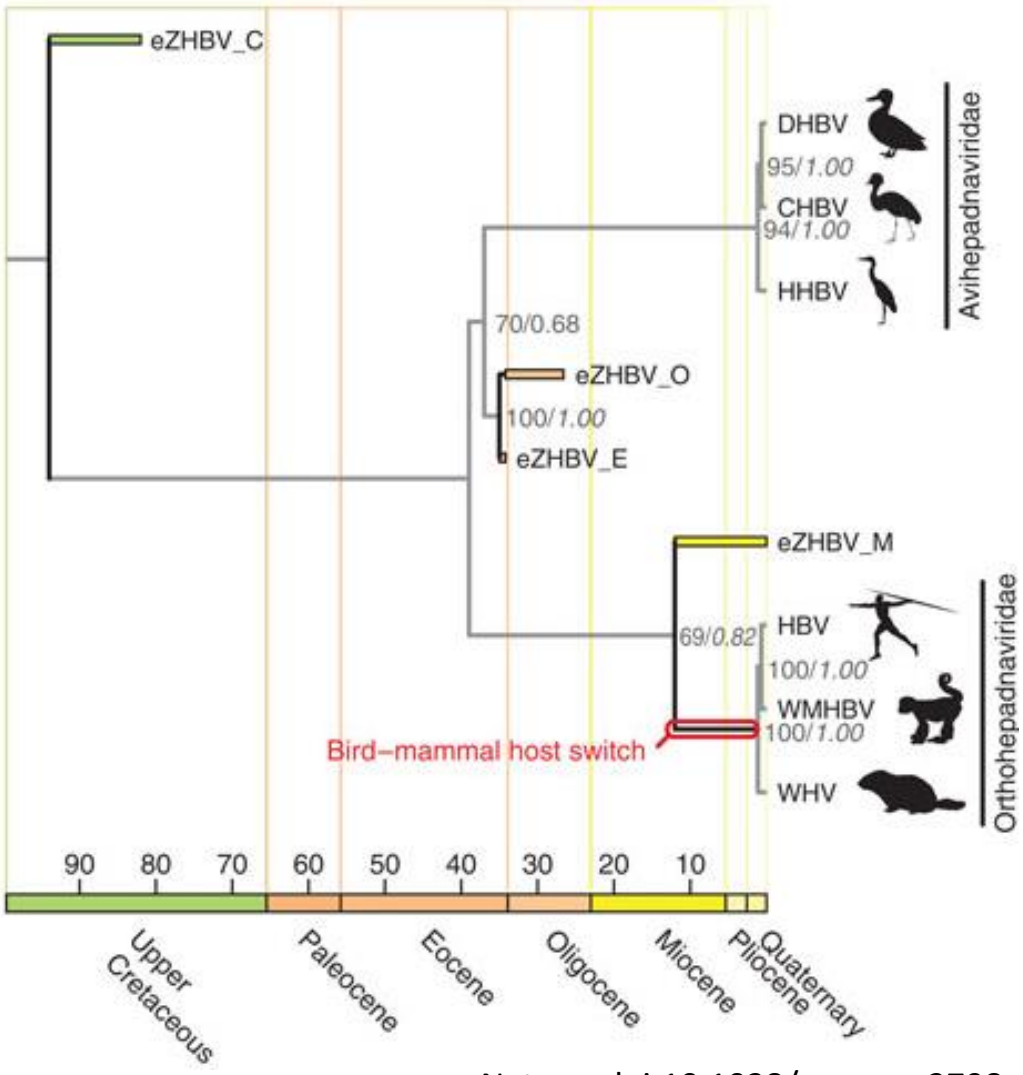


Dandri M, *et* al. Gut 2012;61(Suppl 1):i6ei17.

Paleovirology (detection of ancient viral elements in eucariotic genome) ;

HBV revers transcriptase: >82 - <12.1 milion year age

- Bird-mammal host switch..
- Hepadnaviridae, Mesozoic - Cenozoic age.



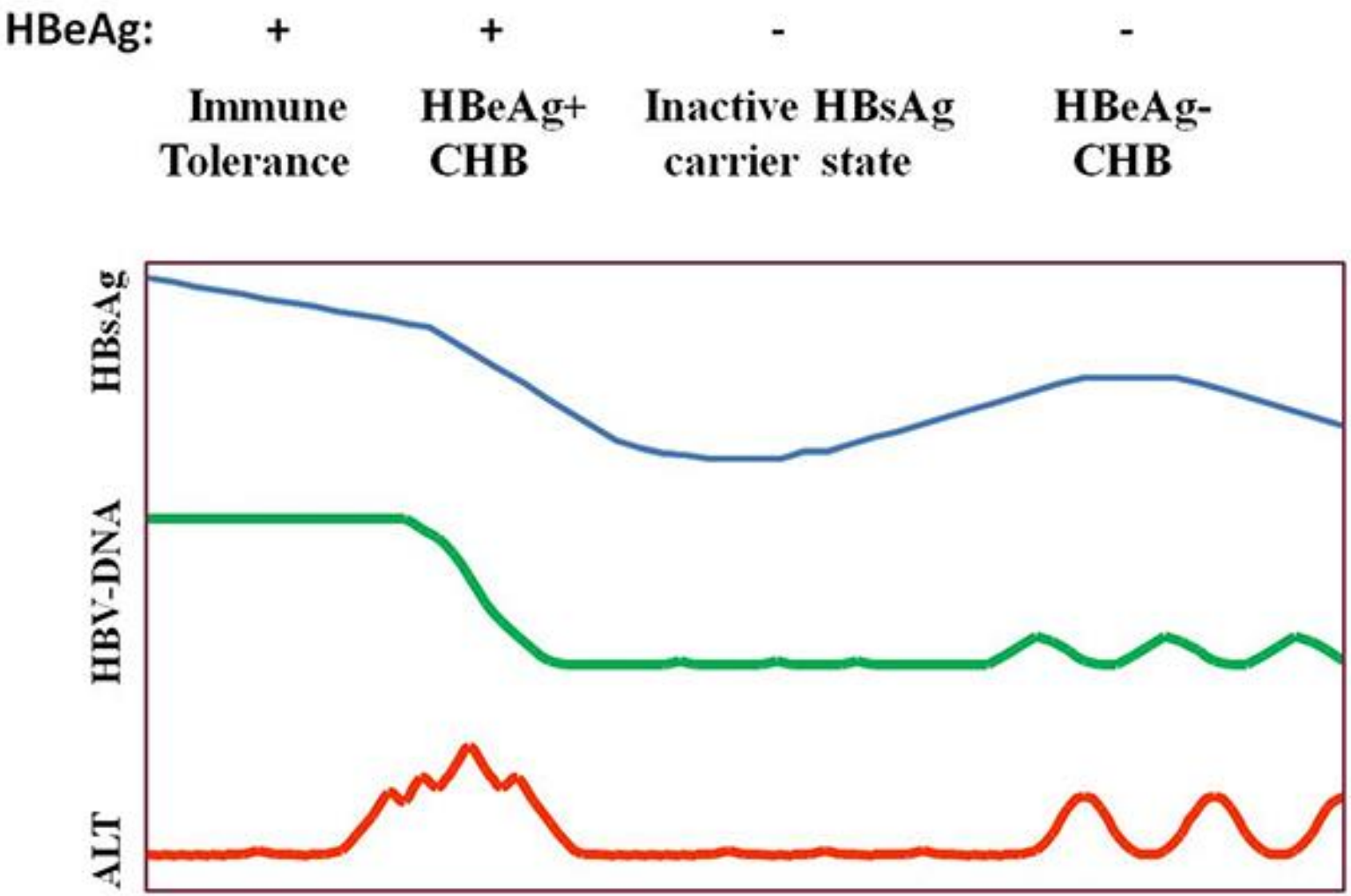
Nature, doi:10.1038/ncomms2798

HBV amplification dynamics

- HBV replication kinetic; 10^{12-13} virion
- HBV viral concentration in a peripheric blood stream; 10^{8} – 10^{10} virion/ml;
- Bayesian prediction; 1/10 000 bp (subsitution/region/year)

Locarnini S, *et* al. Clinics in Liver Diseases. 2010; 14(3):439–459

Clinical phases of hepatitis B



Hepatit B virus pathogenesis; destroing by host immun system

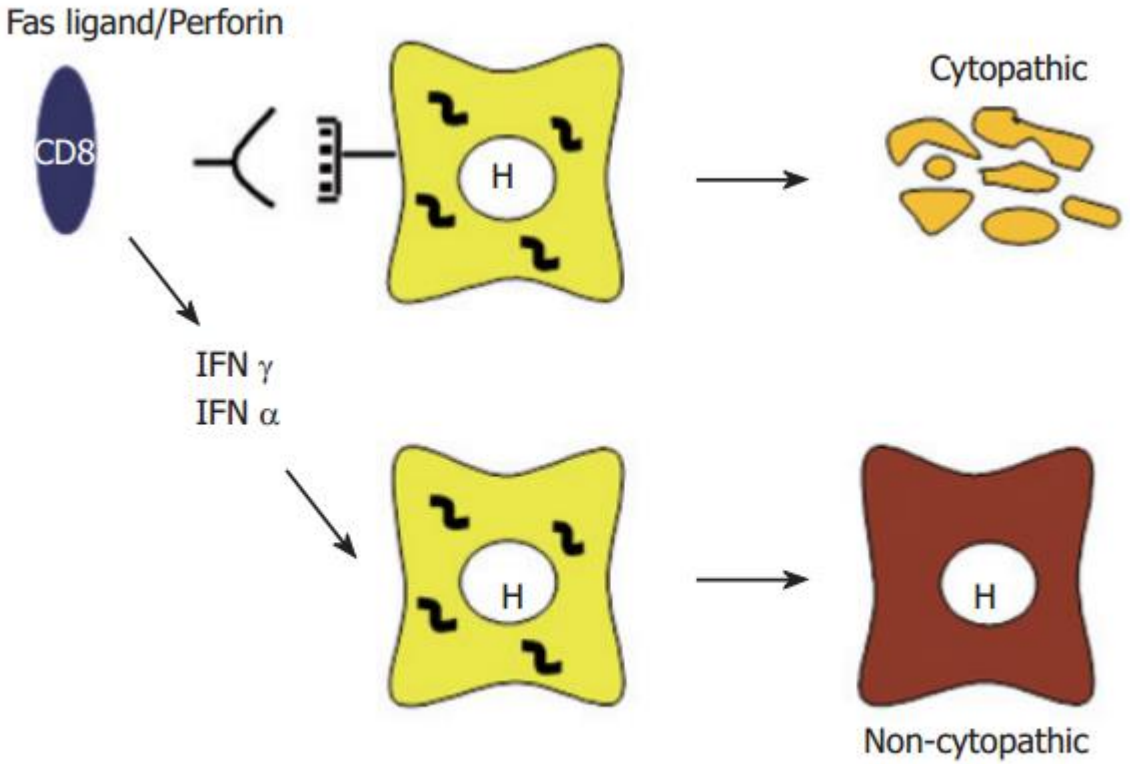


Figure 1 Cytopathic and non-cytopathic T cell responses against HBV infection.

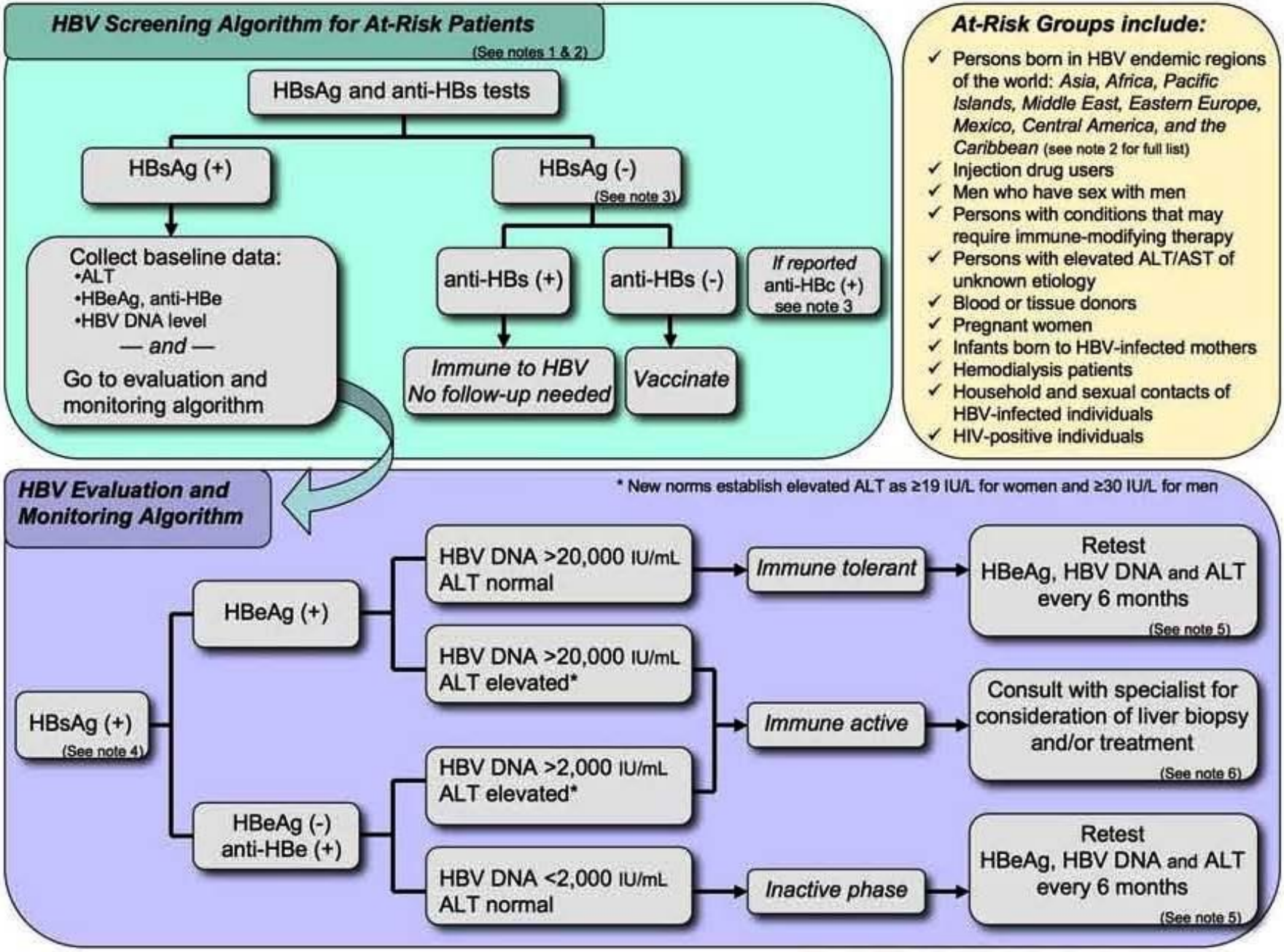
Baumert TF, *et* al. World J Gastroenterol 2007; 13(1): 82–90

Viral antigens

- 1) surface antigen (**HBsAg**) surface (envelope) protein of the dane particle Secreted in excess into the blood as 22 nm spheres and tubules presence in serum indicates that virus replication is occurring in the liver
- 2) e antigen (**HBeAg**) secreted protein; shed in small amounts into blood presence in serum indicates that a high level of viral replication is occurring in the liver. May be negative in carriers with mutations in the e antigen gene who nonetheless have high level viraemia.
- 3) core antigen (**HBcAg**) core protein present in infected liver cells, not found in blood

Antibody response

- 1) Surface antibody (sAb, **antiHBs**)) becomes detectable late in convalescence following resolution of infection, remains detectable for life; not found in chronic carriers; indicates immunity
- 2) e antibody (eAb, **antiHBe**) becomes detectable as viral replication falls In a carrier, it indicates low infectivity
- 3) Core IgM rises early in infection indicates recent infection
- 4) Core IgG Rises early present for life in both chronic carriers as well as those who clear the infection indicates exposure to HBV Usually tested as total core antibodies, and implies IgG in the absence of IgM



