MEDICAL UNIVERSITY – PLEVEN
FACULTY OF MEDICINE

DEPARTMENT OF INFECTIOUS DISEASE, EPIDEMIOLOGY, PARASITOLOGY AND TROPICAL MEDICINE

Lecture № 5

SIXTH YEAR MEDICAL STUDENTS - TRAINEE DOCTORS

EPIDEMIOLOGY OF BLOOD-BORN INFECTIOUNS

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HEPATITIS B

Definition

- Hepatitis B is an acute systemic infection with major pathology in the liver, caused by hepatitis B virus (HBV).
- It is clinically characterized by a tendency to a long incubation period (6 weeks to 6 months) and a protracted illness with a variety of outcomes.

- Usually, it is an acute self-limiting infection, which may be either subclinical or symptomatic.

- In approximately 5 to 15% of cases, HBV infection fails to resolve and the affected individuals then become persistent carriers of the virus.

- Persistent HBV infection may casuse progressive liver disease including chronic active hepatitis and hepatocellular carcinoma.
- There is also evidence of a close assotiation between hepatitis B and primary liver cancer.
- Hepatitis B virus can form a dangerous alliance with delta virus and produce a new form of virulent hepatitis which is considered to be a widespread threat for much of the world.

Problem statement

- The virus is transmitted through contact with the blood or other body fluids of an infected person – not through casual contact.
- About 2 billion people worldwide have been infected with the virus and about 350 million live with chronic infection.
- An estimated 600 000 persons die each year due to the acute or chronic consequences of hepatitis B.

- About 25% of adults who become chronically infected during childhood later die from liver cancer or cirrhosis (scarring of the liver) caused by the chronic infection.
- The hepatitis B virus is 50 to 100 times more infectious than HIV.
- Hepatitis B virus is an important occupational hazard for health workers.
- Hepatitis B is preventable with a safe and effective vaccine.

Who is most at risk for chronic disease?

- The likelihood that an HBV infection will become chronic depends upon the age at which a person becomes infected, with young children who become infected with HBV being the most likely to develop chronic infections.
- About 90% of infants infected during the first year of life develop chronic infections; 30% to 50% of children infected between one to four years of age develop chronic infections.

- About 25% of adults who become chronically infected during childhood die from HBV-related liver cancer or cirrhosis.
- About 90% of healthy adults who are infected with HBV will recover and be completely rid of the virus within six months.

Where is hepatitis B most common?

- Hepatitis B is endemic in China and other parts of Asia. Most people in the region become infected with HBV during childhood.
- In these regions, 8% to 10% of the adult population are chronically infected. Liver cancer caused by HBV is among the first three causes of death from cancer in men, and a major cause of cancer in women.

- High rates of chronic infections are also found in the Amazon and the southern parts of eastern and central Europe.
- In the Middle East and Indian sub-continent, an estimated 2% to 5% of the general population is chronically infected.
- Less than 1% of the population in western Europe and North American is chronically infected.

- Endemicity of HBV is defined from the prevalence of HBsAg to the general population in certain geographic region.
- Territories with high endemicity of HBsAg over 8% include the countries from Southeast Asia, tropical Africa,
- territories of intermediate endemicity between 2% and7% (the countries in East Europe, Mediterranean, Middle East, South Africa) and
- territories with low endemicity of HBsAg below 2% (Canada, USA, West Europe, Australia).

- Bulgaria is in the zone of intermediate endemicity.
- The average percent of HBsAg transmission is 3,5% and some more.
- The spread of the transmission among risk groups of population is considerably higher compared to the average values for the country:
- patients with venereal diseases – 15,38%,
- prostituting women – 11,04%,
- homosexuals – 9,32%,
- medical staff – 8,37%, youth blood donors – 17,21%,
- foreign citizens – 13,23% etc. (B. Iliev, 1989).
- The average morbidity for the country is about 3000 persons.

- The mass immunization, applied after delivery, as well other vaccine strategies have led to considerable reduction of HBV in many countries of high endemicity and consecutively to reducing the cases of chronic hepatitis, liver cirrhosis and hepato cellular carcinoma.

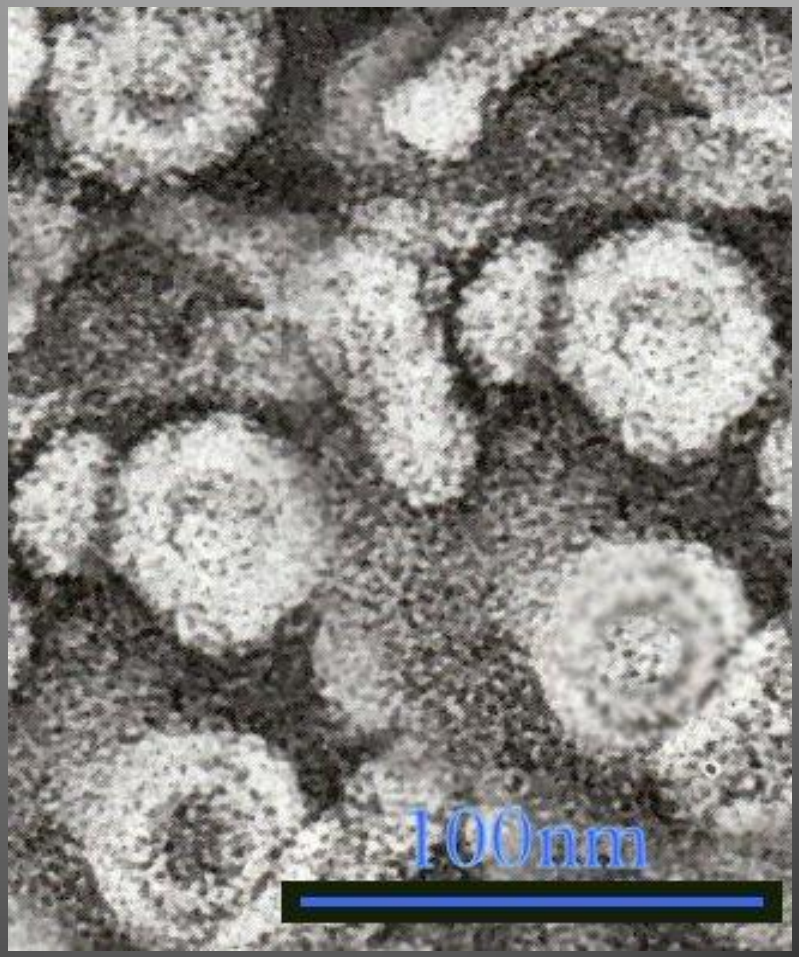
- Towards the end of 2008 177 countries employed anti hepatitis vaccine in their national immunization schedule.
- Reducing the price of the vaccine facilitated its distribution in many developing countries.
- The morbidity rate of the disease includes elder children, youth and adults in the countries with intermediate and low endemicity, born prior to introduction of the vaccine.

- It is appropriate those countries to perform catch-up vaccine campaigns, targeted to bigger age groups in order to increase the herd immunity of the population.
- Other risk groups, should be immunized are health personnel, travelling for endemicity regions, venous addicted to drugs, homosexuals and others.

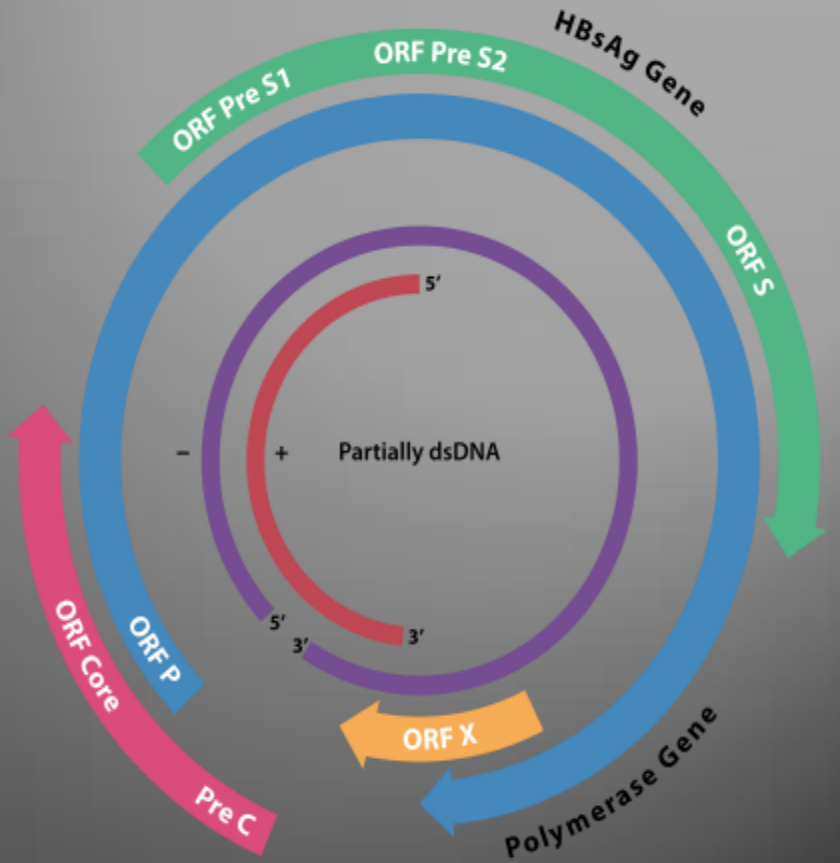
- The continuity of the vaccine immunity is not precisely defined. It was established that the greater values of peak antibody response (1-4 months after last immunization/dosage), the greater time serum antibodies are preserved to relatively high titers.
- The cited with different surveys protective effect of vaccination varies from 10 to 22 years.
- In accordance with number of surveys, related to immunologic memory of organism, WHO do not recommend application of booster doze of the immunization schedule of the countries.

