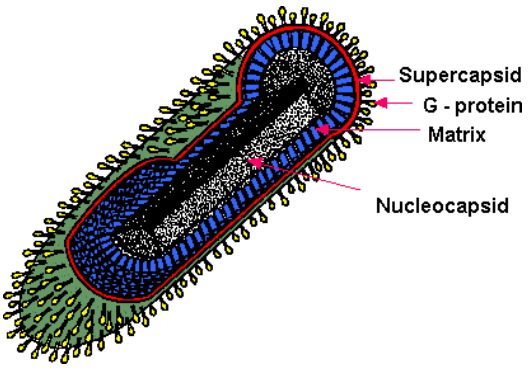
R A B I E S

Rabies is an acute infection of the central nervous system that is almost always fatal.

Rabies virus is in the Rhabdoviridae family(rhabdos-rod), genus Lyssavirus (Greek-lyssa-rabies). It causes mortal infection in animals and humans. The virus is transmitted by the bite of a rabid animal that manifests aggressive, biting behaviour induced by the viral encephalitis.

Structure: The Rhabdoviruses are rod or bullet shaped 50-60x180 nm in size. Nucleocapsid is surrounded by an envelope with protruding spikes. They possess **spiral** nucleocapsid symmetry. The spikes are composed of the viral glycoprotein G. Between supercapsid and capsid **M matrix** protein is present. Virions contain an RNA-dependent RNA polymerase. Rabies virus has a single antigenic type. They possess superficial glycoprotein antigen and cor, nucleoprotein, complement fixing antigen. The antigenicity resides in the envelope **G glycoprotein** spikes.



There are two types of Rabies viruses:

1. Freshly isolated virus from natural human or animal infection is termed **street virus.** It produces fatal encephalitis in laboratory animals, inoculated by any route, after long and variable incubation period of 1-12weeks. Intracytoplasmic inclusion bodies (Negri bodies)can be demonstrated in the brain of animals dying of street virus infection.
2. After several serial intracerebral passages in rabbits, the virus undergoes certain changes and becomes what is called fixed virus. It is used for vaccine production.

Cultivation of viruses is in cell cultures or in experimental animal organism(intradural injection). In brain neurons of experimental animals appearance of eosinophilic intracytoplasmic Babes-Negri inclusions is detected.

Rabies virus survives storage at 4°C for weeks but it is inactivated by CO_2. This virus is killed rapidly by exposure to ultraviolet radiation or sunlight, by heat (one hour at 50°C), by lipid solvents (ether, 0,1% sodium deoxycholate), by trypsin, by detergents, and by extremes of pH.

Virus replication and pathogenesis: Rabies virus attaches to the acetylcholine receptor on the cell surface. After entry into the cell, the virion RNA polymerase synthesizes mRNA that code for viral proteins. After replication of the genome viral RNA by a virus-encoded RNA polymerase, progeny RNA is assembled with virion proteins to form the nucleocapsid, and the envelope is acquired as the virion buds through the cell membrane.

Rabies is a natural infection of dogs, foxes, wolves, skunks, cats and bats. Rabies virus is excreted in the saliva of effected animals. Man acquires infection by the bite of rabid dog or other animals. Rarely, infection can occur following licks on abraded skin and intact mucosa. Infection has occurred through the inhalation of massive virus aerosols generated in bat caves and laboratory accidents.

The virus multiplies locally at the bite site (primary reproduction), infects the sensory neurons, and moves by axonal transport to the central nervous system. The virus multiplies in the central nervous system and then travels down the peripheral nerves to the salivary glands and other organs. From the salivary glands, it enters the saliva to be transmitted by the bite. There is no viremic stage.

Within the central nervous system, encephalitis develops, with the death of neurons, and demyelination. Infected neurons contain an eosinophilic cytoplasmic inclusion called a **Negri body**, which is important in laboratory diagnosis of rabies.

Clinical findings: The incubation period varies, according to the location of the bite. It lasts from 2 weeks to 16 weeks or longer. It is shorter when bites are sustained on the head rather than on the leg, because the virus has a shorter distance to travel to reach the central nervous system.

Clinically there are three phases.

1. Prodromal period lasting 2-10 days. This period is with non-specific symptoms such as fever, anorexia, malaise, headache, photophobia, nausea, vomiting and sore throat. Usually there is an abnormal sensation around the site of infection**. 2. Acute neurologic phase**. During this phase the signs of nervous system dysfunction such as nervousness, apprehension, and hallucinations occur.

General sympathic overactivity is observed, including lacrimation, pupillary dilatation, and increased salivation and perspiration. A large fraction of patients will exhibit hydrophobia (fear of water). Most notable is painful spasm of the throat muscles on swallowing. This phase is followed by convulsive seizures or coma and death usually in 2-7 days after onset **(the third period – coma phase).** The major cause of death is respiratory paralysis. Progressive paralytic symptoms may develop before death. The disease course is slower, with some patients surviving 30 days. Death is preceded by coma and is due to respiratory arrest.