HBV viral load and hepatitis B profile

- HBV viral load measures level of HBV DNA in blood. This is the most reliable marker of infectivity. It is more reliable than e antigen which can be negative in some carriers due to mutations in the e antigen gene

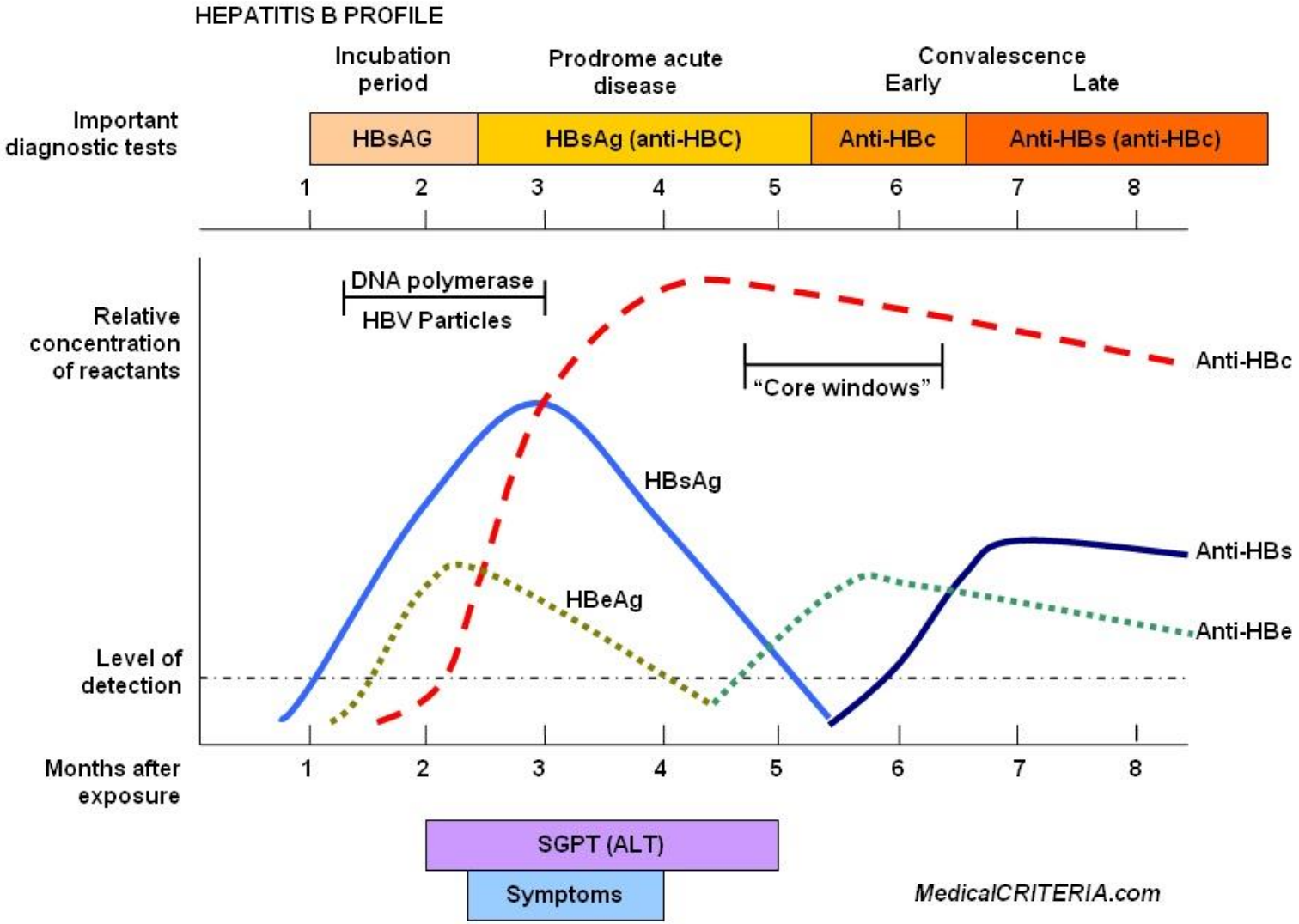


Table 3.1: Serological, virological and biochemical profiles of hepatitis B virus								
	HBsAg	Anti-HBs	Anti-HBc (total)	Anti-HBc IgM	HBeAg	Anti-HBe	HBV DNA (IU/mL)	ALT
Acute HBV	+	-	+	+	+	+/-	High	↑
Natural HBV immunity (resolved infection)	-	+	+	-	-	+/-	Absent	N
Vaccination	-	+	-	-	-	-	Absent	N
Chronic HBeAg positive								
Immune tolerance phase	+	-	+	-	+	-	>20,000 IU/mL	N
Immune clearance phase	+	-	+	-	+	-/+	>20,000 IU/mL (fluctuating)	↑
Chronic HBeAg negative								
Immune control phase	+	-	+	-	-	+	<2, 000 IU/mL*	N
Immune escape phase	+	-	+	-	-	+	>2 000 IU/mL*	↑
Occult HBV	-	-	+	-	-	+/-	Very low	N
Reactivation of HBV	+	-	+	+/-	+	+/-	>20,000 IU/mL	↑

+=positive, -=negative, N=normal, ↑=elevated.
* HBV DNA cut-off levels may change in the future.

Mark Danta, Chapter 3. HBV testing and Interpretating test results. The University of New South Wales, NSW.

Table 2. Geographic distribution of HBV genotypes and subtypes

Genotype	Subtype	Geographic location
A	A1	Sub-Saharan Africa
	A2	Northern Europe
	A3	Western Africa
B	B1	Japan
	B2-B5	Taiwan, China, Indonesia, Vietnam, Philippines
	B6	Alaska, Northern Canada, Greenland
C	C1-C3	Taiwan, China, Japan, Korea, Southeast Asia
	C4	Australia
	C5	Philippines, Vietnam
D	D1-D5	Africa, Europe, Mediterranean basin, India
E		Restricted to West Africa
F	F1-F4	Central and South America
G		France, Germany, United States
H		Central America
I		Laos, Vietnam
J		Japan

Ural O, Mikr Bult 2013, 47(3);550–55
Sayan M, Hepat Mon 2012,12(2):118–121
Sayan M, Hepat Mon 2010, 10(4):302–305

Genotype

Current 8 types (A – H).

Differency; 8%

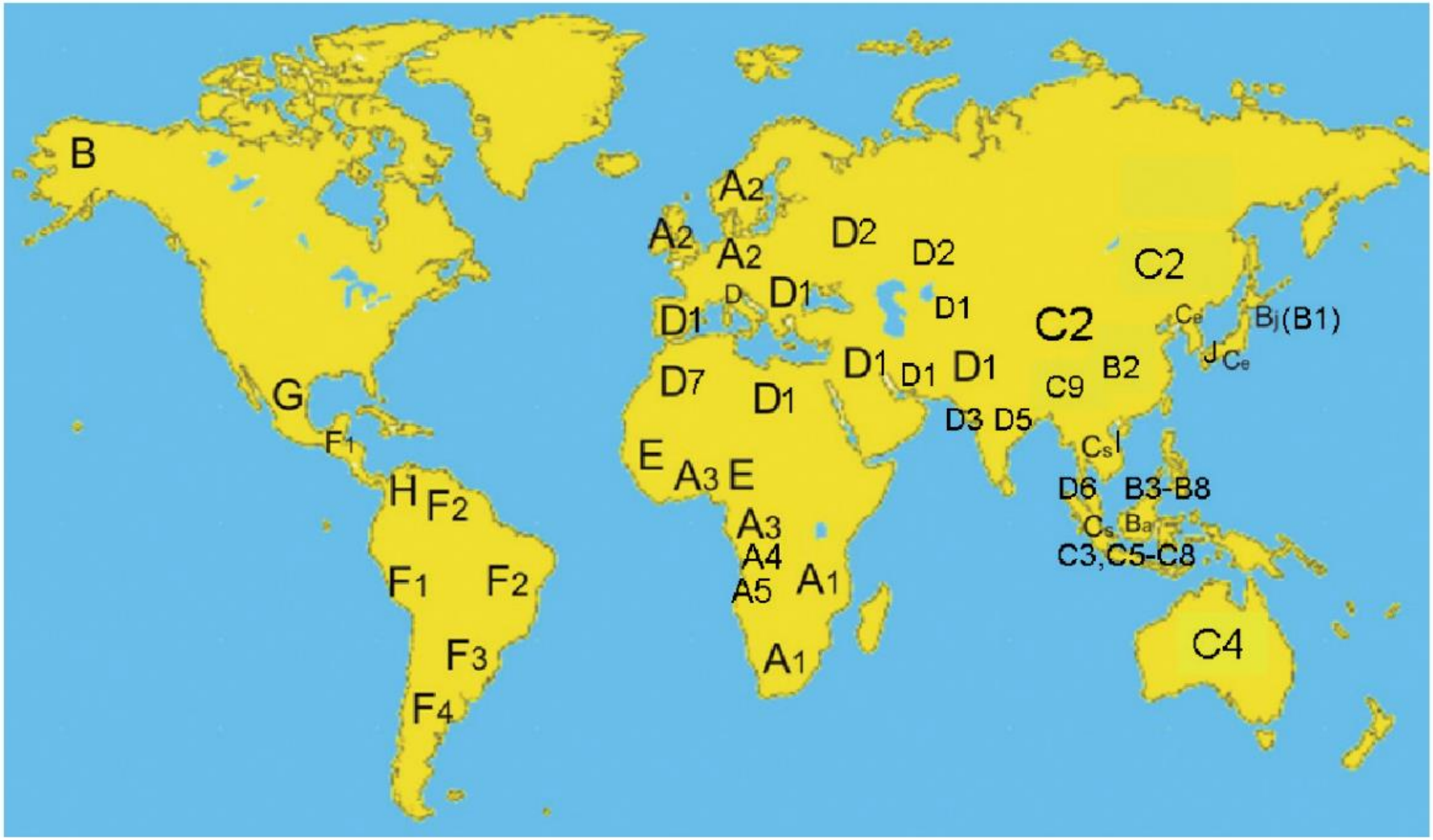
Subgenotype

24 types

Differency; 4%

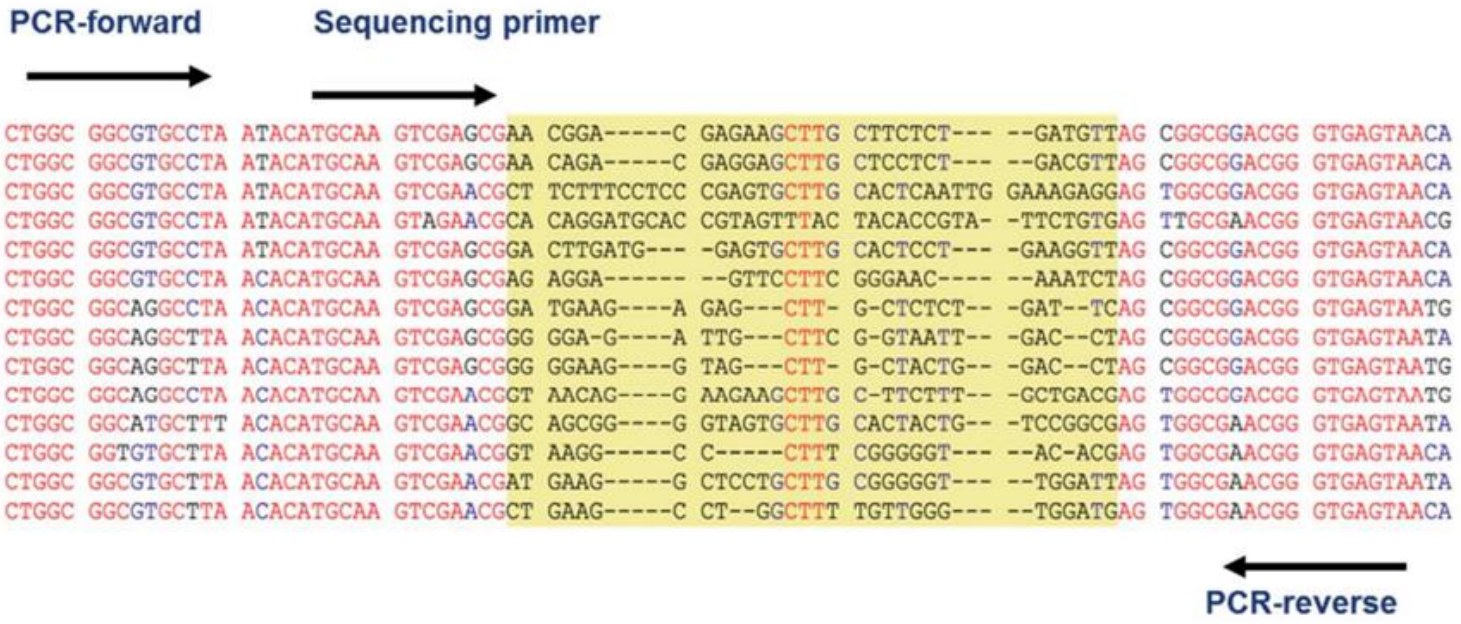
Allain J-P, *et* al., Biologicals (2011); doi:10.1016/j.2011.09.014
Zakim and Boyer's Hepatology (6th Ed.) 2012, P 86–96.
Ma Y, *et* al. Virol J 2011; 8: 315.

HBV subtypes epidemiology



Applied Biosystem 3130

Sanger sequencing system



Phylogenetic analysis is gold standart technique for genotyping

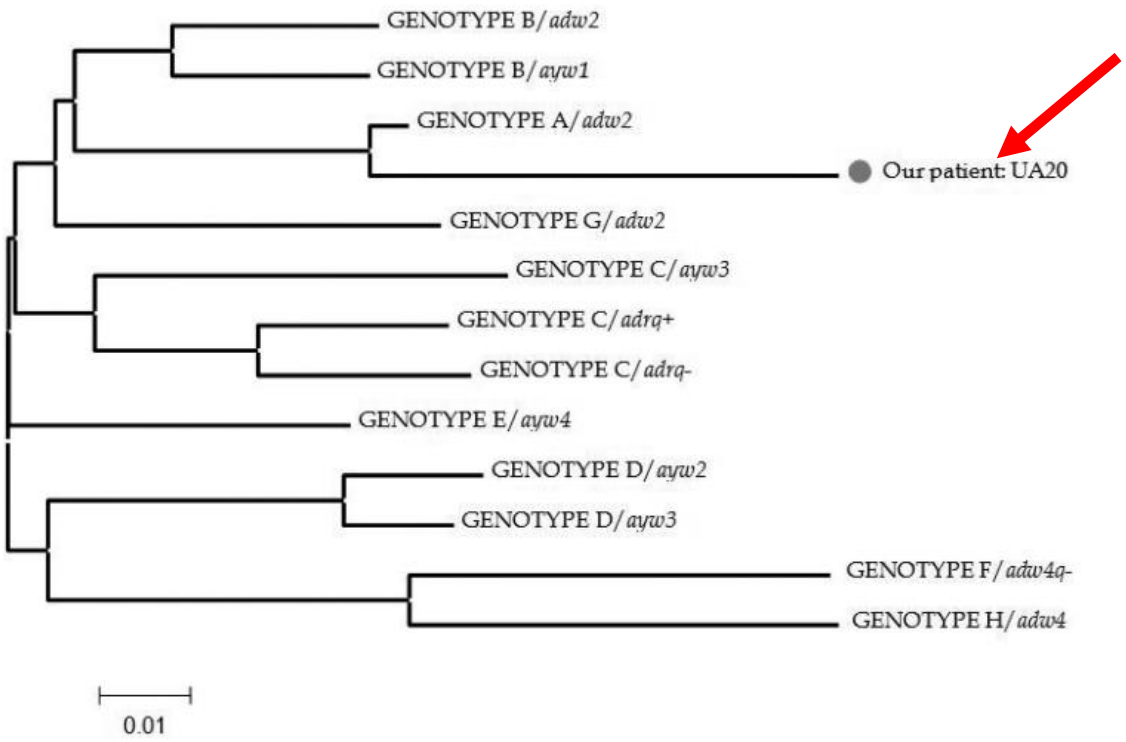


Figure 1. Phylogenetic tree obtained by distance matrix/UPGMA comparison (with Kimura-2 correction) after bootstrapping 1000 replicates of sequence segment at amino acid positions between 80-250 from the reverse transcriptase domain of the polymerase region of hepatitis B virus (UA20 is our patient, the others are from the gene bank)