Agent factor

- **Agent:**
- Hepatitis B virus (HBV)
- genus – Orthohepadnavirus
- family – Hepadnaviridae



- The virus is divided into four major serotypes
- (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins,
- and into eight genotypes (A–H) according to overall nucleotide sequence variation of the genome.



- The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus.
- Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.

- Hepatitis B virus was discovered by Blumberg in 1963.

- Efforts to grow this virus have been so far unsuccessful. HBV is a complex, 42 nm, double-shelled DNA virus, known as the “Dane particle”. It replicates in the liver cells.

- HBV occurs in three morphological forms in the serum of a patient:

a/ small spherical particles with an average diameter of 22 nm. These particles are antigenic and stimulate production of surface antibodies. The purified 22 nm parcticles are used in the preparation of hepatitis B vaccine.

b/ tubules of varying length and diameter

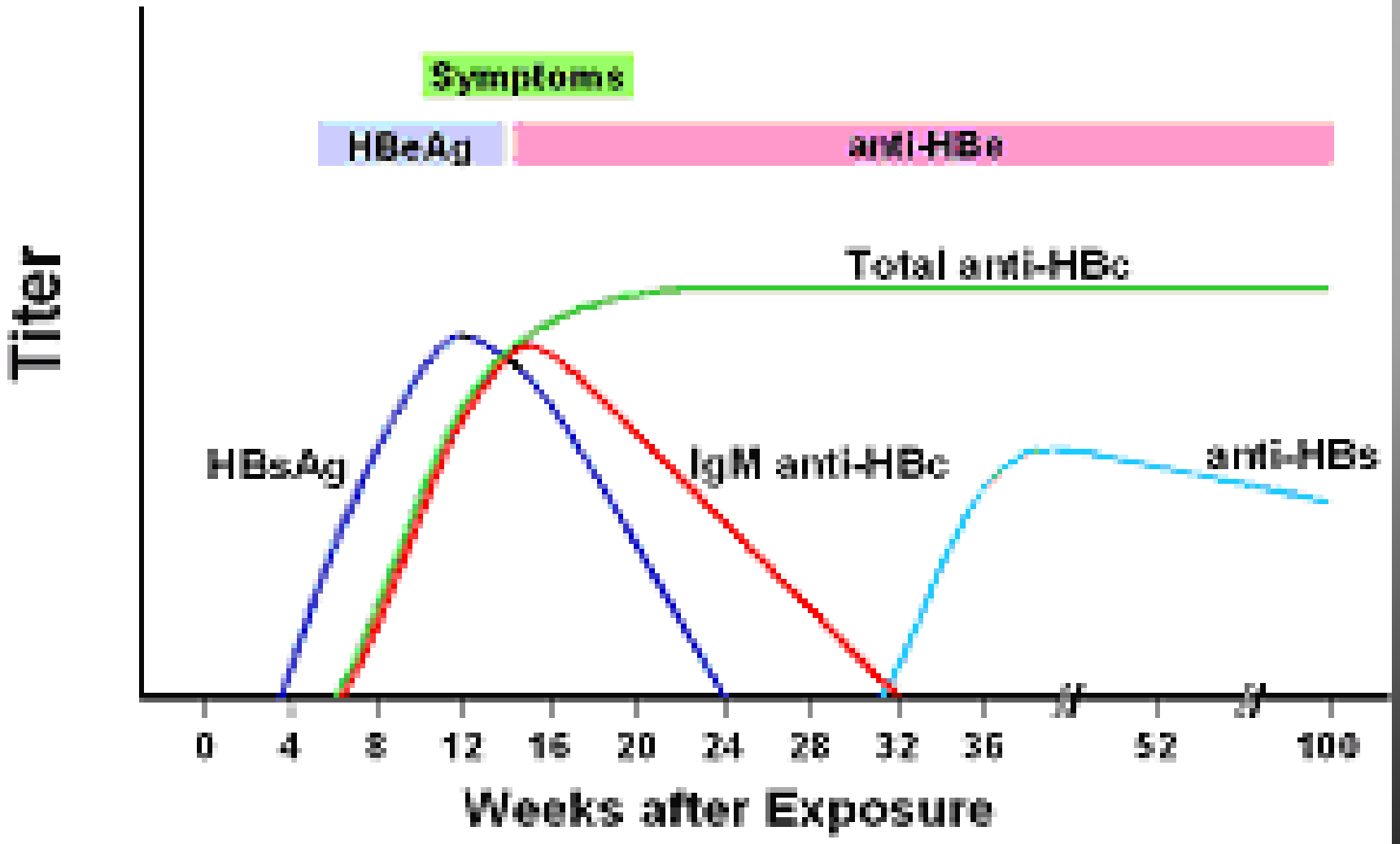
c/ the Dane parcticle which corresponds morphologically to hepatitis B virus.

- A person who is serologically positive for surface antigen is circulating all morphological forms, of which 22 nm parcticles constitute the bulk.

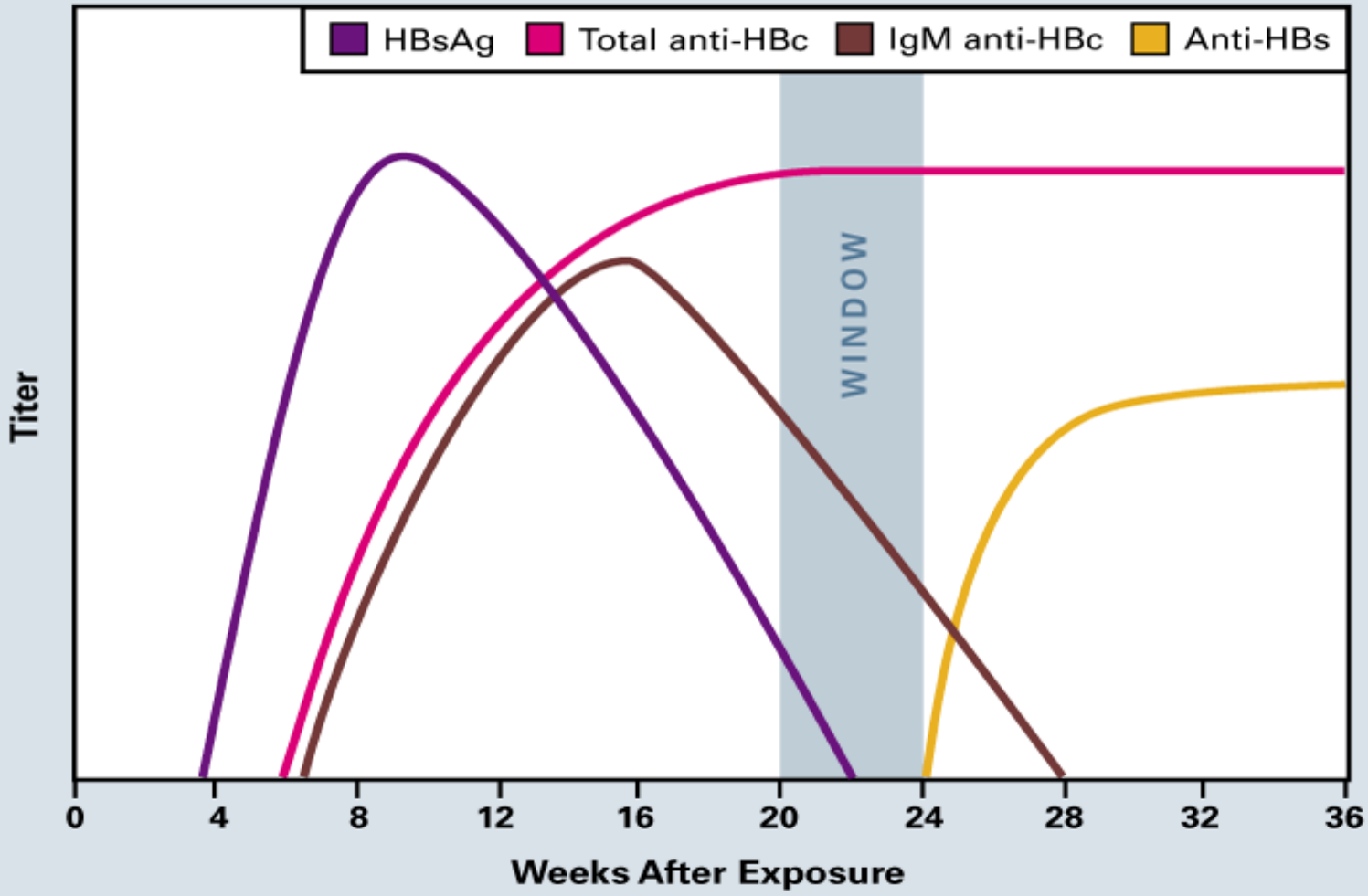
- Of the three morphological forms, only the Dane particle is considered infectious, the other circulating morphological forms are not infectious.

- HBV has three antigens:
- a surface antigen, known as “Australia antigen” (HBsAg),
- a core antigen (HBcAg) and
- an “e” antigen (HBeAg).
- They stimulate the production of corresponding antibodies e.g. surface antibody (anti-HBs), core antibody (anti-HBc) and “e” antibody (anti-HBe).
- These antibodies and their antigens constitute very useful markers of HBV infection.
- Patients with HBV infection are expected to have one or more HBV markers.

Acute Hepatitis B Virus Infection with Recovery
Typical Serologic Course



Isolated Anti-HBc During Window Period Following Acute Infection



- The surface antigen is the first to be detected. It appears in the serum during the incubation period before biochemical evidence of liver damage or the onset of jaundice.
- It persists during acute illness, and is usually cleared from the blood stream during convalescence.
- This may take 4 to 6 months.
- The next to appear are the “e” antigen and DNA polymerase.