The usual incubation period in dogs ranges from 3 to 8 weeks, but it may be short as 10 days. Clinically, the disease in dogs is divided into the same three phases as human rabies.

Immunity, treatment and prevention: Because so few individuals have survived rabies, there is no information regarding immunity to disease upon being bitten again. There is no antiviral therapy for a patient with rabies. Only supportive treatment is available.

There are two approaches to prevent rabies in human: **pre-exposure** and **postexposure**. Pre-exposure immunization with rabies vaccine should be given to individuals in high risk groups, such as veterinarians, zoo keepers, and travellers to areas of hyperendemic infection.

The rabies vaccine is the only vaccine that is routinely used postexposure. The long incubation period of the disease allows the virus in the vaccine sufficient time to induce protective immunity. The rabies vaccine contains inactivated virus grown in human diploid cells (Vaccine grown in monkey lung cells or chick embryo cells is also available).

The duck embryo vaccine or various nerve tissue vaccine are available as well. Duck embryo vaccine has low immunogenicity, and the nerve tissue vaccines can cause an allergic encephalomyelitis as a result of a cross-reaction with human myelin; for these reasons, the human diploid cell vaccine is preferred.

Postexposure immunization involves the use of both the vaccine and human rabies immune globulin (RIG, obtained from hyperimmunized persons) plus immediate cleaning of wound. This is an example of passive-active immunization.

The decision to give postexposure immunization depends on a variety of factors, such as: **1.** Type of animal (all wild animal attacks demand immunization); **2.** Whether an attack by a domestic animal was provoked, whether the animal was immunized adequately, and whether the animal is available to be observed; **3.** Whether, rabies is endemic in the area. The advice of local public health officials should be sought. Hospital personnel exposed to a patient with rabies need not be immunized unless a significant exposure has occurred, eg, a traumatic wound to the health care worker.

HEPATITIS VIRUSES

Viral hepatitis is a systemic disease primarily involving the liver. Medically important hepatitis viruses are: Hepatitis A virus (HAV); Hepatitis B virus (HBV); non-A, non-B viruses, of which Hepatitis C virus (HCV); Hepatitis D virus (HDV, delta agent) and Hepatitis E virus (HEV), Hepatitis G virus (table). Hepatitis viruses are taxonomically unrelated. Except for type B, which is a DNA virus, all the others are RNA viruses. The features common to them are their hepatotropism and ability to cause a similar icteric illness, ranging in severity from the unapparent to the fulminate fatal forms. As all types of hepatitis viruses cause a clinically indistinguishable acute illness, their differentiation is based on their serological and molecular markers. By epidemiological and clinical criteria, two types of viral hepatitis had been recognized for long:

1. One type occurred sporadically or as epidemics, affecting mainly children and young adults, and transmitted by the fecal- oral route. This was called infectious hepatitis (Hepatitis A).
2. A second type of viral hepatitis, transmitted mainly by inoculation, was originally observed in persons receiving serum inoculation or blood transfusion. This had been given various names such as homologous serum jaundice, serum hepatitis and transfusion hepatitis (Hepatitis B).

HEPATITIS A VIRUS

Hepatitis A (infectious hepatitis) is a subacute disease of global distribution, affecting mainly children and young adults.

HAV is a distinct member of the Picornavirus family. HAV is a 27-32nm, non-enveloped, spherical virus. It has single stranded RNA positive polarity genome, icosahedral nucleocapsid and replicates in the cytoplasm of the cell. Only one serotype is known. HAV is stable to treatment with 20 per cent ether, acid and heat (60°C for one hour) and its infectivity can be preserved for at least one month after being dried and stored at 25^0 C and 42% relative humidity or for years at -20° C. The virus is destroyed by autoclaving (121°C for 20 minutes), by boiling in water for 5 minutes.

Transmission and Epidemiology:

Humans are the reservoir for HAV. Virus appears in the faeces roughly 2 weeks before the appearance of symptoms. Children are the most frequently infected group, and outbreaks occur in special living situations (kinder gardens, summer camps). Common-source outbreaks arise from faecal contaminated water or food such as oysters grown in polluted water and eaten raw. HAV is rarely transmitted via the blood, because the level of viremia is low and chronic infection does not occur.