Hepat Mon. 2010; 10(4): 302-305

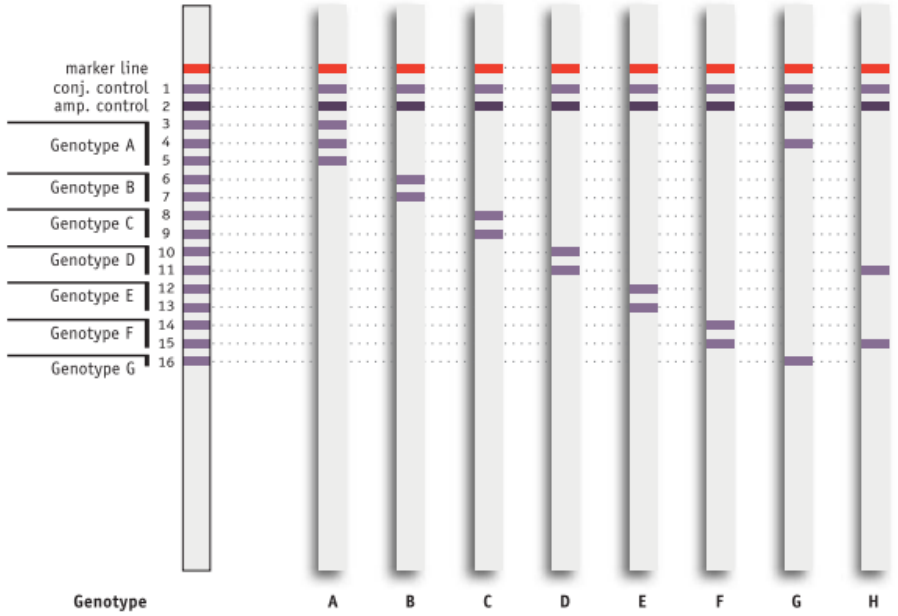
Genotype A2/*adw2* Strain of Hepatitis B Virus in Turkey: A Case Report

Murat Sayan [1*], Sila Cetin Akhan [2], Mithat Bozdayi [3,4]

Line immuno probe assay (LiPA)

INNO-LiPA HBV Genotyping

Line probe assay for the identification of hepatitis B virus genotypes A to H.



Note: Genotype H indicated by reactivity of lines 11 and 15

Features and benefits

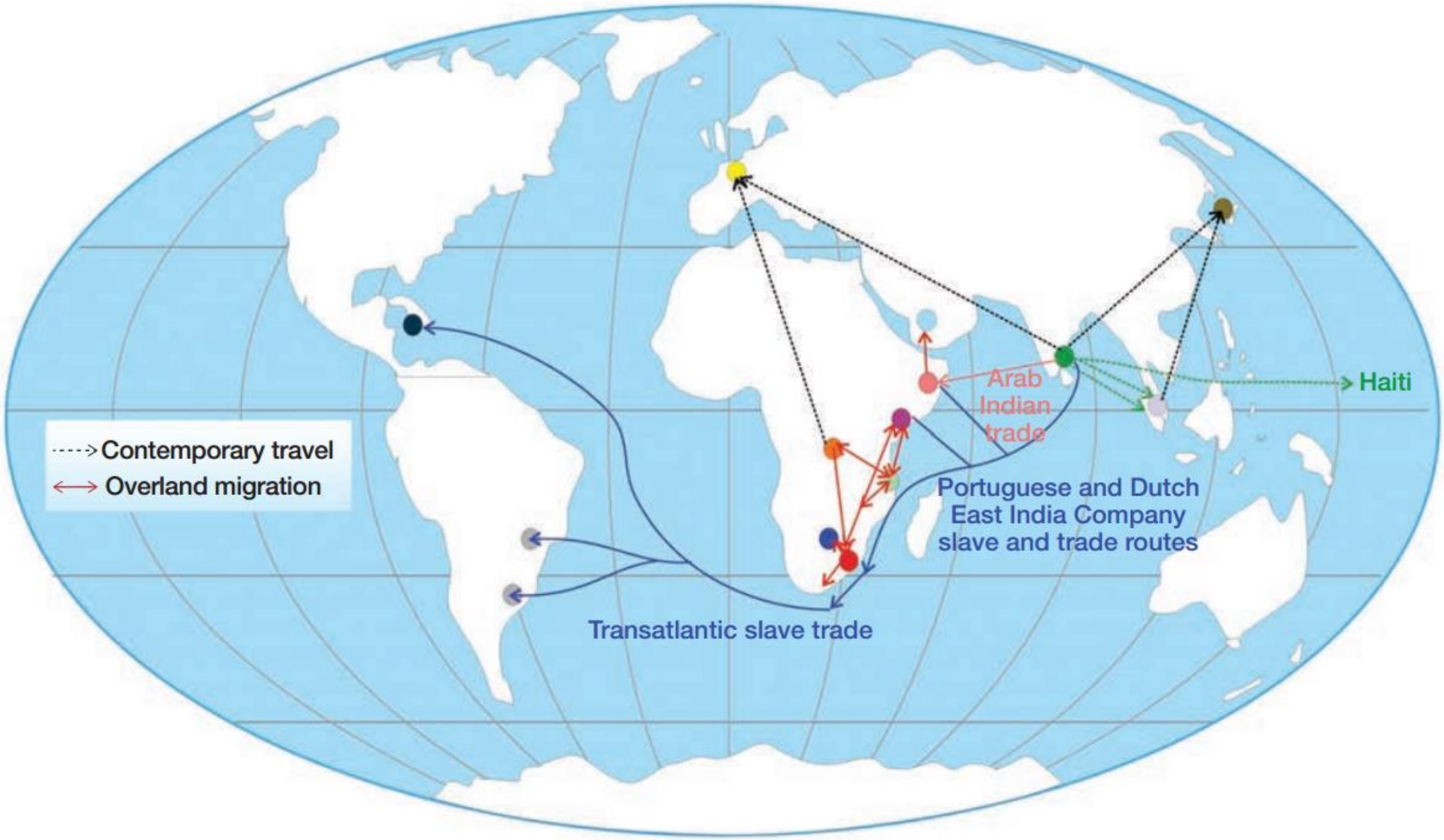
- **Distinct advantage in detection of mixed genotype infections**
  LiPA technology detects up to 16.3% more mixtures than direct sequencing: confirmed by clonal analysis.[1]

Pas *et* al. J Clin Virol 2002;25:63-71

HBV genotype/subgenotype distribution in Turkey

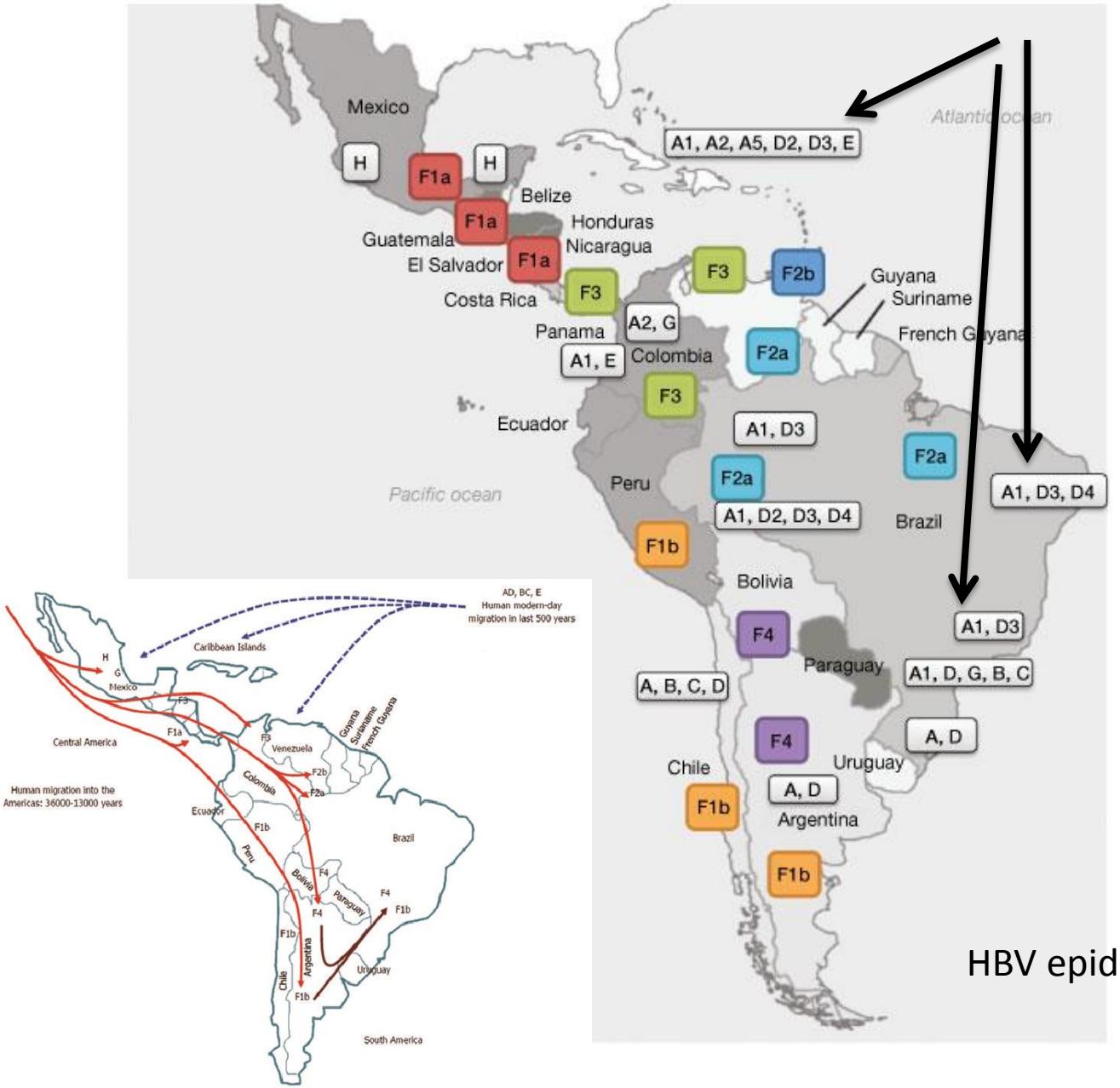
HBV (+)	Naive group	Treatment group
Patient	n=249	n=150
Genotype	D; 248 (%99.6) H; 1(%0.4)	D; 150 (%100)
Subgenotype	**D1**; 220 (%88.8) D3; 17 (%6.8) D2; 10 (%4) D4; 1 (%0.4)	**D1**; 129 (%86) D3; 11 (%7.3) D2; 9 (%6) D4; 1 (%0.6)

Origin and migration of HBV subgenotype A1, 15.- 19. century slave trade



Kramvis A, Paraskevis D. Antiviral Therapy 2013;18:513-521

HBV epidemiology in East-North Europe and Baltic countries:
Dispersion of soviets and raising of IVDU's



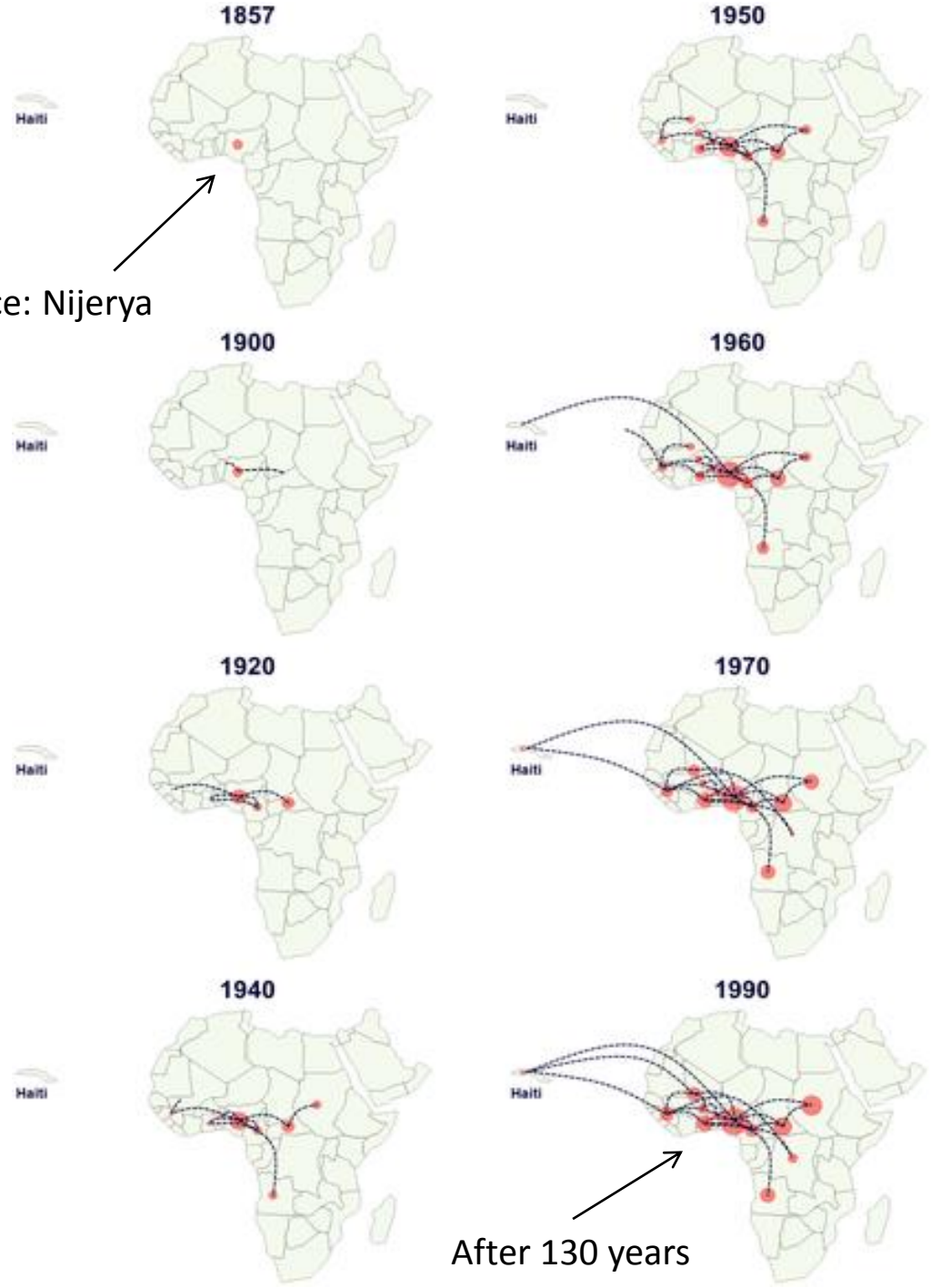
Imported of HBV genotpes bySpanish colonist

HBV epidemiology in Latin America

World J Gastroenterol.2014 June 21; 20(23): 7181-7196.