- All these three markers precede the onset of disease. The “e” antigen (HBeAg) is a marker of virus replication and therefore a marker of infectivity.
- Detected within 3 to 5 days following the appearance of the surface antigen, it persists for 2 to 6 weeks.
- In carriers, the “e” antigen may persist for years without seroconversion.
- The presence of “e” antigen indicates that the patient is highly infectious.
- The seroconversion of “e” antigen into “e” antibody is considered a good prognostic feature.

Interpretation of test results

Test Results	Interpretation
HBsAg (-) Total anti HBc (-) Anti HBs (-)	Susceptible

Test Results	Interpretation
HBsAg (-) Total anti HBc (+) Anti HBs (+)	Immune due to natural infection

Test Results	Interpretation
HBsAg (-) Total anti HBc (-) Anti HBs (+)	Immune due to hepatitis B vaccination

Test Results	Interpretation
HBsAg (+) Total anti HBc (+) IgM anti HBc (+) Anti HBs (-)	Acutely infected

Test Results	Interpretation
HBsAg (+) Total anti HBc (+) IgM anti HBc (-) Anti HBs (-)	Chronically infected

Test Results	Interpretation
HBsAg (-) Total anti HBc (+) Anti HBs (-)	Four interpretations possible: 1/ Recovering from acute HBV infection 2/ Distantly immune and test not sensitive enough to detect very low level of serum anti HBs 3/ Susceptible with a falce positive anti HBc 4/ Chronis HBV infection with rare circumstance where HBV does not produce detectible HBsAg

- **Source of infection** are carriers or cases.
- The continued survival of infection is due to the large number over 350 million worldwide.
- The persistent carrirer state has been defined as the presence of HBsAg for more than 9 months.
- Cases may range from inapparent to symptomatic cases.
- The risk of an adult becoming a carrier following acute infection is 5 to 10%; in infants, it may exceed 50%.

- **Infective material**: contaminated blood, in body secretions such as saliva, vaginal secretions and semen of infected person.

- **Resistance**: The virus is quite stable and capable of surviving for days on environmental surfaces. It can be readily destroyed by disinfectants and by heat sterilization in an autoclave for 30 to 60 minutes.

- **Period of communicability:**
- The virus is present in the blood during yhe incubation period (for a month before jaundice) and acute phase of the disease.
- Period of communicability is usually several months (occasionally years in chronic carriers) or until disappearance of HBsAg and surface antibody.

Prevention

- All infants should receive the hepatitis B vaccine: this is the mainstay of hepatitis B prevention.
- The vaccine can be given as either three or four separate doses, as part of existing routine immunization schedules.
- In areas where mother-to-infant spread of HBV is common, the first dose of vaccine should be given as soon as possible after birth (i.e. within 24 hours).

- The complete vaccine series induces protective antibody levels in more than 95% of infants, children and young adults.
- After age 40, protection following the primary vaccination series drops below 90%.
- At 60 years old, protective antibody levels are achieved in only 65 to 75% of those vaccinated.
- Protection lasts at least 20 years and should be lifelong.

- All children and adolescents younger than 18 years old and not previously vaccinated should receive the vaccine.
- People in high risk groups should also be vaccinated, including:
- persons with high-risk sexual behaviour;
- partners and household contacts of HBV infected persons;

- injecting drug users;
- persons who frequently require blood or blood products;
- recipients of solid organ transplantation;
- those at occupational risk of HBV infection, including health care workers; and
- international travellers to countries with high rates of HBV.

- The vaccine has an outstanding record of safety and effectiveness.
- Since 1982, over one billion doses of hepatitis B vaccine have been used worldwide.
- In many countries where 8% to 15% of children used to become chronically infected with HBV, vaccination has reduced the rate of chronic infection to less than 1% among immunized children.

- As of December 2006, 164 countries vaccinate infants against hepatitis B during national immunization programmes – a major increase compared with 31 countries in 1992, the year that the World Health Assembly passed a resolution to recommend global vaccination against hepatitis B.

- **Hepatitis B immunoglobulin (HBIG)**
- For immediate protection, HBIG is used for those acutely exposed to HBsAg-positive blood, for example surgeons, nurses or laboratory workers, newborn infants of carrier mothers, and sexual contacts of acute hepatitis B patients.

- The HBIG should be given as soon as possible after an accidental inoculation (ideally within 6 hours and preferably not later than 48 hours).
- At the same time the victim,s blood is drawn for HBsAg testing. If the test is negative, vaccination should be started immediately and a full course given.
- If the test is positive for surface antibody, no further action is needed.

- The recommended dose is 0.05 to 0.07 ml/kg of body weight; two doses should be given 30 days apart.
- HBIG provides shourt-term passive protection which lasts approximately 3 months.
- Since the median incubation periods is said to be lower than 100 days, two doses of HBIG gives one month apart should suffice.
- The general use of HBIG for long-term prophylaxis has not been recommended because of its limited availability, its high cost and risk (although remote) of complications through repeted use over a long period of time.

Passive – active immunization

- The simultaneous administration of HBIG and hepatitis B vaccine is more efficacious than HBIG alone.
- HBIG does not interfere with the antibody response to the hepatitis B vaccine.
- This combined procedure is ideal both for prophylaxis of persons accidentally exposed to blood known to contain hepatitis B virus and for prevention of the carrier state in the newborn babies of carrier mothers.
- HBIG (0.05–0.07 ml/kg) should be given intramuscularly within 7 days of exposure and second and third doses given one and six months, respectively, after the first dose.

Other measures

- All blood donors should be screened for HBV infection, and those positive for HBsAg should be rejected.
- Voluntary blood donation should be encouraged because purchased blood has shown a higher risk of post-transfusion hepatitis.
- Health personnel should be alerted to the importance of adequate sterilization of all instruments and to the practice of simple hygienic measures.
- Carriers should be told not to share razors or tooth brushes and use barrier methods of contraception; they should not donate blood.

HEPATITIS D

Definition

- Hepatitis D, also referred to as hepatitis D virus (HDV) and classified as Hepatitis delta virus, is a disease caused by a small circular enveloped RNA virus.
- HDV is considered to be a subviral satellite because it can propagate only in the presence of the hepatitis B virus (HBV).

- Transmission of HDV can occur either via simultaneous infection with HBV (coinfection) or superimposed on chronic hepatitis B or hepatitis B carrier state (superinfection).
- Both superinfection and coinfection with HDV results in more severe complications compared to infection with HBV alone.

- These complications include a greater likelihood of experiencing liver failure in acute infections and a rapid progression to liver cirrhosis, with an increased chance of developing liver cancer in chronic infections.
- In combination with hepatitis B virus, hepatitis D has the highest mortality rate of all the hepatitis infections of 20%.

History

- Hepatitis D virus was first reported in the mid – 1977, by an Italian researcher, Mario Rizzetto, as a nuclear antigen in patients infected with HBV who had severe liver disease. This nuclear antigen was then thought to be a hepatitis B antigen and was called the delta antigen.

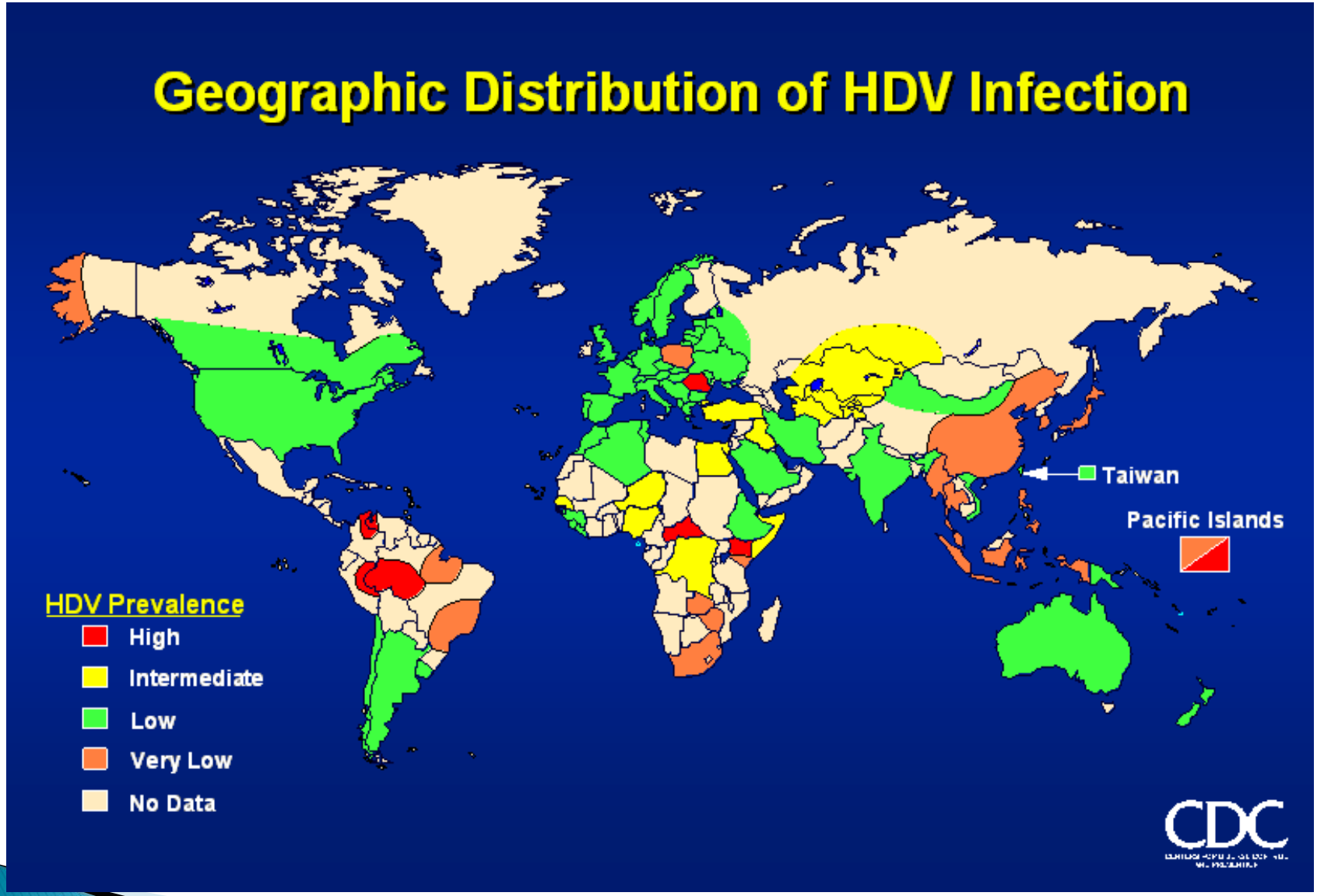
- Subsequent experiments in chimpanzees showed that the hepatitis delta antigen (HDAg) was a structural part of a pathogen that required HBV infection to replicate. The entire virus was cloned and sequenced in 1986, and obtained its own genus deltavirus.