Hepatitis A is an anthroponose infection. The source of infection is patient. HAV is transmitted by the faecal-oral route (infection is by ingestion). The primary reproduction in small intestine is occurs. Later the virus enters into the blood and spreads to the liver via the blood. For these viruses the target cells are hepatocytes and these cells are infected. The incubation period is 21-28 days but can last 50 days. The prodromal period lasts 5-7 days. Fever, anorexia, nausea, vomiting, and jaundice are typical. After 2-4 days urine becomes dark (like beer), the faeces is pale **(acholia),** and elevated transaminase levels are seen, increasing of Specific M immunoglobulins is detected which have differential diagnostic meaning. During this period the enlargement of liver is detected. The next is specific illness period, which lasts from 1 week to 1.5 months, jaundice develops, and the intoxication becomes weak. Jaundice begins from the oral cavity mucous layer, palate, frenula, sclera and then skin. The urine becomes darker **(urobilinuria, choluria)**, acholia is intensive.

Disappearance of the jaundice shows convalesces and it lasts from 2 to 6 months.

Post infectious immunity is stable depends on virus neutralizing antibodies, memory cells, and intestinal local immunity. There is no cross immunity between HAV and any of the other hepatitis viruses.

Prevention: General prophylaxis consists of improved sanitary practices and prevention of fecal contamination of food and water.

Specific prophylaxis: Specific **passive prophylaxis** by pooled normal human immunoglobulin IM, before exposure or in the early incubation period, can prevent or attenuate clinical illness, while not necessarily preventing infection and virus excretion. Specific **active prophylaxis** by inactivated cultural vaccine and recombinative vaccine are used.

Treatment is symptomatic. No specific antiviral drug is available.

Laboratory diagnosis: The detection of IgM antibody is the most important test. IgM and anti- HAV

antibody appears during the late incubation period, reaches peak levels in 2-3weeks and disappears after 3-4 months. The IgG antibody appears at about the same time, peaks in 3-4 months and persists much longer, perhaps for life. Demonstration of IgM antibody in serum indicates current or recent infection, while the IgG antibody denotes recent or remote infection and immunity. ELISA kits for detection of IgM and IgG antibodies are available.

HEPATITIS B VIRUS (HBV)

HBV is the cause of serum hepatitis. HBV is a member of the **Hepadnaviridae** family. Hepatitis B is an anthroponose, viral infection, which infects especially liver and causes development of liver disease and hepatocellular carcinoma. Type B hepatitis is the most widespread and the most important type of viral hepatitis.

Structure: It is enveloped virion (known as a Dane particle named after the scientist, who first discovered the virus-under the electron microscope, sera from type B hepatitis patients show three types of particles: spherical, filamentous, double –walled spherical structure), with an icosahedral nucleocapsid. The size is 35-42 nm in diameter. The viral genome consists of double stranded circular DNA. One of the strands is incomplete (15-60 defective genome), so that the DNA appears partially double stranded and partially single stranded. This part doesn't have infectious properties. In nucleocapsid viral DNA polymerase is present, which has both DNA-dependent DNA polymerase and RNA-dependent reverse transcriptase functions. This polymerase can repair the gap strand and render the genome fully double stranded. The genome contains four genes that encode the following properties: surface (envelope) protein, core (nucleocapsid) protein, DNA polymerase, antigens.

Antigenic structure: They contain four antigens:

HBs antigen (HBsAg was known as **Australian antigen**, because it was first found in the serum of an Australian aborigine): It is glycoprotein-lipid complex. This antigen is on the surface of virion. HBsAg consists of two parts: preS2 which is poly-globulin receptor by which the virus is absorbed on the host cell receptors (on the hepatocytes). PRES1 ensures immunogenic properties. This antigen is discovered in blood.

HBc antigen: it is a nucleoprotein, which is in the core of virion. They can be found in the hepatocyte's nuclei, but they do not enter into the blood.

HBe antigen: This antigen is separated from HBc antigen, when HBc antigen crosses hepatocytes and HBe antigen is detected in the blood.

HBx antigen: This antigen takes part in cancer transformation of hepatocytes.

In patient's organism immunoglobulins against HBc, HBe, HBs antigens are synthesized.

Reproduction (picture1): After entry of the virion into the cell and its uncoating, the virion DNA polymerase synthesizes the missing portion of DNA and a double-stranded closed circular DNA is formed in the nucleus. This DNA is a template for mRNA synthesis by cellular RNA polymerase. Hepadnaviruses are the only viruses that produce genome DNA by reverse transcription with mRNA as the template.

Pathogenesis: The portal of entry for this virus: blood, sexual and perinatally from mother to newborn. The virus enters into the blood, and dissemination of the virus in the organism occurs. Primary HBV fixes on the hepotocytes and reproduced, but reproduction of the virus in the hepatocytes is not accompanied by cytolysis of these cells. It shows that virus doesn't have direct cyto-pathic effect and pathogenic process occurs when immunocytes recognize the antigens of the HBV on the surface of the hepatocytes. So, the damaging of the liver cells is immune dependent. The pathological variety of infection (acute, subacute, chronic, and persistent) depends on the antigens of the virus and interaction with host cell. In **acute forms** suppression of T helpers occurs. Inhibition of T helpers means disturbing of recognition of viral antigens and at least inhibition of immunoglobulin synthesis occurs.