Phylogeographie of HBV genotype E in Subsaharan Africa

Source: Nijerya

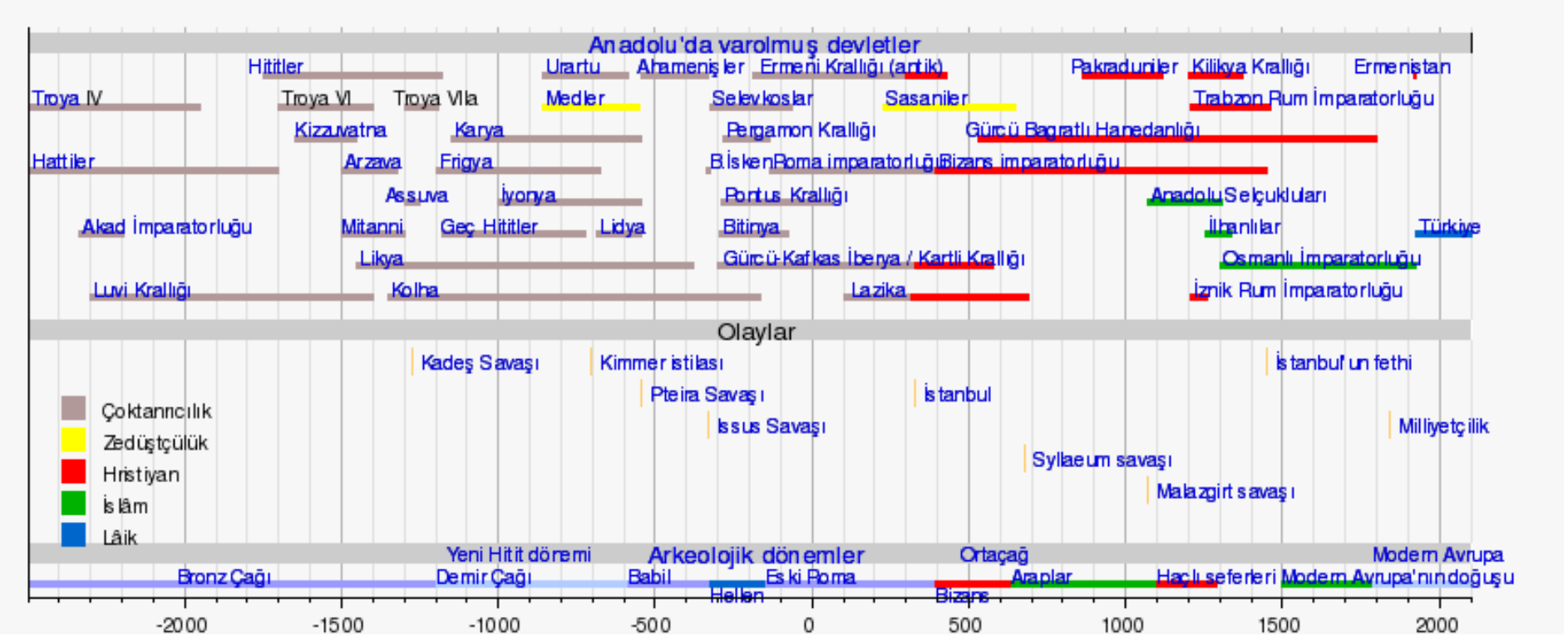


Diversity of genotype E is lower.

After 130 years

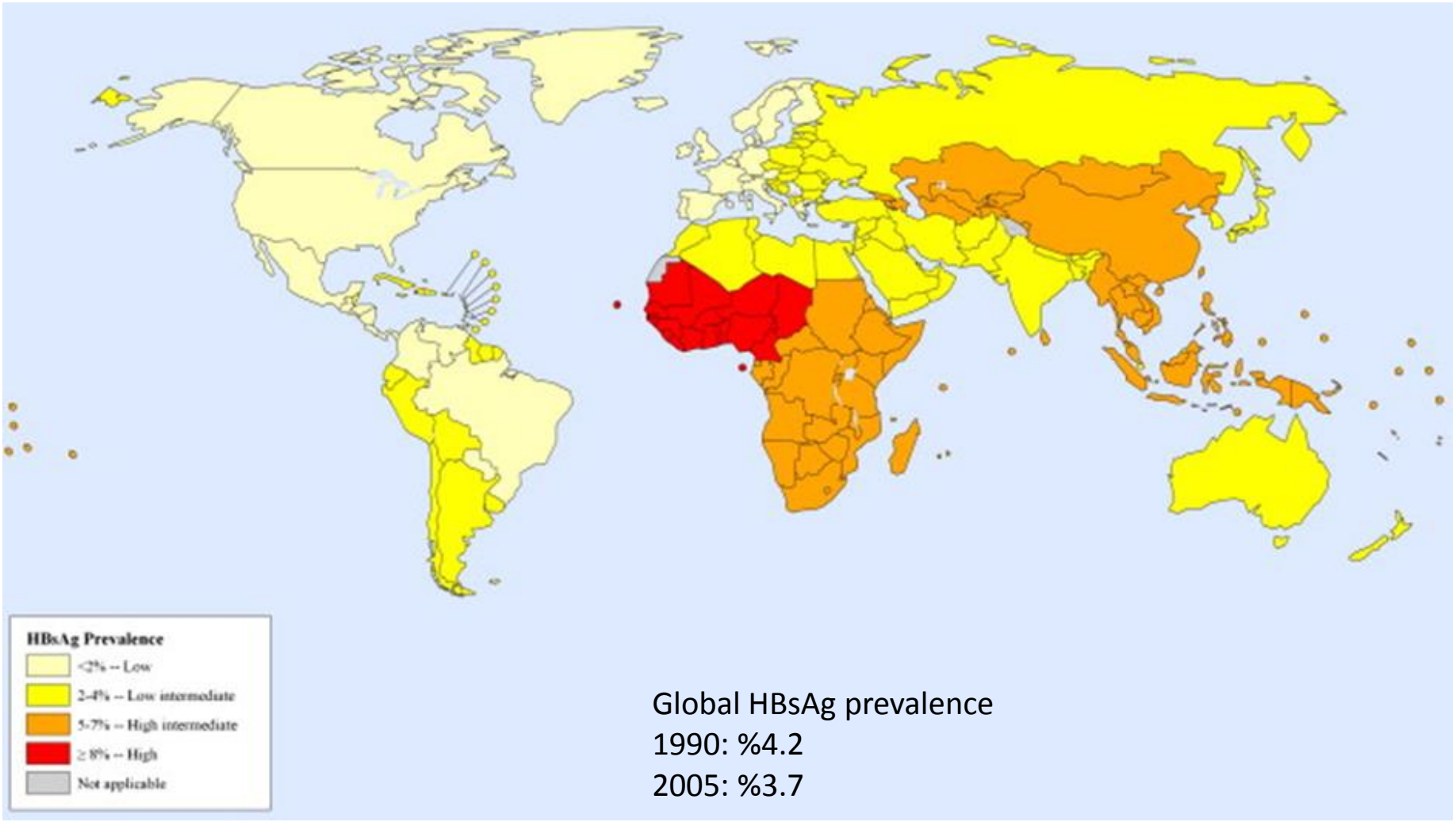
Andernach IE, *et* al. Bayesian Inference of the Evolution of HBV/E. PLoS ONE 2013; 8(11): e81690.

Why not vary HBV genotype diversity in Turkey?



HBV global epidemiyology

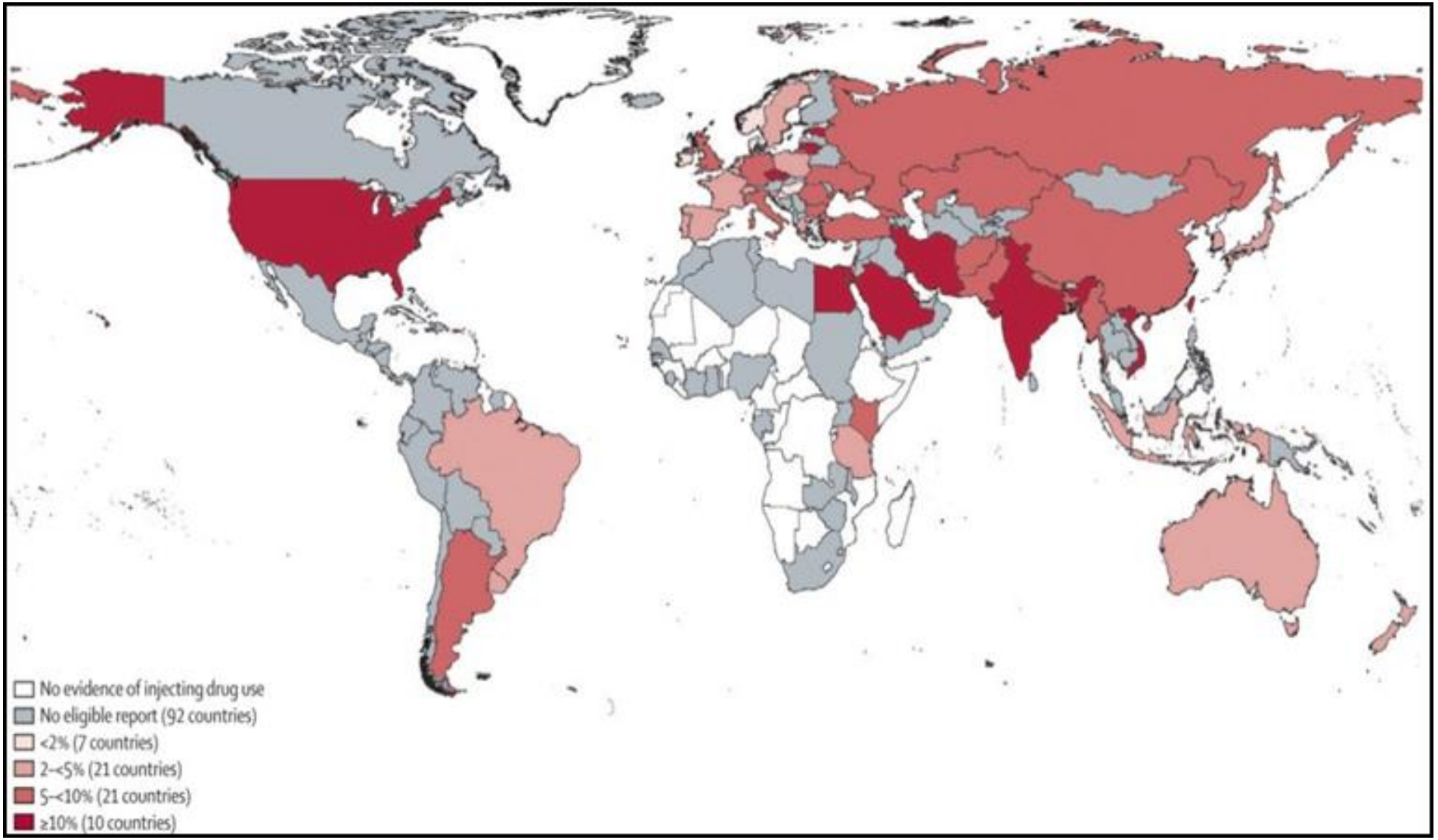
- 2 bilion individuals infected with HBV
- 240 milion are chronical diseases
- 600 000 dead; HBV related diseases and hepatocelular carsinom



Global HBsAg prevalence
1990: %4.2
2005: %3.7

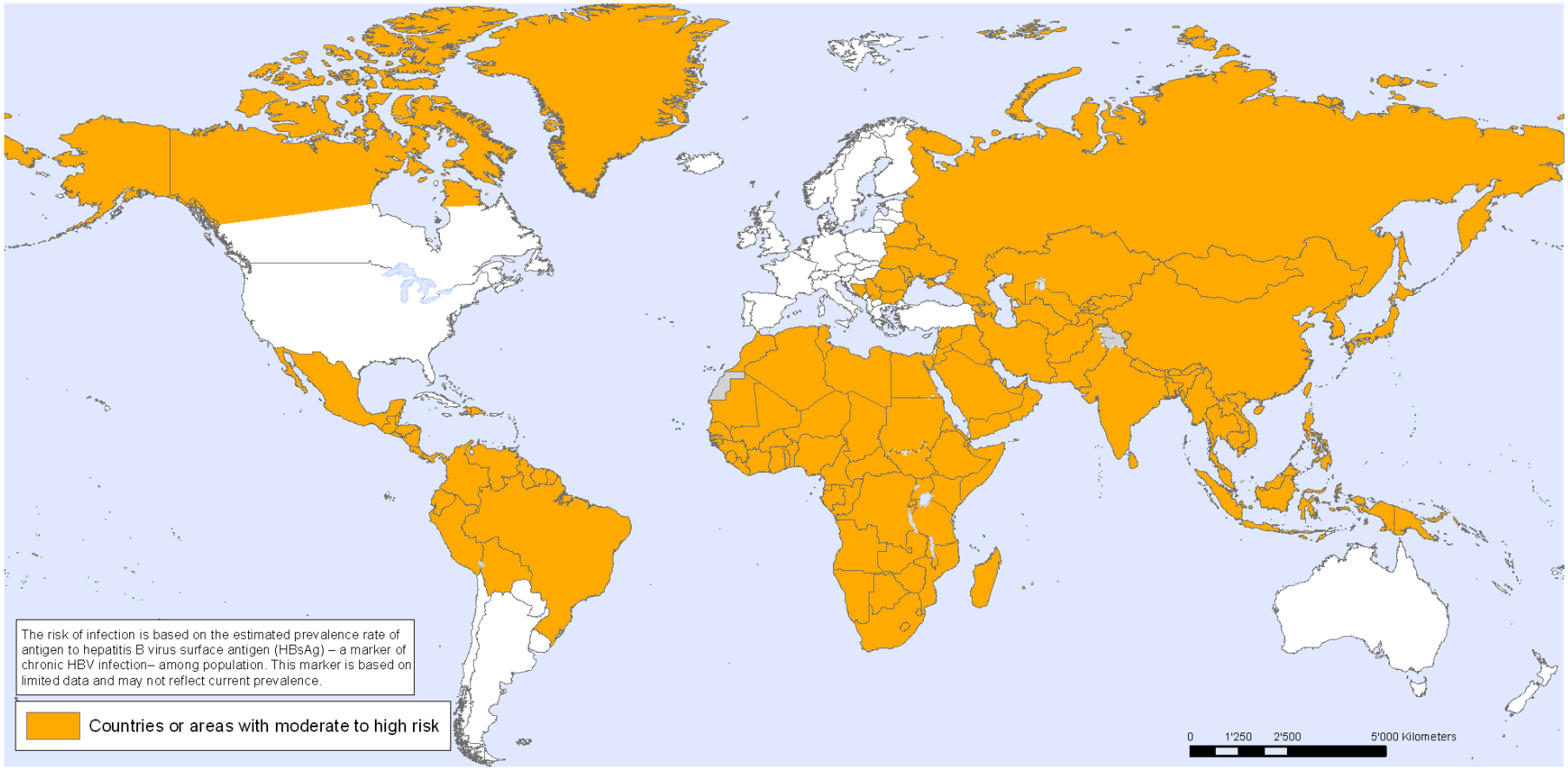
Ott JJ, Vaccine 2012;30:2212-9.