Problem statement

- Hepatitis Delta Virus infections are found worldwide, but the prevalence varies in different geographical areas.
- Anti-HDV antibodies are found in 20-40% of HBsAg carriers in Africa, the Middle East, and Southern Italy.
- HDV infection in the United States is relatively uncommon, except in drug addicts and hemophiliacs, who exhibit prevalence rates of 1-10%.

- Homosexual men and health care workers are at high risk for contracting HBV, but are surprisingly at low risk for HDV infection for unclear reasons.
- Additionally, HDV infection is uncommon in the large population of HBsAG carriers in Southeast Asia and China.

- Additional high-risk groups for contracting HDV include hemodialysis patients, sex contacts of infected individuals, and infants born to infected mothers (rare). Worldwide, over 10 million people are infected with HDV.


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

- It is estimated that approximately 5% of the people chronically infected with the hepatitis B virus are also infected with the D virus, which represents some 15 millions of infected people worldwide.
- The infection is not always directly related to the incidence of infection by the hepatitis B virus.

- Transmission routes are similar to those of hepatitis B, is parenteral.
- It is interesting to note that its apparent transmission is lower in homosexuals, being predominant in intravenous drug addicts and hemophiliacs.

- The infection is endemic in the Mediterranean basin, in particular in southernItaly(where the virus was first described).
- It has also been described in the Far East, thePacificIslandsand in some regions ofSouth America.
- It has not been reported in Chile.
- The incidence of infection is probably decreasing.

Structure and Genome

- HDV is the only virus in the genus, Deltaviridae.
- HDV is not classified into a viral family because it is a unique virus dependent on HBV.
- HDV is a co-infection of HBV.
- The envelope of HDV particles contains the Hepatitis B surface antigen (HBsAg).
- The production and transmission of HDV is entirely dependent on HBV to provide HBsAg.

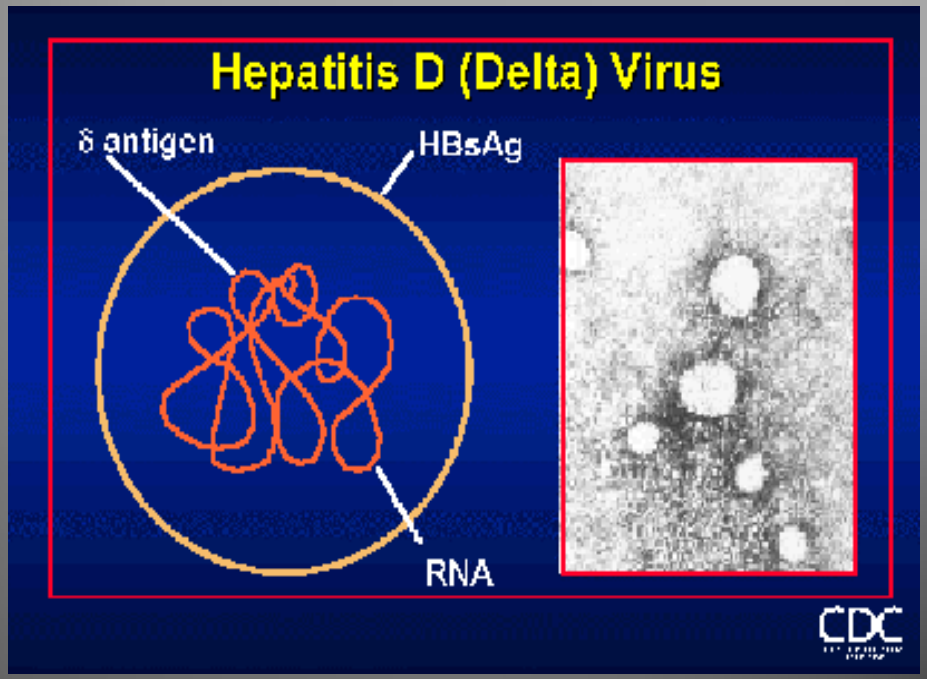
- Thus, HDV is considered a satellite virus of HBV.
- Unlike a classical satellite virus, however, HDV does not share sequence similarity with HBV, and it can replicate independently of HBV.

- There are at least three HDV genotypes: I, II, and III.
- HDV isolates of Genotype I have been reported in every part of the world, and the pathogenesis of Genotype I infections varies from fulminant hepatitis to asymptomatic chronic liver disease.

- The milder HDV II genotype is found primarily in Asia, including Japan, Taiwan, and Russia. Some sequences from Taiwan and the Okinawa islands have been assigned to a subtype of Genotype II, called Genotype IIB.
- HDV genotype III has been isolated only in northern South America (Peru, Venezuela, and Columbia) and is associated with severe acute hepatitis.

- Furthermore, HDV genotype I is the only genotype found in some locations, including Europe and North America. Multiple genotypes have been detected in Africa and in Asia.
- Mixed infections of genotypes I and II or II and IIb have been reported in Taiwan. Furthermore, 15 of 22 recently characterized African sequences formed new lineages and the other 7 are scattered within genotype I.

- The HDV is a small, spherical virus with a 36 nm diameter.
- It has an outer coat containing three HBV envelope proteins (called large, medium, and small hepatitis B surface antigens, and host lipids surrounding an inner nucleocapsid.



- The nucleocapsid contains single-stranded, circular RNA of 1679 nucleotides and about 200 molecules of hepatitis D antigen (HDAg) for each genome.
- The central region of HDAg has been shown to bind RNA.
- Several interactions are also mediated by a coiled-coil region at the N terminus of HDAg.

- The hepatitis D circular genome is unique to animal viruses because of its high GC nucleotide content.
- The HDV genome exists as an enveloped negative sense, single-stranded, closed circular RNA nucleotide sequence is 70% self-complementary, allowing the genome to form a partially double stranded RNA structure that is described as rod-like.

- With a genome of approximately 1700 nucleotides, HDV is the smallest "virus" known to infect animals.
- It has been proposed that HDV may have originated from a class of plant viruses called viroids.

Life Cycle

- The receptor that HDV recognizes on human hepatocytes has not been identified; however it is thought to be the same as the HBV receptor because both viruses have the same outer coat. HDV recognizes its receptor via the N-terminal domain of the large hepatitis B surface antigen, HBsAg.
- Mapping by mutagenesis of this domain has shown that aminoacid residues 9-15 make up the receptor binding site.

- After entering the hepatocyte, the virus is uncoated and the nucleocapsid translocated to the nucleus due to a signal in HDAg.
- Since the nucleocapsid does not contain an RNA polymerase to replicate the virus' genome, the virus makes use of the cellular RNA polymerases.
- Initially just RNA pol II, now RNA polymerases I and III have also been shown to be involved in HDV replication.

- Normally RNA polymerase II utilizes DNA as a template and produces mRNA.
- Consequently, if HDV indeed utilizes RNA polymerase II during replication, it would be the only known pathogen capable of using a DNA-dependent polymerase as an RNA-dependent polymerase.

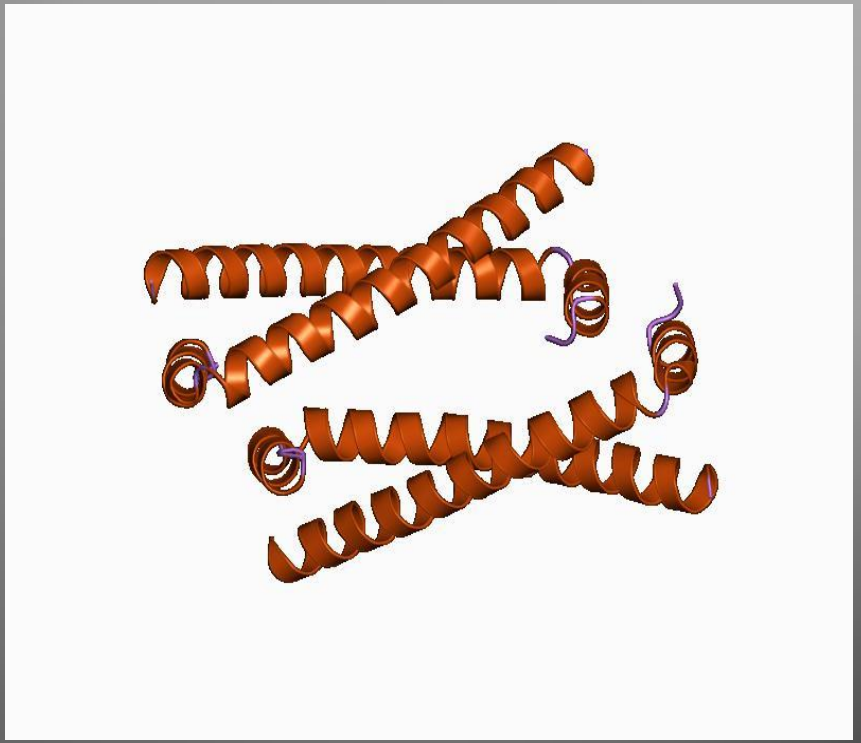
- The RNA polymerases treat the RNA genome as double stranded DNA due to the folded rod-like structure it is in.
- Three forms of RNA are made:
- circular genomic RNA,
- circular complementary antigenomic RNA,
- and a linear polyadenylated antigenomic RNA, which is the mRNA containing the open reading frame for the HDAg.