HBV epidemiology in IVDU population



Nelson PK, Lancet 2011;378:571-83

Hepatitis B, countries or areas at risk



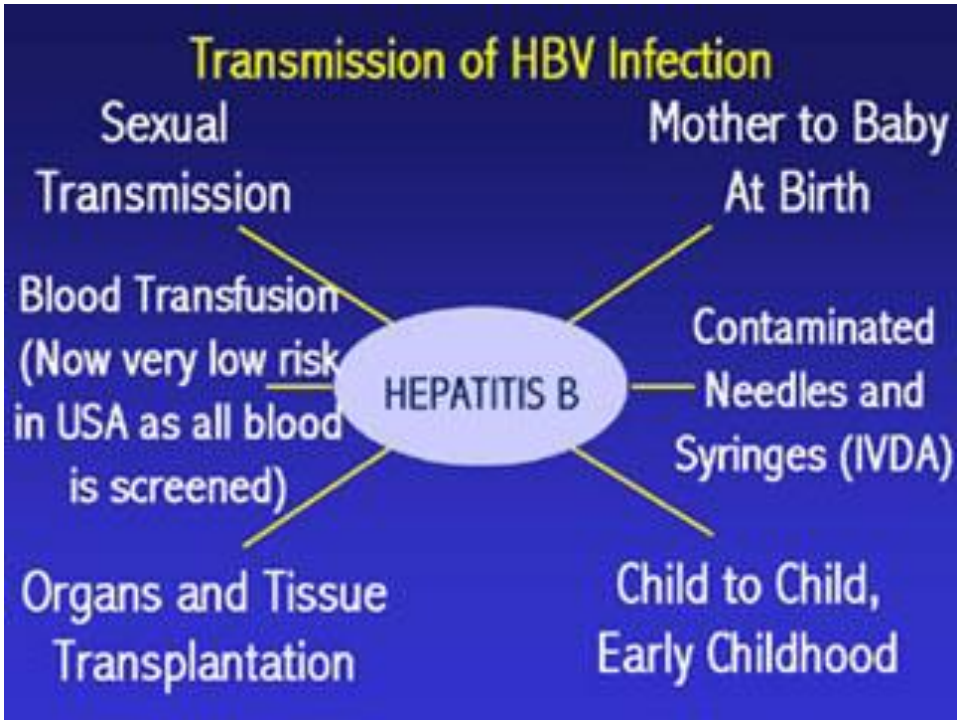
The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

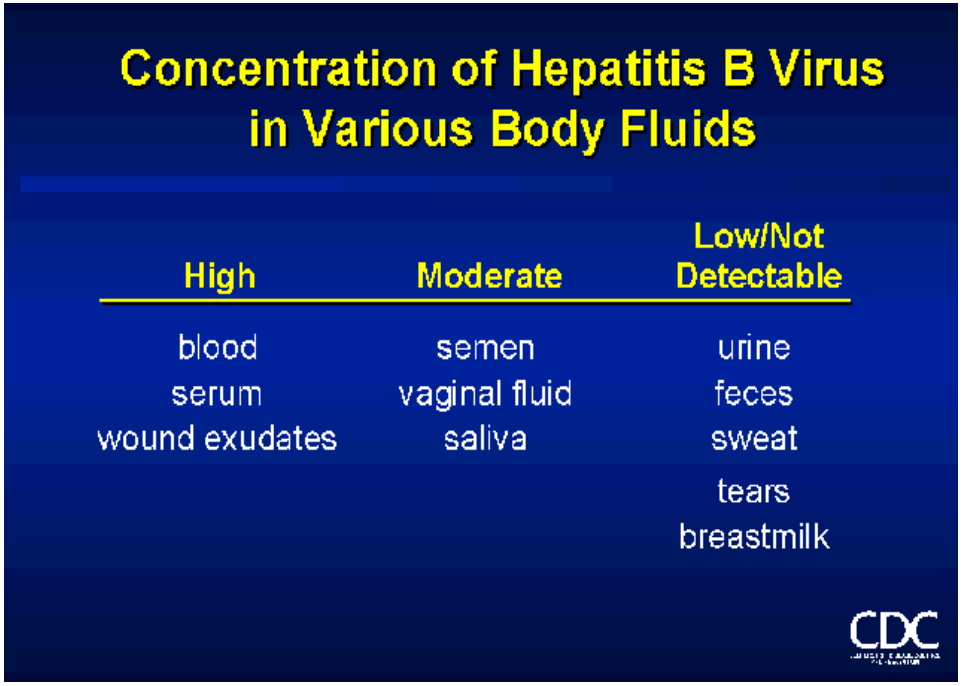
Data Source: World Health Organization/CDC
Map Production: Public Health Information and Geographic Information Systems (GIS)
World Health Organization

World Health Organization

Hepatitis B is parenterally transmitted

- **Sexual intercourse**: Predominant mode of spread amongst adults
- **Close personal contact**: spread amongst children and in families, often called "horizontal" spread. This is the most common mode of transmission in areas of high HBV prevalence, where infection is acquired early in life
- **Vertical transmission:** Perinatal transmission from a carrier mother to her baby Transplacental (rare), During delivery Post natal ??, breast feeding ?? close contact. This is a major mode of transmission in South East Asia.
- **Blood**: Blood transfusions, serum products
- **Sharing of needles, razors**
- **Tattooing, acupuncture**
- **Renal dialysis**
- **Organ donation**





Prevention

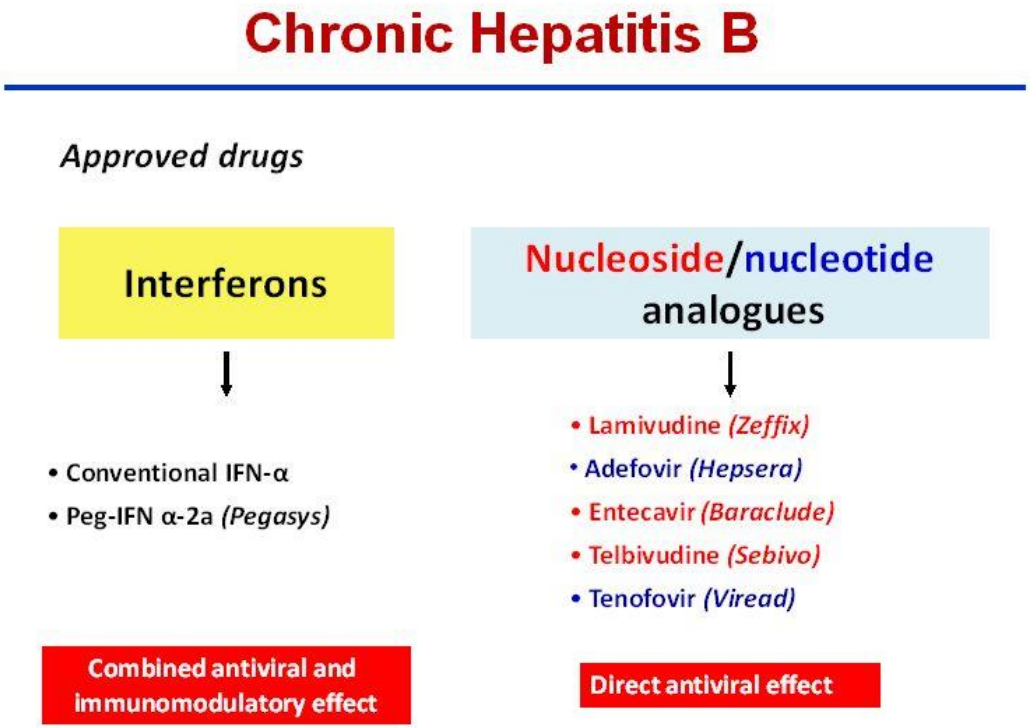
Vaccine

- **Active Immunization :**Four types of vaccine are available **Serum derived** - prepared from s Ag purified from the serum of HBV carriers **Recombinant sAg** - made by genetic engineering in Saccharomyces cerevisiae, also known as Brewer's yeast Third generation vaccines genetically engineered producing different size surface antigens
  - **Health care workers**
  - **Sexual partners of chronic carriers**
  - **Infants of HBV carrier mothers**
  - **Post exposure prophylaxis**
- **Passive Antibody** Both Hepatitis B immune globulin and vaccine should be administered to non immune individuals following single episode exposure to HBV-infected blood.

Treatment

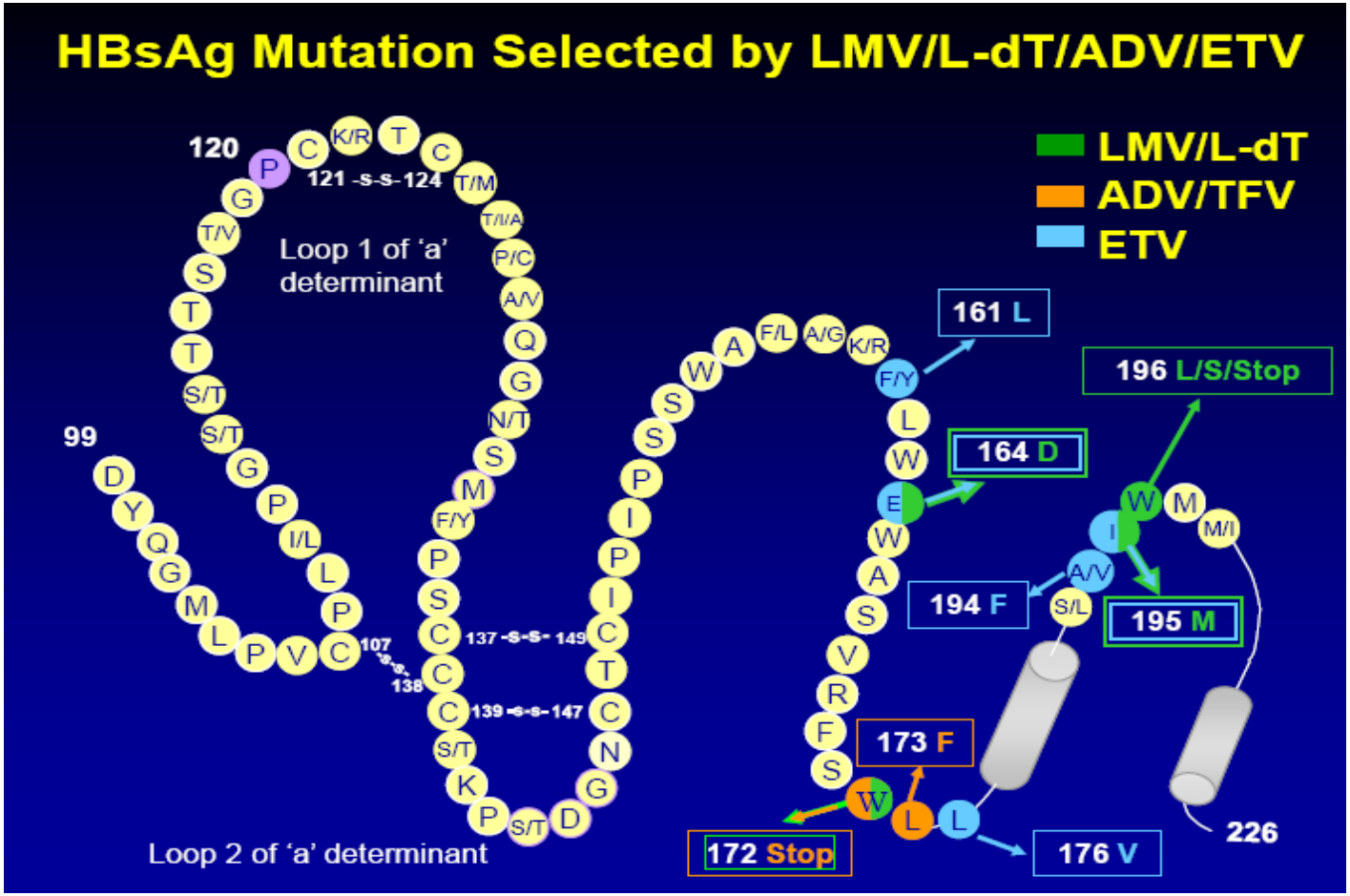
Two classes of drugs are used to treat chronic HBV infection

- **1. Interferons:** Interferon α2a Pegylated interferon α2a Interferon-α enhances the host immune response to HBV and improves immune control of the virus. Clearance of infection (and immunity) is the best outcome, but is achieved in only around 25% cases (after a six month course of treatment).
- **2. Nucleoside reverse transcriptase inhibitors (oral antivirals):** These drugs interfere with viral replication, but cannot clear HBV infection. They need to be taken life long (as for HIV) to control infection..



Thesis proposal:

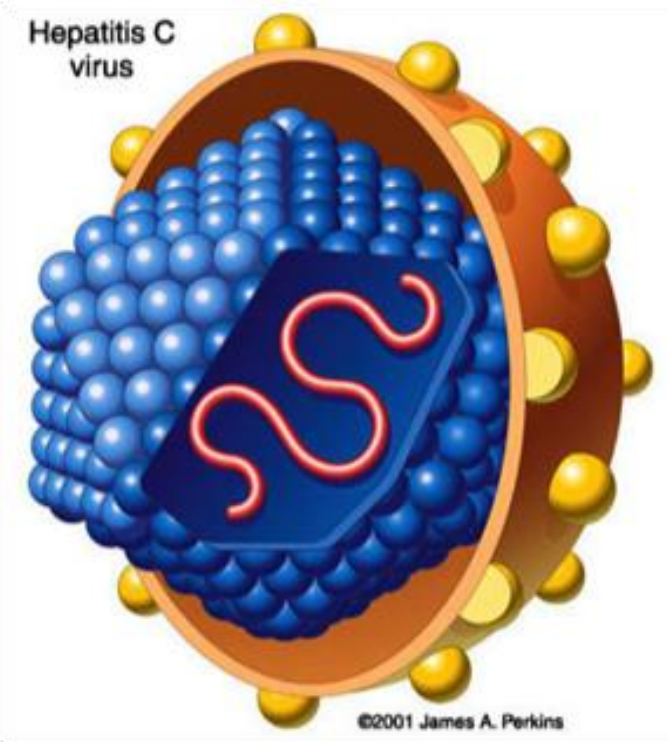
Antiviral drug-associated potential vaccine-escape mutant (ADAPVEM)



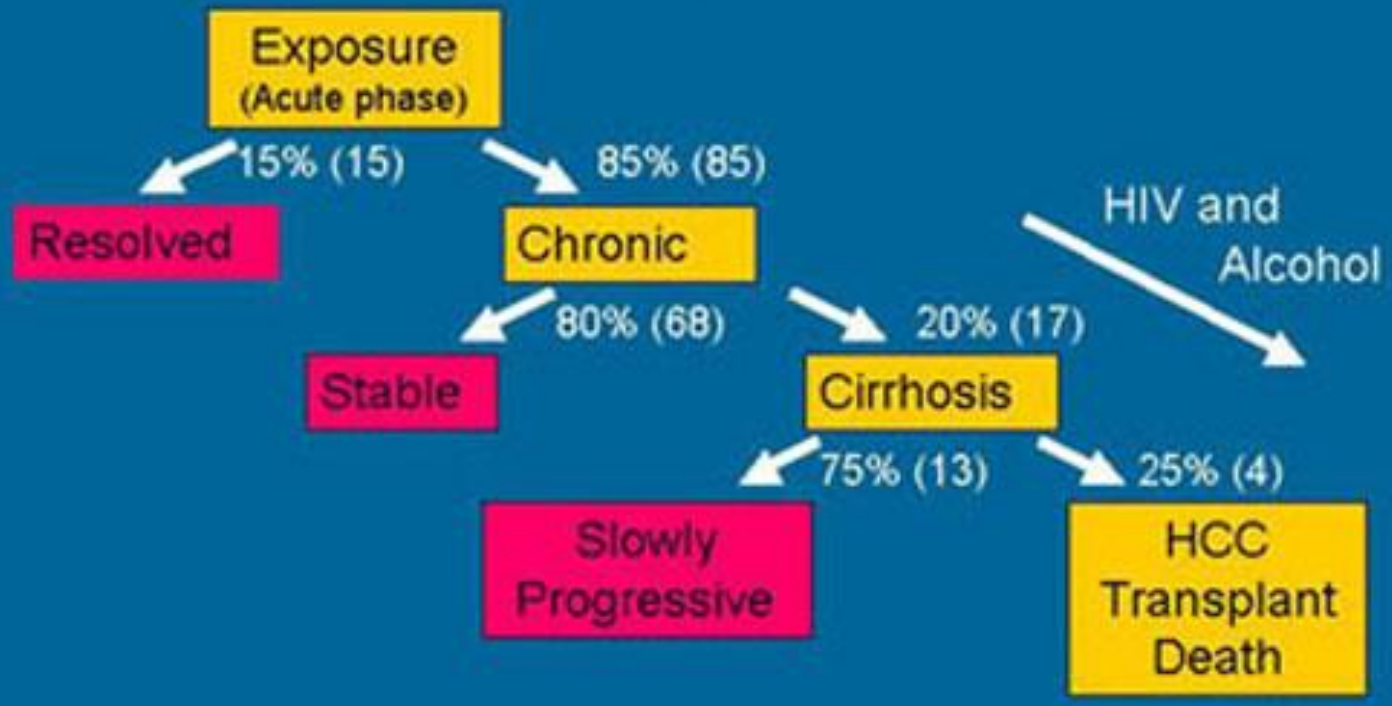
Hepatitis C

HCV Virology

- Order: none
- Family: Flaviviridae
- Genus: Hepacivirus
- Species: Hepatitis C virus
- Structure: 55-65 nm in diameter, enveloped
- Genome: +ssRNA (positive sense, single stranded RNA) Genome has a high mutation rate Viruses do not grow in cell culture, and only infect humans and chimpanzees

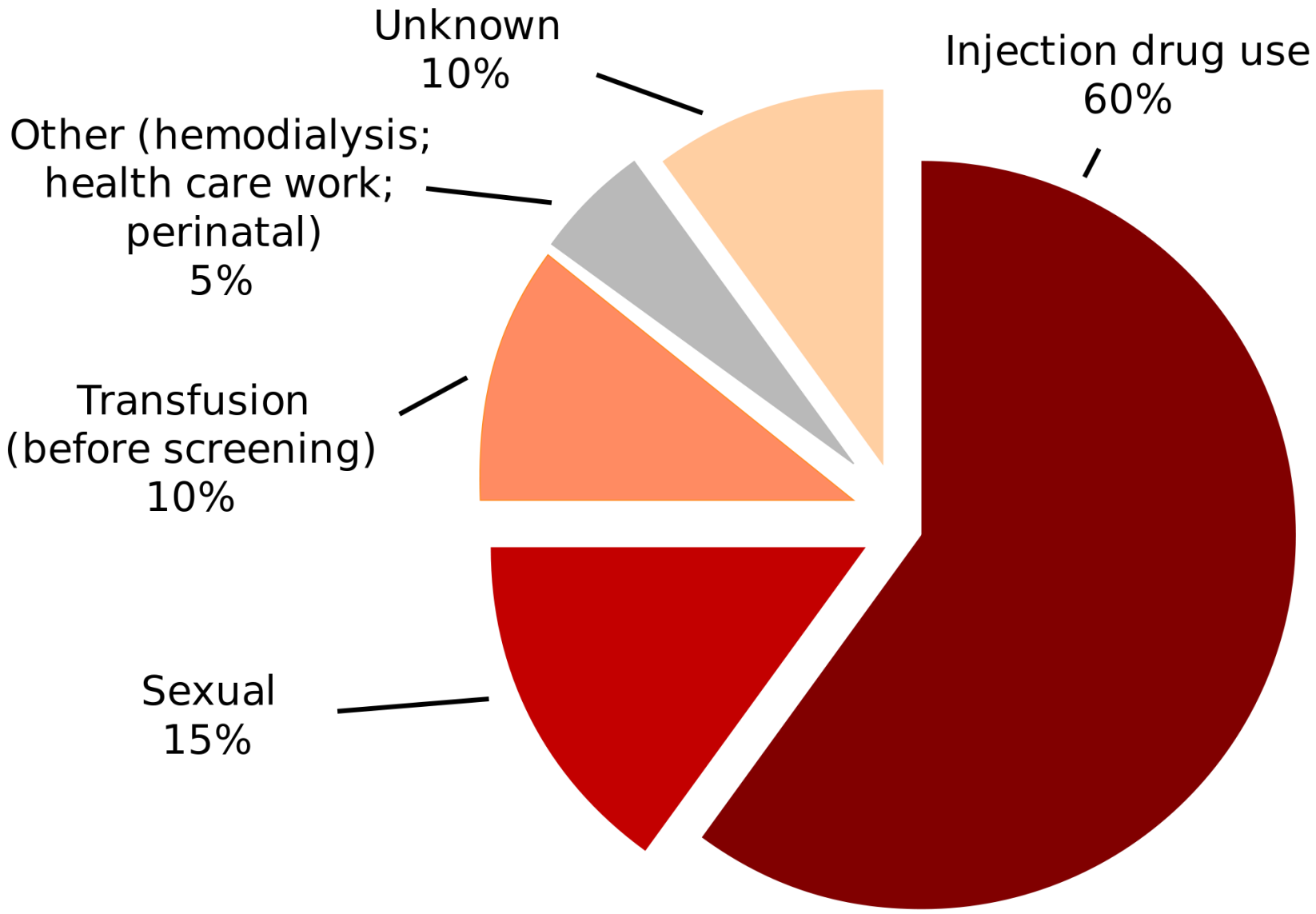


Natural History of HCV Infection

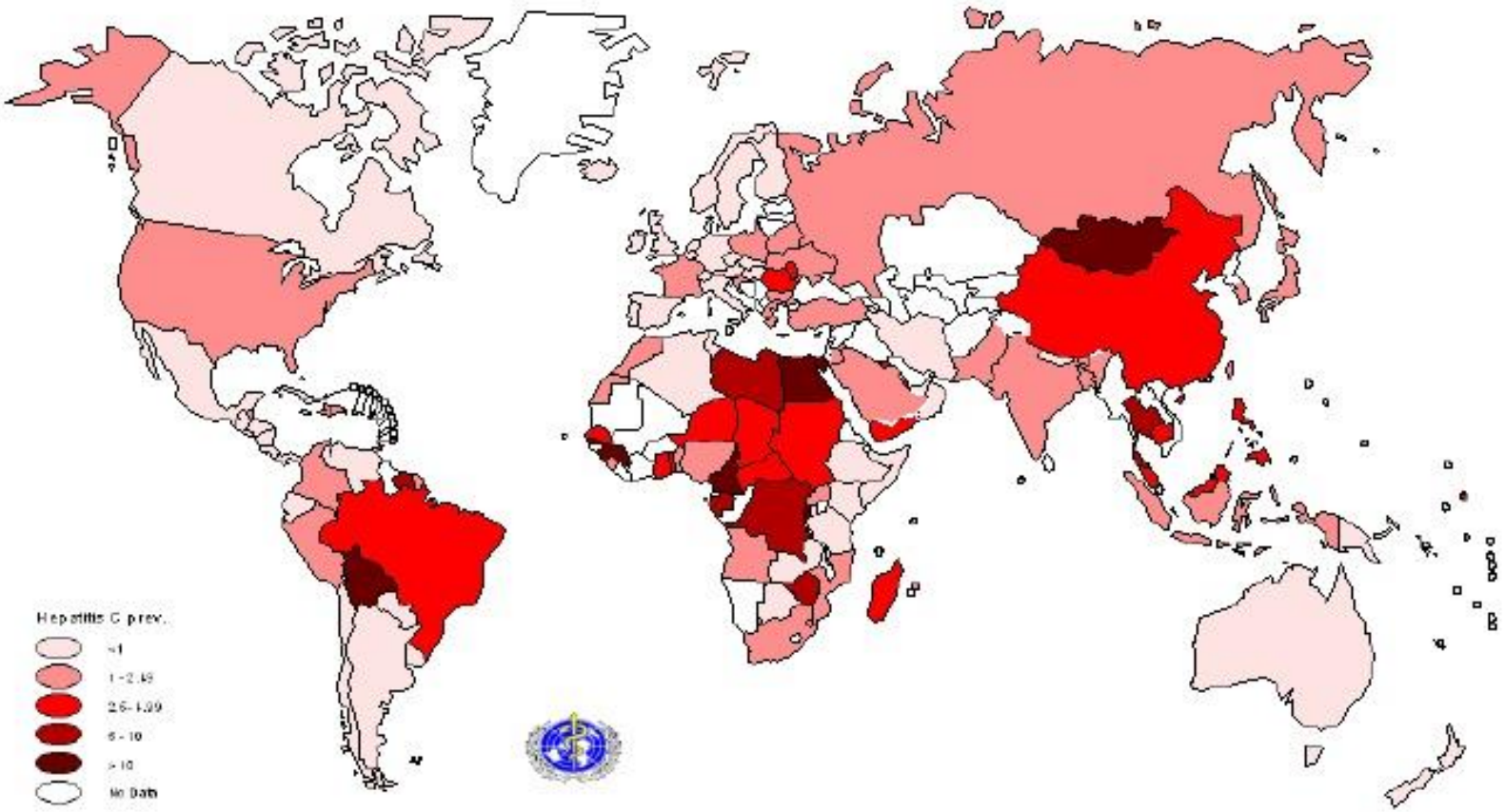


Alter, MJ. Epidemiology of Hepatitis C in the West. Semin Liver Dis. 1996; 15:5-14.
Management of Hepatitis C. NIH Consensus Statement. 1997 March 24-26;15(3).

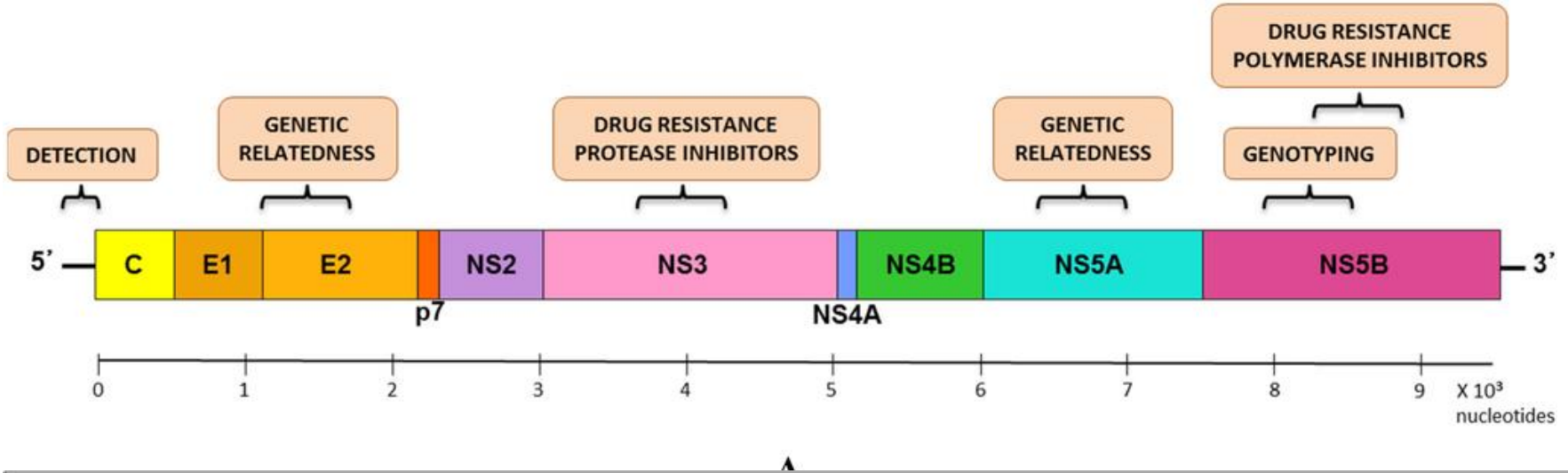
Transmission of HCV

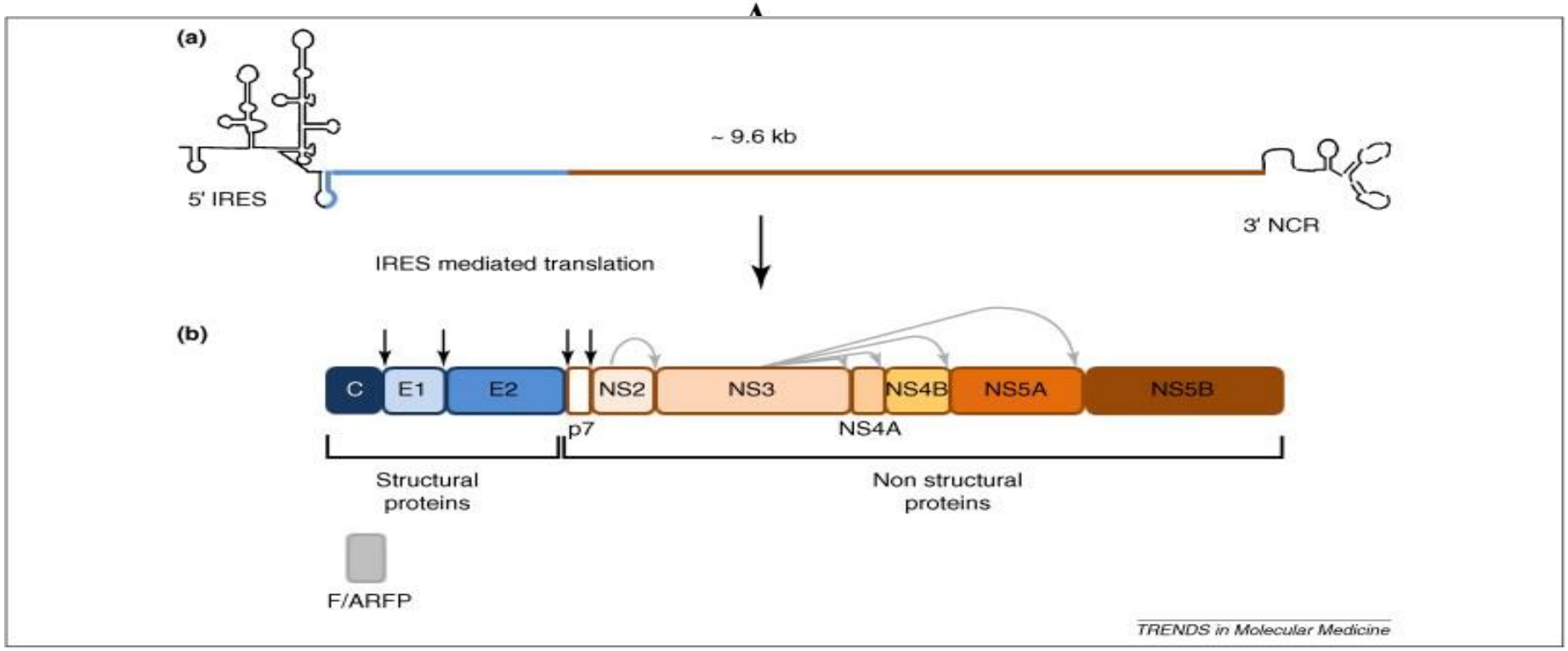


GLOBAL PREVALENCE OF HEPATITIS C

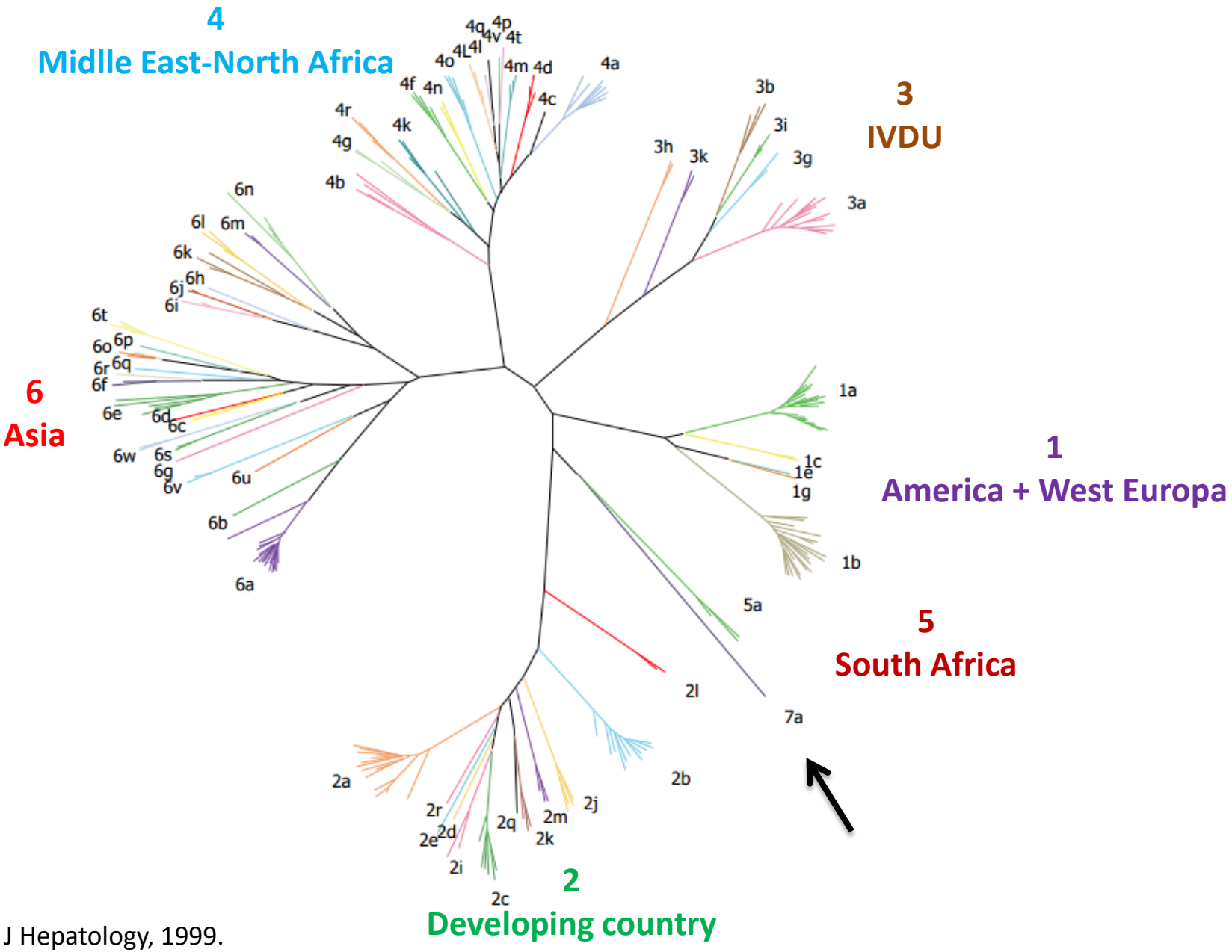


HCV genome organisation





HCV genotypes



Simmonds P, J Hepatology, 1999.
Simmonds P, Hepatology, 2014.