- HDV RNA is synthesized first as linear RNA that contains many copies of the genome.
- The genomic and antigenomic RNA contain a sequence of 85 nucleotides that acts as a ribozyme, which self-cleaves the linear RNA into monomers.
- This monomers are then ligated to form circular RNA.

- Synthesis of antigenomic RNA occurs in the nucleous, mediated by RNA Pol I, whereas synthesis of genomic RNA takes place in the nucleoplasm, mediated by RNA Pol II.

Delta antigens

- A significant difference between viroids and HDV is that, while viroids produce no proteins, HDV is known to produce one protein, namely HDAg.



- It comes in two forms; a 27kDa large-HDAg, and a small-HDAg of 24kDa.
- The N-terminals of the two forms are identical, they differ by 19 more amino acids in the C-terminal of the large HDAg.
- Both isoforms are produced from the same reading frame which contains an UAG stop codon at codon 196, which normally produces only the small-HDAg.

- However, editing by cellular enzyme adenosine deaminase-1 changes the stop codon to UCG, allowing the large-HDAg to be produced.
- Despite having 90% identical sequences, these two proteins play diverging roles during the course of an infection.
- HDAg-S is produced in the early stages of an infection and enters the nucleus and supports viral replication.

- HDAg-L, in contrast, is produced during the later stages of an infection, acts as an inhibitor of viral replication, and is required for assembly of viral particles.
- Thus RNA editing by the cellular enzymes is critical to the virus' life cycle because it regulates the balance between viral replication and virion assembly.

Clinical Features

- Although variable, the clinical course of HDV is typically more severe than that of the other hepatitis viruses.
- After an incubation period of 3–7 weeks, nonspecific clinical symptoms, including fatigue, lethargy, nausea, and anorexia, begin and last for about 3–7 days.

- Viral replication is usually diminished during this phase.
- Jaundice occurs in the next phase of symptoms.
- Fatigue and nausea usually continue, and the serum bilirubin level becomes abnormal.
- At the same time, the infected person may have clay-colored stool and dark urine.
- This is evidence of the liver , s diminished ability to excrete bilirubin.

Diagnosis

- Type D hepatitis should be considered in individuals who are HBsAg positive or who have evidence of recent HBV infection.
- The diagnosis for Hepatitis D infection is made following serologic tests for the virus.
- Total anti-HDV antibodies are detected by radioimmunoassay (RIA) or enzyme immunoassay (EIA) kits.

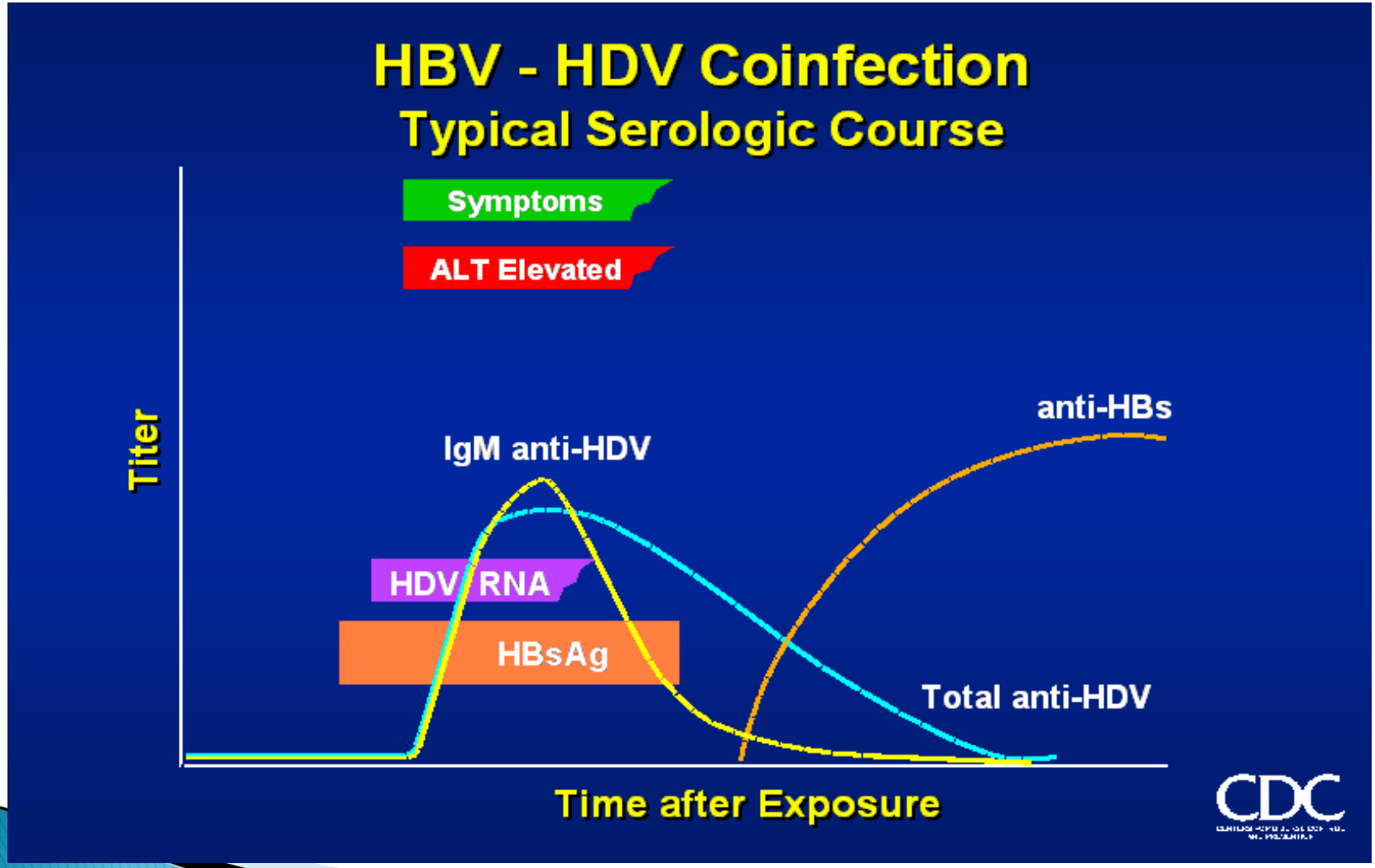
- To monitor ongoing HDV infection, reverse transcriptase-polymerase chain reaction (RT-PCR) should be used.
- RT-PCR can detect 10 to 100 copies of the HDV genome in infected blood serum.
- Each of the markers of HDV infection, including IgM and IgG antibodies, disappears within months after recovery.
- In chronic Hepatitis D infection, on the other hand, HDV RNA, HDAg, IgM anti-HD antibodies, and IgG anti-HD antibodies persist.

Outcomes

- The outcome of disease depends on whether HDV is contracted as a co-infection or a superinfection.

- **Co-infection**:
- Co-infection occurs when both HDV and HBV are contracted simultaneously.
- This results in acute HDV and HBV infection.
- Depending on the relative amounts of HBV and HDV, one or two episodes of hepatitis occurs.