Different Virus Replication Strategies: Different Treatment Goals

HBV
(Latent Reservoir)

Long-term reduction of viral replication to lowest possible level

HIV
(Latent Reservoir)

Lifelong suppression of viral replication

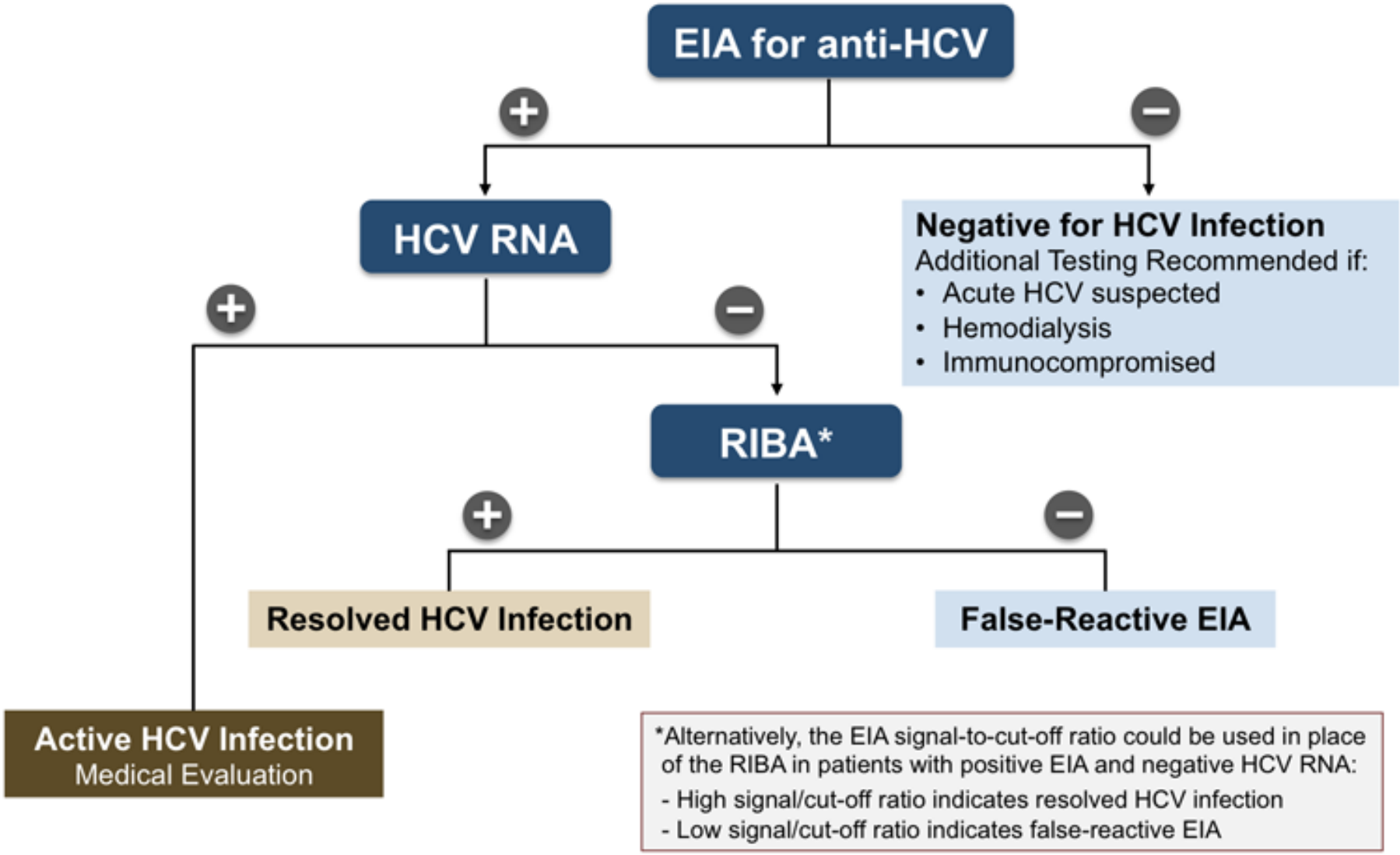
HCV
(No Latent Reservoir)

Definitive viral clearance:
SVR possible for HCV

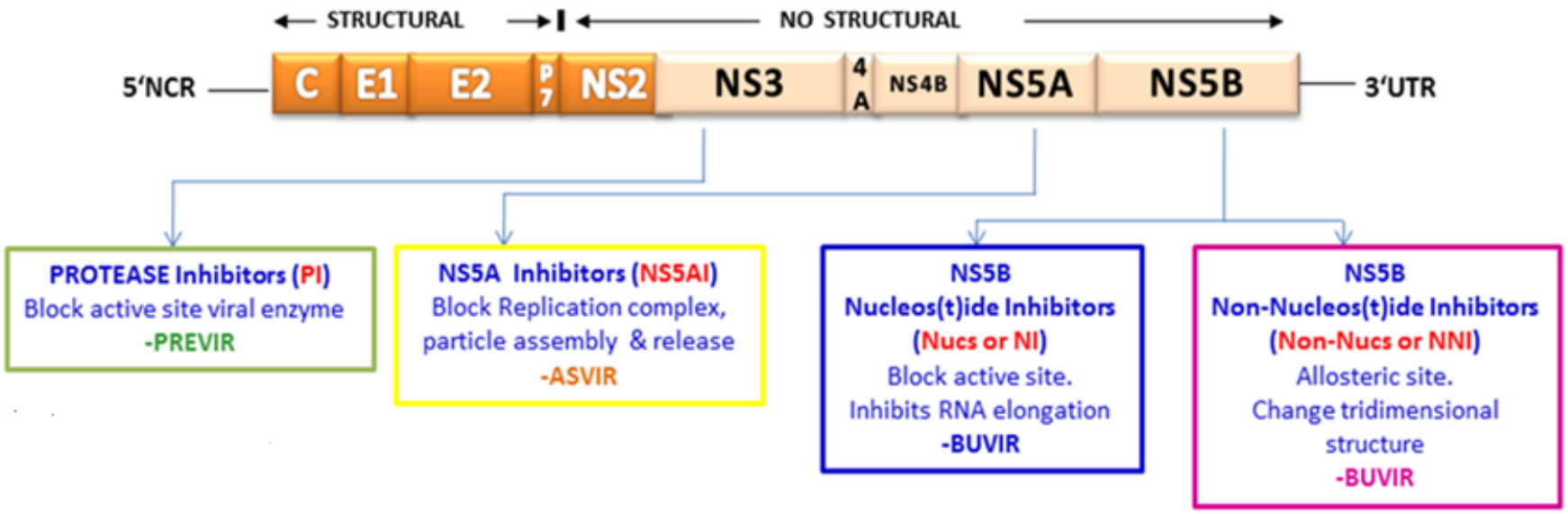
SVR = sustained virologic response

Kieffer TL, et al. *J Antimicrob Chemother*. 2010;65:202-212.

Diagnosis of HCV

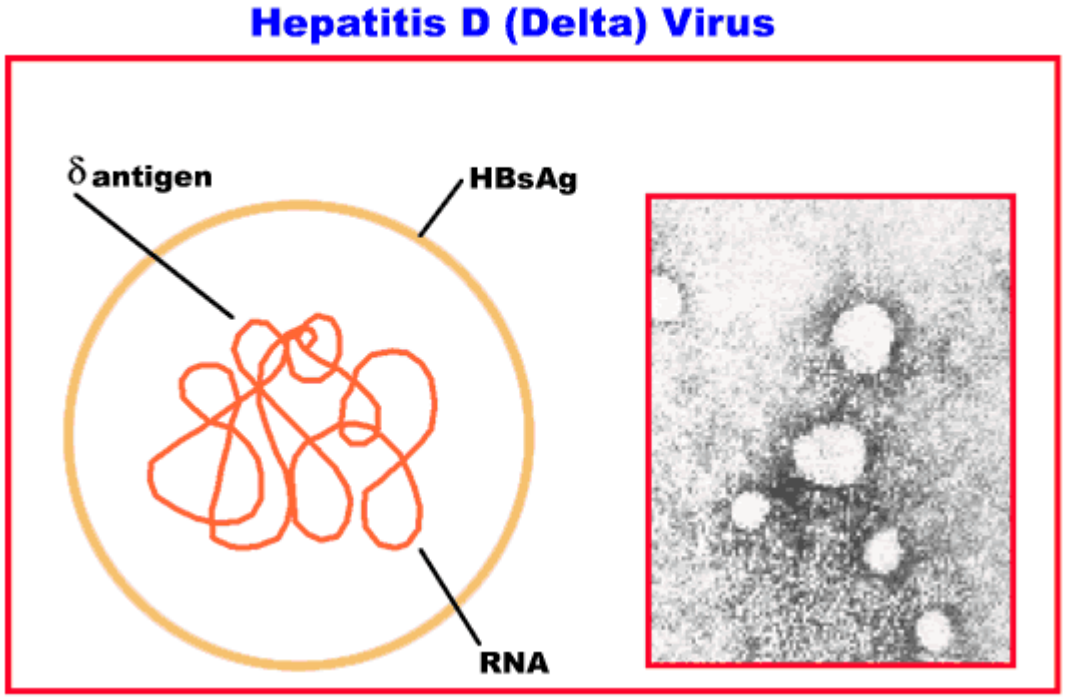


Antiviral treatment in chronical hepatitis C



Hepatitis Delta

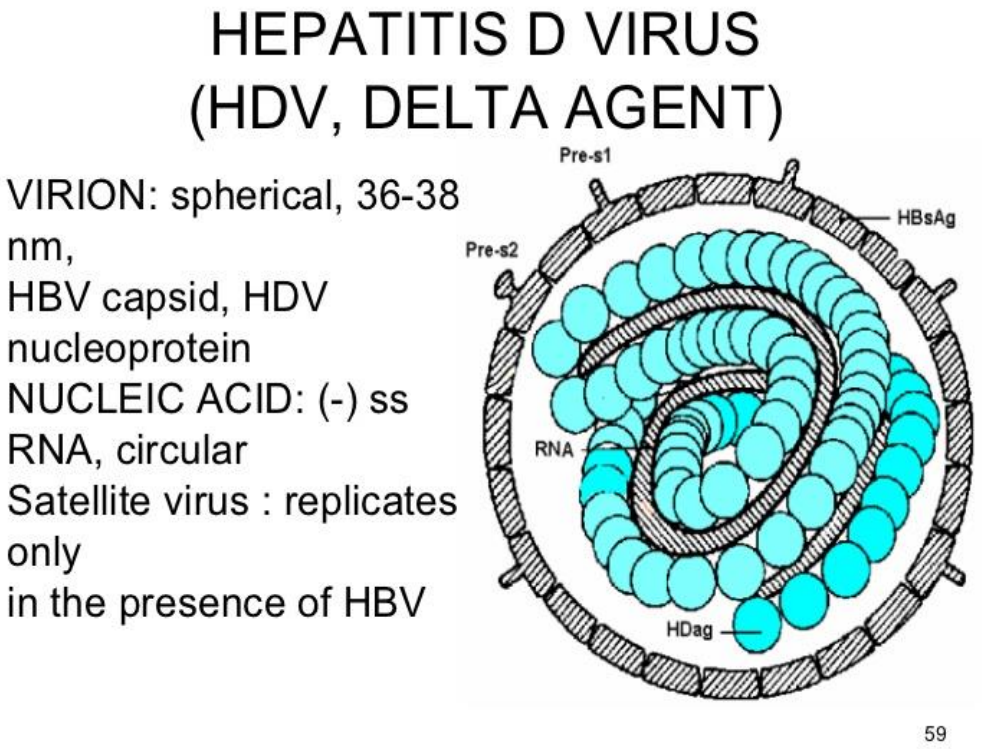
- In 1977 an novel protein was discovered in the serum of some patients who were infected with Hepatitis B.
- It was named the delta antigen.
- Subsequent investigation showed that the protein was encoded by a new virus, now called the hepatitis D virus (HDV).
- It is a defective virus which requires Hepatitis B as a helper virus in order to replicate.
- Infection therefore only occurs in patients who are already infected with Hepatitis B.