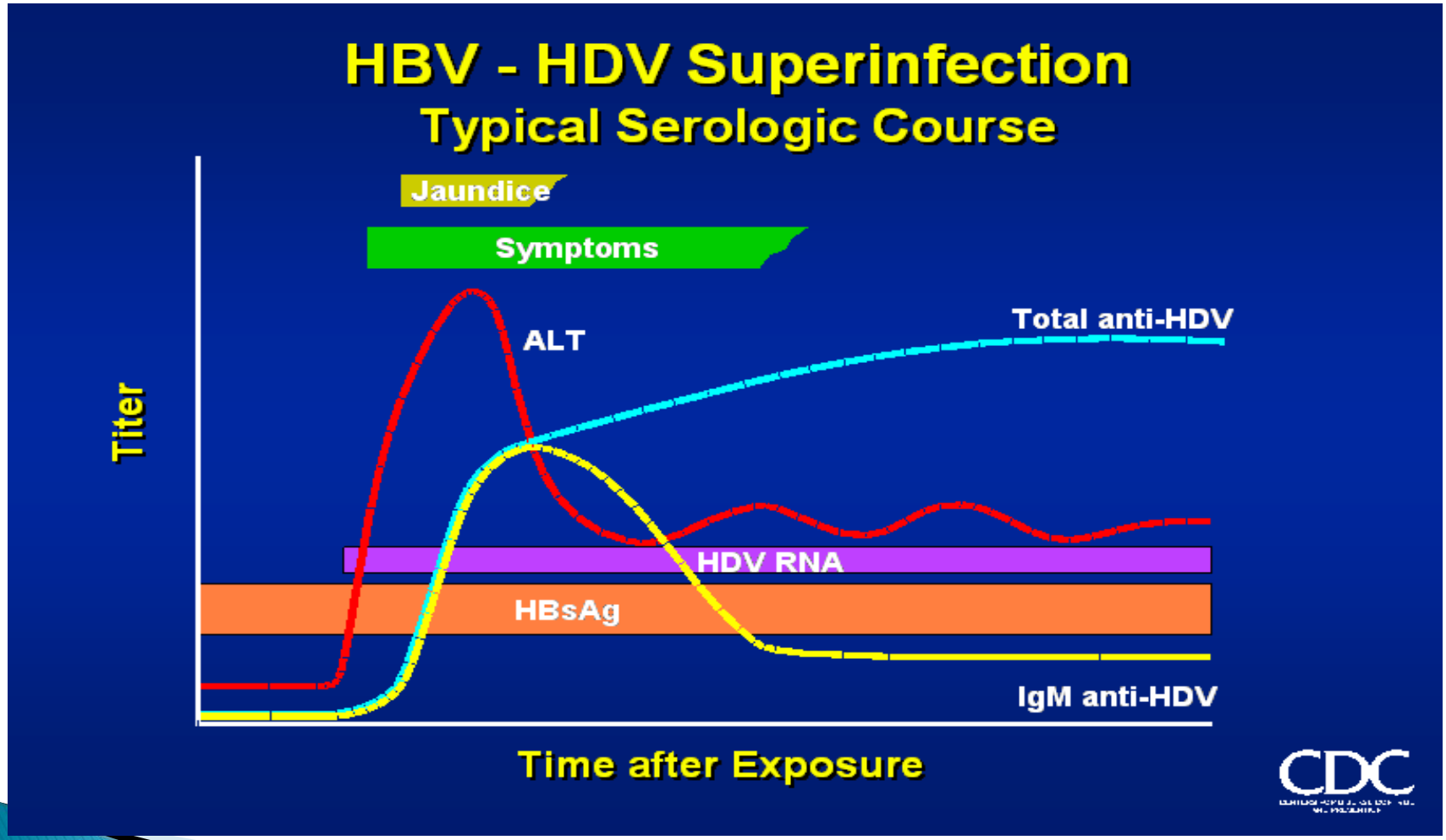
- Co-infections of HDV and HBV are usually acute and self-limiting infections.
- HBV/HDV co-infections cause chronic HDV infections in less than 5% of co-infected patients.
- Although clinical symptoms disappear, fatigue and lethargy may persist for weeks or months.


HBV - HDV Coinfection
Typical Serologic Course
Symptoms
ALT Elevated
Titer
IgM anti-HDV
anti-HBs
HDV RNA
HBsAg
Total anti-HDV
Time after Exposure
CDC

- **Superinfection**:
- Superinfection occurs when chronic HBV carriers are infected with HDV.
- This leads to severe acute hepatitis and chronic Hepatitis D infection in 80% of the cases.
- Superinfection is associated with the fulminant form of viral hepatitis.
- Fulminant viral hepatitis, the most severe form of acute disease, is about ten times more common in HDV infections than in the other types.

- It is characterized by hepatic encephalopathy that is manifested by changes in personality, disturbances in sleep, confusion, difficulty concentrating, and sometimes abnormal behavior and coma.
- The mortality rate of fulminant hepatitis is about 80%.
- Chronic hepatitis D infection progresses to liver cirrhosis in about 60-70% of patients.

- Cirrhosis takes about 5-10 years to develop, but can appear two years after the onset of infection.
- Hepatocellular carcinoma occurs in chronically infected HDV patients with the same frequency as in patients with ordinary HBV.
- Overall, the mortality rate for HDV infections lies between 2% and 20%, values ten times greater than the mortality rates for HBV.


HBV - HDV Superinfection
Typical Serologic Course
Jaundice
Symptoms
ALT
Total anti-HDV
Titer
HDV RNA
HBsAg
IgM anti-HDV
Time after Exposure
CDC

Transmission

- The routes of transmission of hepatitis D are similar to those for hepatitis B.
- Infection is largely restricted to persons at high risk of hepatitis B infection, particularly injecting drug users and persons receiving clotting factor concentrates.
- Worldwide more than 15 million people are co-infected.

- HDV is rare in most developed countries, and is mostly associated with intravenous drug use.
- However, HDV is much more common in the immediate Mediterranean region, sub-Saharan Africa, the Middle East, and the northern part of South America.
- In all, about 20 million people may be infected with HDV.

Prevention and Treatment

- Prevention of Hepatitis Delta Virus infection is based on prevention of HBV, as HDV requires the surface antigen of HBV to cause infection.
- There is no vaccine for HDV, but there is an effective vaccine for HBV.
- In order to prevent HDV-HBV co-infection, the HBV vaccine or post exposure prophylaxis (Hepatitis B Immune Globulin) can be used to prevent infection.

- The only way to prevent HBV-HDV superinfection is to educate chronic HBV carriers about transmission and risky behaviors.
- HDV can be transmitted via blood exchange, sexual contact, sharing needles, and from mother-to-child.

- There is no specific treatment for HDV infections.

HEPATITIS C

Definition

- **Hepatitis C** is an infectious disease affecting primarily the liver, caused by the hepatitis C virus (HCV).

- The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis, which is generally apparent after many years.

- In some cases, those with cirrhosis will go to develop liver failure, liver cancer or on life-threatening esophageal and gastric varices.
- HCV is spread primarily by blood-to-blood contact associated with intravenous drug use, poorly sterilized medical equipment and transfusion.

Problem statement

- An estimated 130–170 million people worldwide are infected with hepatitis C.
- The existence of hepatitis C (originally "non-A non-B hepatitis") was postulated in the 1970s and proven in 1989.
- Hepatitis C only infects humans and chimpanzees.
- The virus persists in the liver in about 85% of those infected.

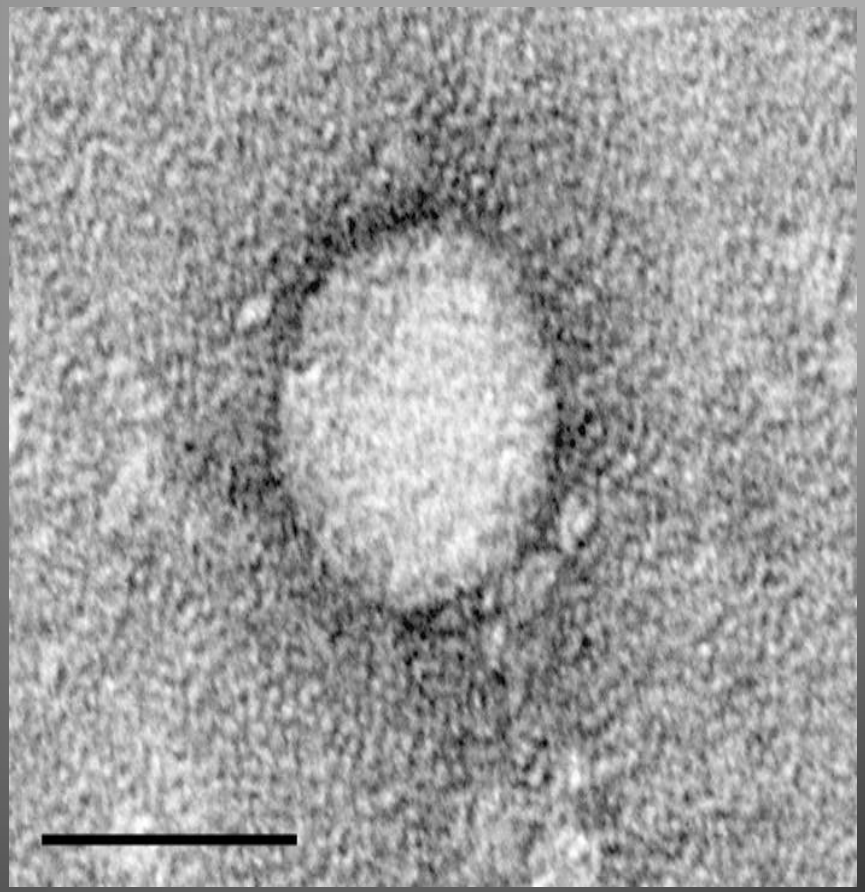
- This persistent infection can be treated with medication: the standard therapy is a combination of Peginterferon and Ribavirin, with either Boceprevir or Telaprevir added in some cases.
- Overall, 50–80% of people treated are cured.
- Those who develop cirrhosis or liver cancer may require a liver transplant.
- Hepatitis C is the leading cause of liver transplantation, though the virus usually recurs after transplantation.

- The annual incidence of HCV infection in SEAR countries is largely unknown, primarily because over 50% of infectious cases are asymptomatic.
- In addition, many symptomatic acute HCV cases are not laboratory-confirmed since testing of patients for HCV markers is not commonly done.

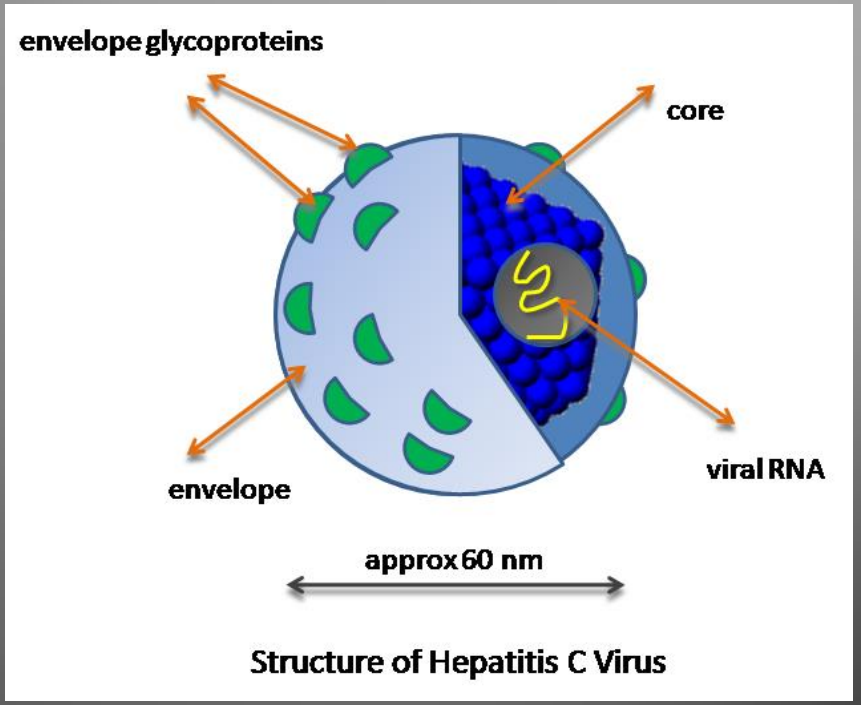
- In India HCV antibodies have been found in 2% of voluntary blood donors.
- Testing of blood samples from patients with hepatocelular carcinoma has shown that 42% of the patient in India, 29% in Indonesia and 35% in Myanmar had markers of HCV infection.
- A high prevalence of HCV markers have also been detected in patients with chronic liver disease.

Agent factor

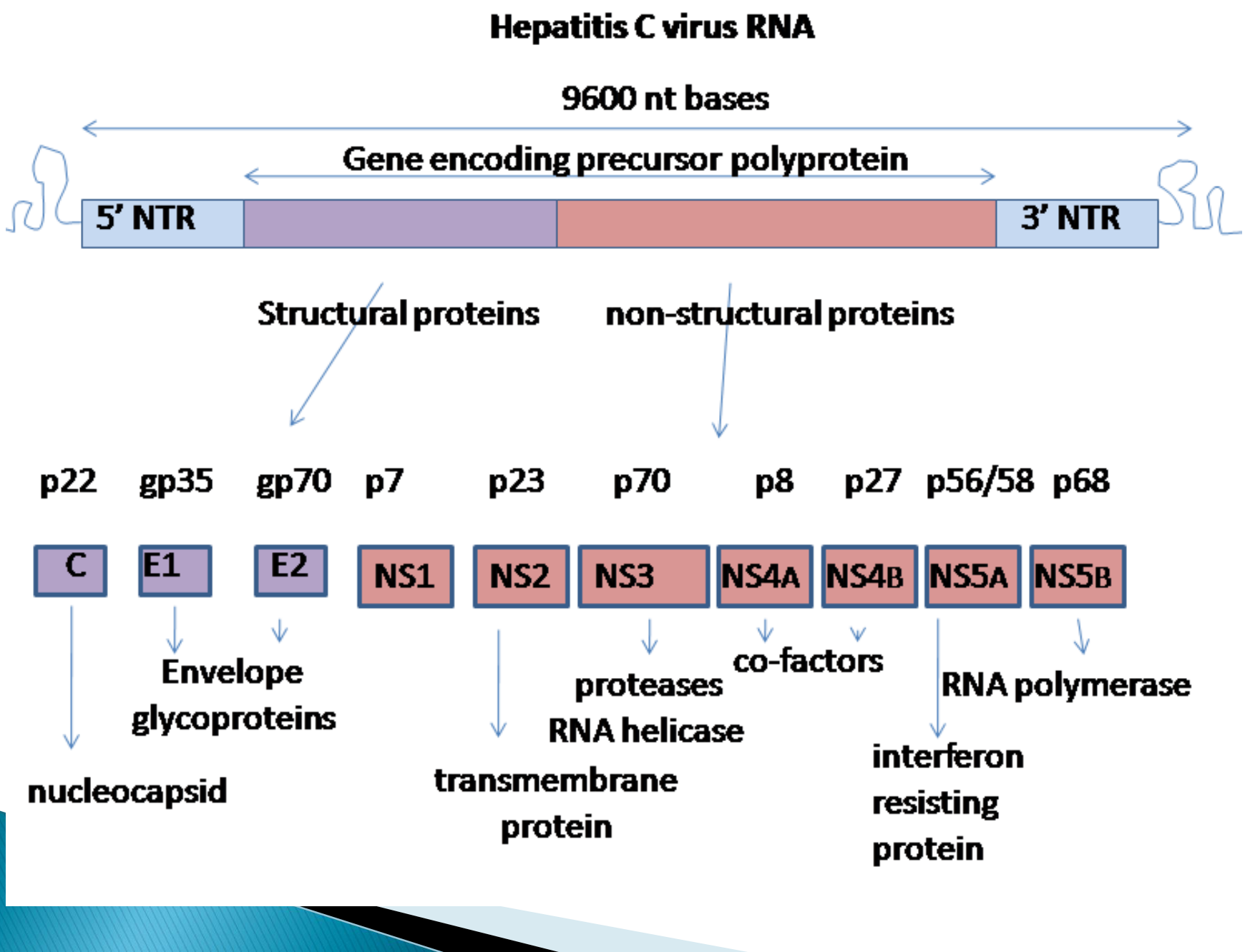
- **Agent:**
- Hepatitis C virus (HCV) is a small (55–65 nm in size),
- enveloped,
- Positive-sence single-stranded RNA virus
- of the family Flaviviridae.



- The hepatitis C virus particle consists of a core of genetic material (RNA), surrounded by an icosahedral protective shell of protein, and further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoproteins, E1 and E2, are embedded in the lipid envelope.



Structure of Hepatitis C Virus

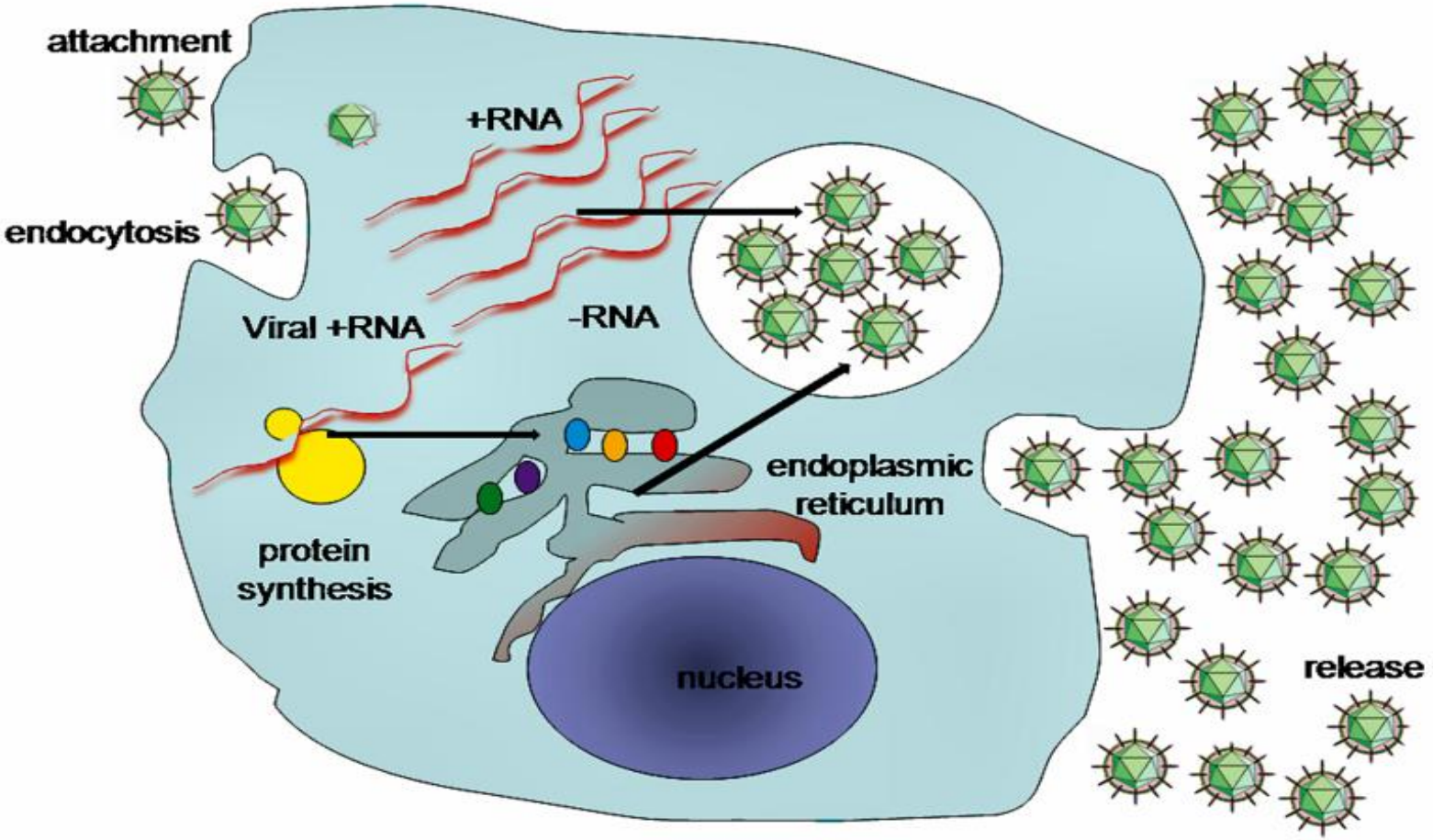

Hepatitis C virus RNA
9600 nt bases
Gene encoding precursor polyprotein
5' NTR
3' NTR
Structural proteins
non-structural proteins
p22
gp35
gp70
p7
p23
p70
p8
p27
p56/58
p68
C
E1
E2
NS1
NS2
NS3
NS4A
NS4B
NS5A
NS5B
nucleocapsid
Envelope glycoproteins
transmembrane protein
proteases
RNA helicase
co-factors
interferon resisting protein
RNA polymerase

- Hepatitis C virus has a positive sense single-stranded RNA genome.
- The genome consists of a single open reading frame that is 9600 nucleotide bases long.
- This single open reading frame is translated to produce a single protein product, which is then further processed to produce smaller active proteins.