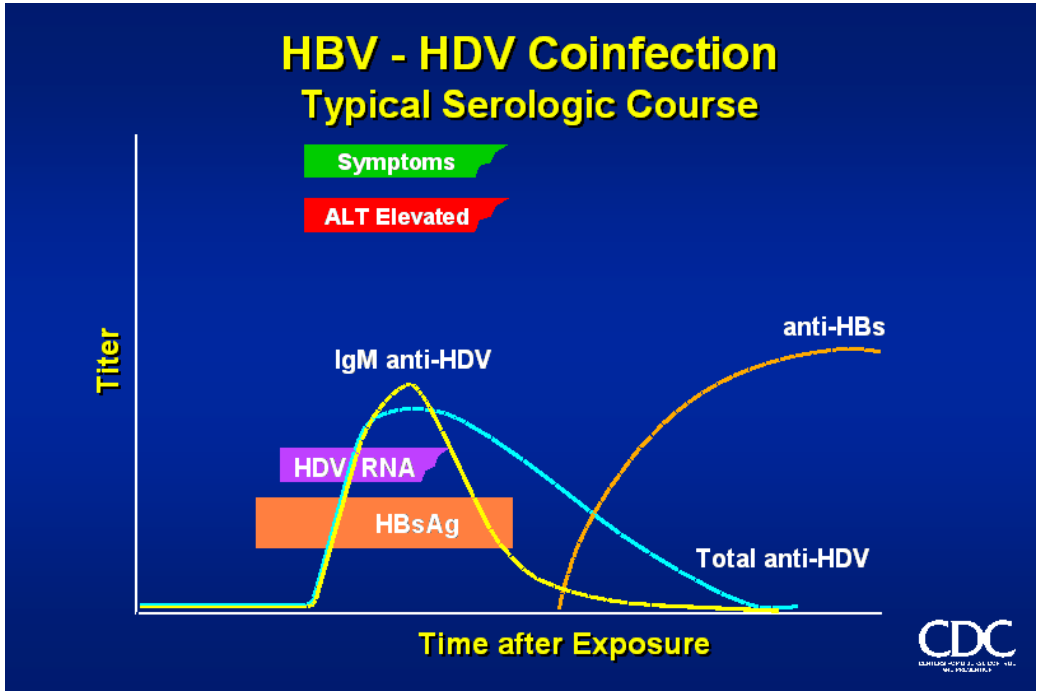


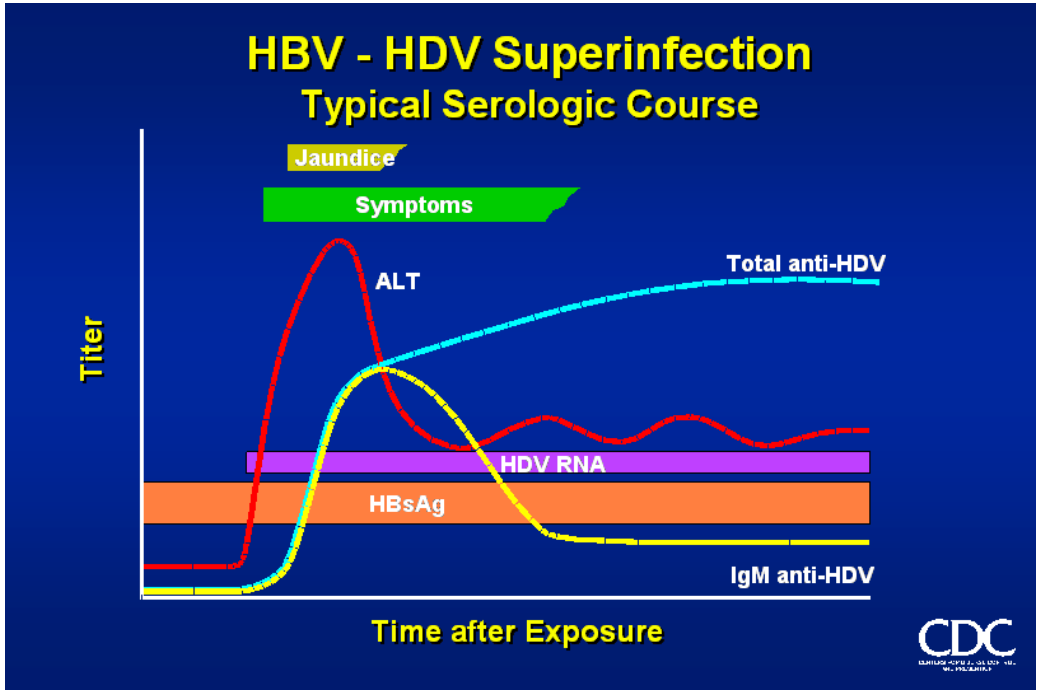
HDV

Virology

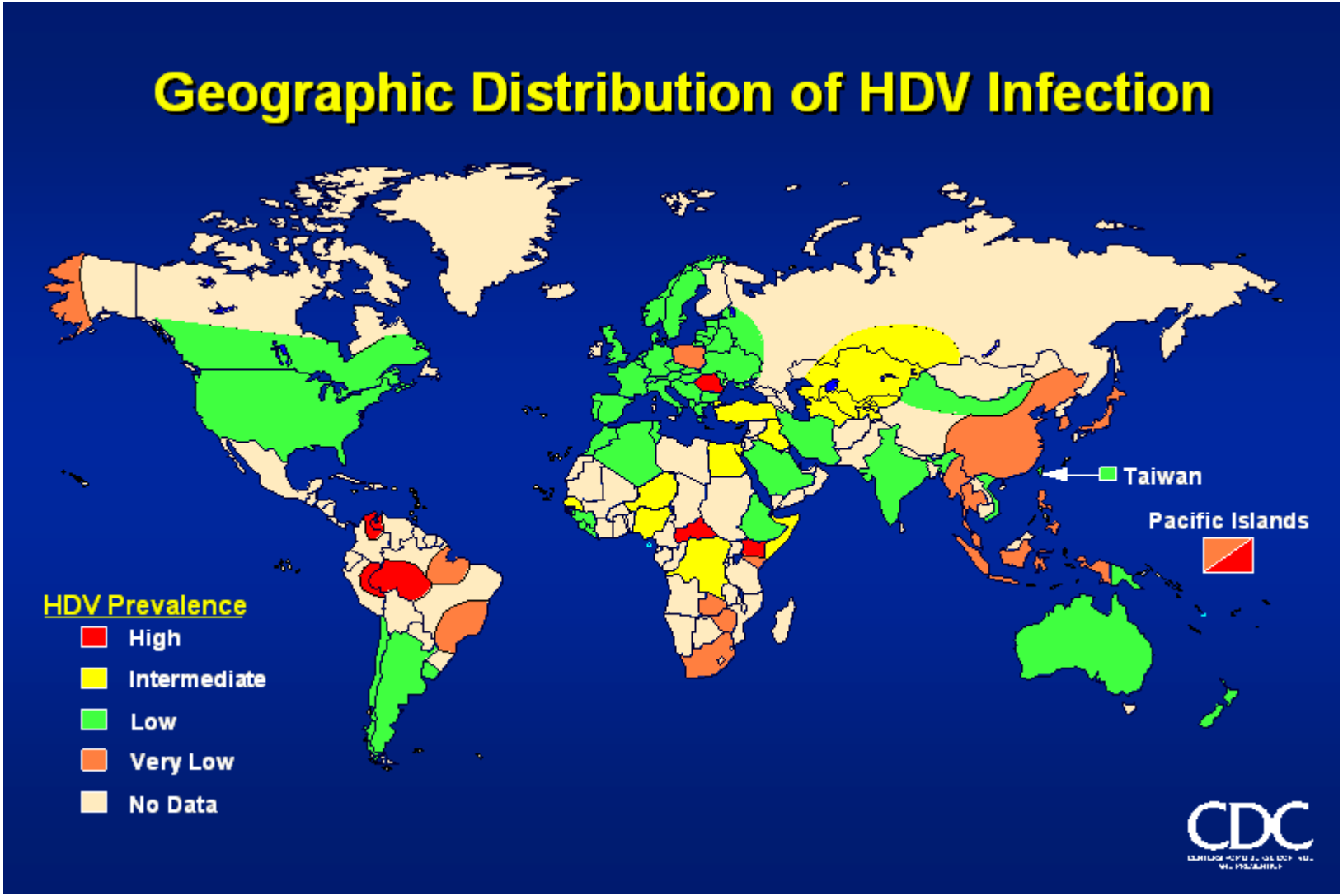
- Order: none
- Family: none
- Genus: Deltavirus
- Species: Hepatitis delta virus
- Structure: 36 nm in diameter, encapsulated with sAg derived from HBV
- Genome: circular -ssRNA (negative sense, single stranded RNA), 1700 nucleotides in length The genome is the smallest genome of any virus known to infect humans. Genome encodes only one protein, namely the delta antigen







What is difference
between two HDV infections status?


Geographic Distribution of HDV Infection
Taiwan
Pacific Islands
HDV Prevalence
High
Intermediate
Low
Very Low
No Data
CDC

HDV genotype

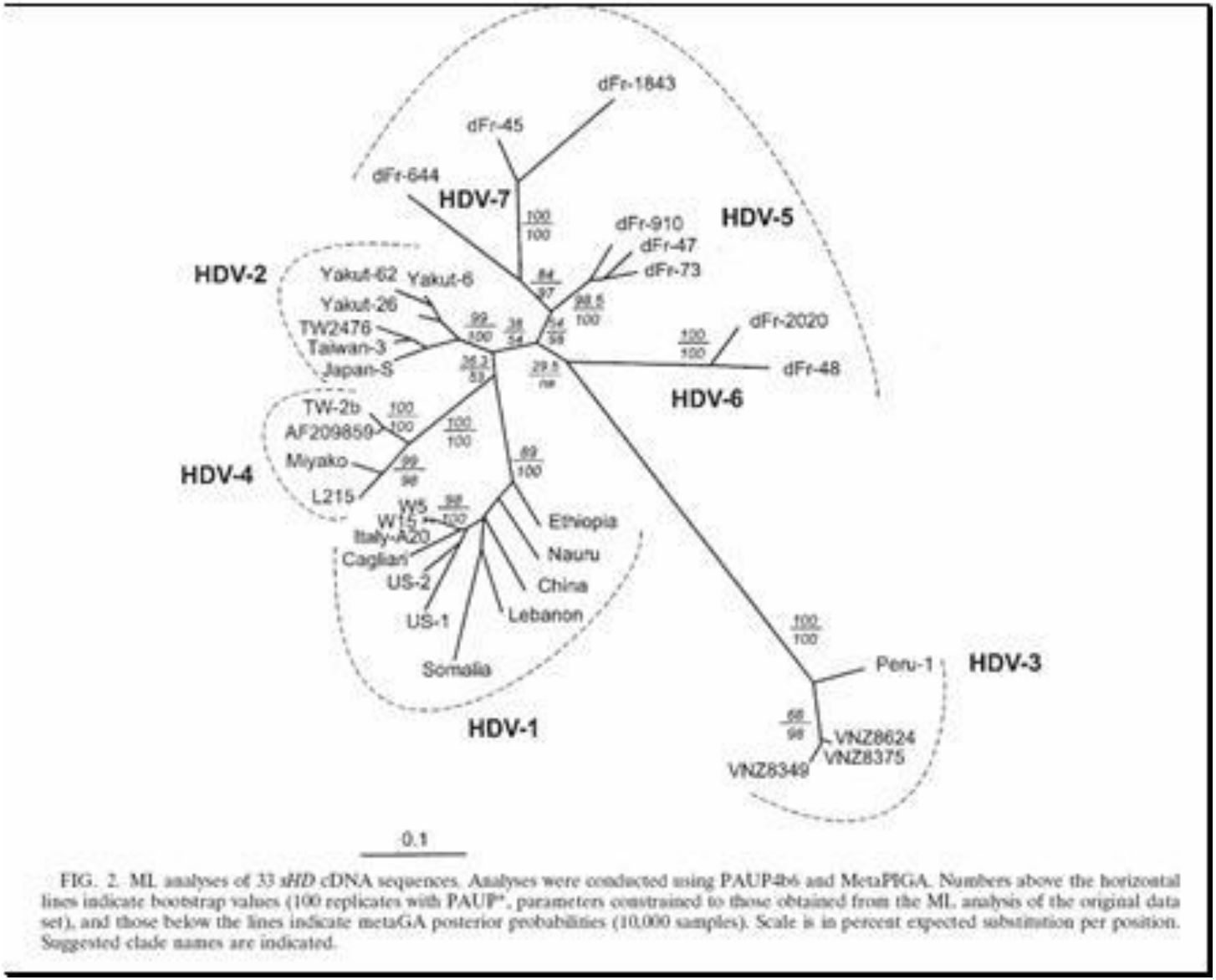
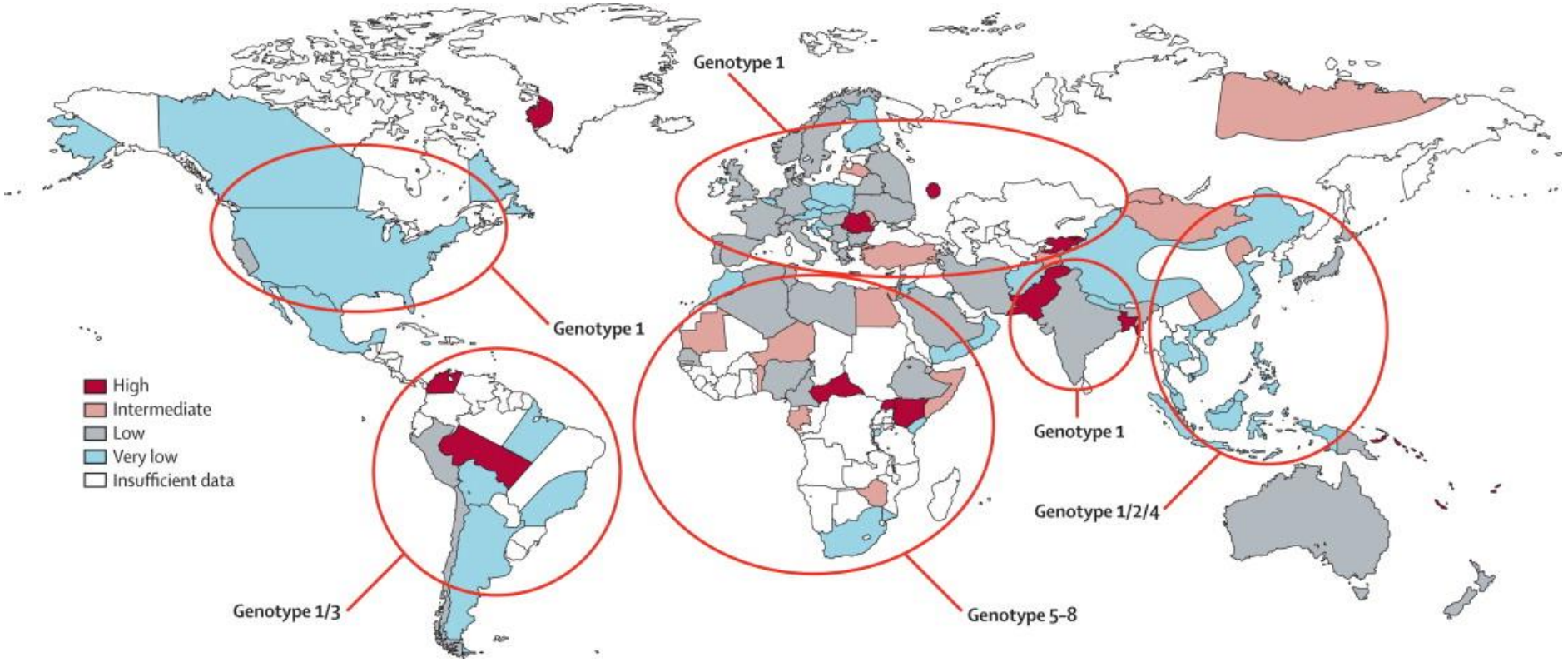


FIG. 2. ML analyses of 33 *sHD* cDNA sequences. Analyses were conducted using PAUP4b6 and MetaPIGA. Numbers above the horizontal lines indicate bootstrap values (100 replicates with PAUP*, parameters constrained to those obtained from the ML analysis of the original data set), and those below the lines indicate metaGA posterior probabilities (10,000 samples). Scale is in percent expected substitution per position. Suggested clade names are indicated.

Geographic distribution of HDV genotypes



Hepatitis D treatment

Treatment and prevention

- No specific treatment is available.
- HDV infection may be controlled by preventing HBV surface antigen production, but this may not always be possible even with complete suppression of HBV replication.
- HDV infection can be prevented by preventing infection with HBV, for which there is a successful vaccine available.

Interferon treatment

- Treatment with Peg-IFN-alfa 2b produced HDV RNA negativitiy in only 17-19% of patients.

Viral Hepatitis - Overview

	Type of Hepatitis				
	A	B	C	D	E
Source of virus	feces	blood/ blood-derived body fluids	blood/ blood-derived body fluids	blood/ blood-derived body fluids	feces
Route of transmission	fecal-oral	percutaneous permucosal	percutaneous permucosal	percutaneous permucosal	fecal-oral
Chronic infection	no	yes	yes	yes	no
Prevention	pre/post-exposure immunization	pre/post-exposure immunization	blood donor screening; risk behavior modification	pre/post-exposure immunization; risk behavior modification	ensure safe drinking water

CDC