- At the 5' and 3' ends of the RNA are the UTR, that are not translated into proteins but are important to translation and replication of the viral RNA. The 5' UTR has a ribosome binding site (IRES — Internal ribosome entry site) that starts the translation of a very long protein containing about 3,000 amino acids.
- The core domain of the hepatitis C virus (HCV) IRES contains a four-way helical junction that is integrated within a predicted pseudoknot.

- The conformation of this core domain constrains the open reading frame's orientation for positioning on the 40S ribosomal subunit.
- The large pre-protein is later cut by cellular and viral proteases into the 10 smaller proteins that allow viral replication within the host cell, or assemble into the mature viral particles.
- Structural proteins made by the hepatitis C virus include Core protein, E1 and E2; nonstructural proteins include NS2, NS3, NS4, NS4A, NS4B, NS5, NS5A, and NS5B.

A simplified diagram of the HCV replication cycle



- Replication of HCV involves several steps.
- The virus replicates mainly in the hepatocytes of the liver, where it is estimated that daily each infected cell produces approximately fifty virions (virus particles) with a calculated total of one trillion virions generated.
- The virus may also replicate in peripheral blood mononuclear cells, potentially accounting for the high levels of immunological disorders found in chronically infected HCV patients.

- HCV has a wide variety of genotypes and mutates rapidly due to a high error rate on the part of the virus' RNA-dependent RNA polymerase.
- The mutation rate produces so many variants of the virus it is considered a quasispecies rather than a conventional virus species
- Entry into host cells occur through complex interactions between virions and cell-surface molecules CD81, LDL receptor, SR-BI, DC-SIGN, Claudin-1, and Occludin.

- Once inside the hepatocyte, HCV takes over portions of the intracellular machinery to replicate.
- The HCV genome is translated to produce a single protein of around 3011 amino acids.
- The polyprotein is then proteolytically processed by viral and cellular proteases to produce three structural (virion-associated) and seven nonstructural (NS) proteins.

- Alternatively, a frameshift may occur in the Core region to produce an Alternate Reading Frame Protein (ARFP).
- HCV encodes two proteases, the NS2 cystein autoprotease and the NS3-4A serine protease.
- The NS proteins then recruit the viral genome into an RNA replication complex, which is associated with rearranged cytoplasmic membranes.

- RNA replication takes places via the viral RNA-dependent RNA polymerase NS5B, which produces a negative strand RNA intermediate.
- The negative strand RNA then serves as a template for the production of new positive strand viral genomes. Nascent genomes can then be translated, further replicated or packaged within new virus particles.
- New virus particles are thought to bud into the secretory pathway and are released at the cell surface.

- The virus replicates on intracellular lipid membranes.
- The endoplasmic reticulum in particular are deformed into uniquely shaped membrane structures termed 'membranous webs'.
- These structures can be induced by sole expression of the viral protein NS4B.
- The core protein associates with lipid droplets and utilises microtubules and dyneins to alter their location to a perinuclear distribution.
- Release from the hepatocyte may involve the very low density lipoprotein secretory pathway.

Genotypes

- Based on genetic differences between HCV isolates, the hepatitis C virus species is classified into seven genotypes (1–7) with several subtypes within each genotype (represented by lower-cased letters).
- Subtypes are further broken down into quasispecies based on their genetic diversity.
- Genotypes differ by 30–35% of the nucleotide sites over the complete genome.
- The difference in genomic composition of subtypes of a genotype is usually ~20–25%. Subtypes 1a and 1b are found worldwide and cause 60% of all cases.

Clinical importance

- Genotype is clinically important in determining potential response to interferon-based therapy and the required duration of such therapy. Genotypes 1 and 4 are less responsive to interferon-based treatment than are the other genotypes (2, 3, 5 and 6).

- Duration of standard interferon-based therapy for genotypes 1 and 4 is 48 weeks, whereas treatment for genotypes 2 and 3 is completed in 24 weeks.
- Sustained virological responses occur in 70% of genotype 1 cases, ~90% of genotypes 2 and 3, ~65% of genotype 4 and ~80% of genotype 6.

- Infection with one genotype does not confer immunity against others, and concurrent infection with two strains is possible.
- In most of these cases, one of the strains removes the other from the host in a short time.
- This finding opens the door to replace strains non-responsive to medication with others easier to treat.

Evolution

- Identifying of the origin of this virus has been difficult but genotypes 1 and 4 appear to share a common origin.
- A Bayesian analysis suggests that the major genotypes diverged about 300–400 years ago from the ancestor virus.
- The minor genotypes diverged about 200 years ago from their major genotypes.
- All of the extant genotypes appear to have evolved from genotype 1 subtype 1b.

- An study of genotype 6 strains suggests an earlier date of evolution: ~1,100 to 1,350 years before the present (95% credible region, 600 to >2,500 years ago).

- The estimated rate of mutation was $1.8 \times 10{-}4$ (95% credible region $0.9 \times 10{-}4$ to $2.9 \times 10{-}4$). This genotype may be the ancestor of the other genotypes.

- A study of European, USA and Japanese isolates suggested that the date of origin of genotype 1b was ~1925.
- The estimated dates of origin of types 2a and 3a were 1917 and 1943 respectively.
- The time of divergence of types 1a and 1b was estimated to be 200–300 years.

- A study of genotype 1a and 1b estimated the dates of origin to be 1914–1930 (95% credible interval: 1802–1957) for type 1a and 1911–1944 (95% credible interval: 1806–1965) for type 1b.
- Both types 1a and 1b underwent massive expansions in their effective population size between 1940 and 1960.
- The expansion of HCV subtype 1b preceded that of subtype 1a by at least 16 years (95% credible interval: 15–17 years).
- Both types appear to have spread from the developed world to the developing world.

- The genotype 2 strains from Africa can be divided into four clades that correlate with their country of origin: (1) Cameroon and Central African Republic (2) Benin, Ghana and Burkina Faso (3) Gambia, Guinea, Guinea-Bissau and Senegal (4) Madagascar.
- Genotype 3 is thought to have its origin in South East Asia. The genotype 2 strains from Africa can be divided into four clades that correlate with their country of origin: (1) Cameroon and Central African Republic (2) Benin, Ghana and Burkina Faso (3) Gambia, Guinea, Guinea-Bissau and Senegal (4) Madagascar.
- Genotype 3 is thought to have its origin in South East Asia.

- These dates from these various countries suggests that this virus may have evolved in South East Asia and was spread to West Africa by traders from Western Europe.
- It was later introduced into Japan once that country's self imposed isolation was lifted.
- Once introduced to a country its spread has been influenced by many local factors including blood transfusions, vaccination programmes, intravenous drug misuse and treatment regimes.

- Given the reduction in the rate of spread once screening for Hepatitis C in blood products was implemented in the 1990s it would seem that at least in recent times blood transfusion has been an important method of spreading for this virus.
- Additional work is required to determine the dates of evolution of the various genotypes and the timing of their spread across the globe.

Vaccination

- Unlike hepatitis A and B, there is currently no vaccine to prevent hepatitis C infection.
- In a 2006 study, 60 patients received four different doses of an experimental hepatitis C vaccine.
- All the patients produced antibodies that the researchers believe could protect them from the virus.

Stability in the environment

- Like many viruses, the hepatitis C virus is gradually inactivated outside the body of a host.
- The presence of heat can have a drastic impact on the virus's lifespan outside the body.
- The virus can remain infectious outside a host for about sixteen days at 25°C and two days at 37°C, while it can remain active for more than six weeks at temperatures less than or equal to 4°C.
- When heated to temperatures of 60°C and 65°C, however, the hepatitis C virus can be inactivated in eight and four minutes, respectively.

Mode of transmission

- The virus is mainly transmitted through transfusion of contaminated blood pr blood products.
- Upto 50% of cases are related to intravenous drug users who share needles.
- The risk of sexual and maternal-neonatal transmission is small.
- A low rate of secondary transmission to household contacts has been recognized.
- For health care workers it is an occupational hazard requiring adherence to universal precautions.

Diagnosis

- Only immunoassays for antibodies to part of the non structural region of HCV (anti- HGV) are available, as well as supplemental recombinant immunoblot assay (RIBA) tests used to confirm anti-HCV positive results.
- Patients with acute parenterally transmitted – nono A non b hepatitis who are anti-HCV negative at the onset of illness should be tested 6 months later, and if they are anti-HCV positive, the diagnosis of acute HCV can be made.

- Most RIBA positive persons are potentially infectious, as confirmed in research laboratories by use of polymerase chain reaction to detect HCV RNA.
- Testing donated blood for HCV has helped reduce the risk of transfusion-associated hepatitis c from 10% to 1% in the industrialized countries.

- Major prevention problems persist in the developing countries.
- Many of them cannot afford the anti-HCV blood test kits, where the use of contaminated equipment for injection and other medical and dental procedures is widespread.
- Efforts are therefore necessary to persuade the manufacturers of tests to lower the costs for developing countries.

- Health education programmes are also needed to inform the general public and health care workers about the risk of transmitting infection with the use of unsterile equipment.
- Surveillance on a global scale needs to be strengthened in order to improve medical knowledge of transmission of the virus.