In **chronic forms** inhibition of T suppressors occurs. It develops autoimmune reactions against hepatocytes. HBV can interact with macrophages. In normal immune response macrophage presents viral antigen and induced normal humoral immune response (HBs, HBc, HBe immunoglobulins synthesised). It means postinfectious immunity is of high duration and life- long. But HBV can infect macrophages and in antigen recognition system defects occur and it develops immunodeficiency, development of persistency results and HBV DNA is integrated into cell DNA. A high rate of

hepatocellular carcinoma occurs. The HBV genome has no oncogene, and hepatocellular carcinoma appears to be the result of persistent cellular regeneration that attempts to replace the dead hepatocytes. Alternatively, malignant transformation could be the result of insertional mutagenesis, which could occur when the HBV genome integrates into the hepatocyte DNA. Integration of the HBV DNA could activate a cellular oncogene, leading to a loss of growth control. Chronic infection is associated with a high risk of hepatocellular carcinoma.

Clinical findings: The incubation period 50-180 days. The prodromal period lasts 4-10 days and the clinical appearance of acute hepatitis B is similar to that of hepatitis A. However, with hepatitis B, symptoms tend to be more severe, and life- threatening hepatitis can occur.

Many HBV infections are asymptomatic and are detected only by the presence of antibody to HBsAg.

Epidemiology: The source of infection is patient. Approximately 5 percent of humanity are carriers, in their blood HBs antigen is present. The routes of transmission are parenteral (blood transfusion, syringe-intravenous injections), sexual, prenatally. Persons who had hepatitis probably should not be used as blood donors.

HBV is sensitive to high temperatures (100° C for 1 min.; 60° C for 10 min). They are sensitive to formalin. HBsAg is not destroyed by ultraviolet irradiation of plasma or other blood products.

Laboratory diagnosis: Specific diagnosis of hepatitis B rests on the serological demonstration of the viral markers. It is therefore necessary to understand the sequence of their appearance in blood. HBsAg is the first marker to appear in blood after infection, being detectable even before elevation of transaminases and onset of clinical illness.

Immunity: depends on humoral and cellular mechanisms. Immunoglobulins participate in elimination of viruses from the host organism and protect intact hepatocytes.T8 cells ensure elimination of viruses from the host organism too.

Prevention: Prevention involves vaccine. Vaccine can be prepared by purifying HBsAg from healthy carriers (treating these particles with virus-inactivating agents-formalin, heat). Now vaccine can be prepared by gene engineering (recombinative HBs vaccine).

Treatment: No specific antiviral treatment is available for acute HBV infection. Interferon alpha alone or in combination with other antiviral agents such as lamivudine and famcyclovir, has been beneficial in some cases of chronic hepatitis. There is no effective treatment for the carrier state, though spontaneous resolution takes place in some of them.

Non-A, Non-B hepatitis viruses

Hepatitis D virus (Delta virus): This virus was discovered in 1977. HDV is spherical virus with single stranded RNA genome of negative polarity. The genome of HDV is very small and encodes only one protein, internal cor protein D (delta) antigen. HDV is **defective virus;** it cannot be replicated by itself because it does not have the genes for its envelope protein. This virus can be replicated only in cells also infected with HBV, because HDV uses the surface antigen of HBV (HBsAg) as its envelope protein. HBV is helper virus for HDV and this connection occurs by two ways: 1. When, both (HBV and HDV) viruses infect the host organism together (at the same time). 2. When HBV infects the organism and then HDV enters (superinfection with HDV). The mechanism of action isn't discovered closely, but there is some evidence that HDV is directed cytopathic for hepatocytes. Portal of entry is similar to HBV. Hepatitis in patients co-infected with HDV and HBV is more severe than in those infected with HBV alone.

Laboratory diagnosis: The diagnosis is made by detecting either delta antigen or IgM antibody to delta antigen in the patient's serum.

No specific prophylaxis exists, but immunisation with the HBV vaccine is effective as HDV cannot infect persons immune to HBV. Screening of blood donors for HBsAg automatically limits bloodborne HDV infection.

Hepatitis C virus (HCV)

HCV is in the family Flaviviridae, Hepacivirus genus. It is enveloped virus. The size is 50-70 nm. The genome is linear, single stranded RNA, positive polarity, enclosed within a core and surrounded by an envelope, carrying glycoprotein spokes. Humans are the reservoir for HCV. The transmission of the HCV into the host organism is mainly by blood or blood products. Sexual

transmission is probably less important. Vertical transmission from mother to baby may take place. HCV infects hepatocytes primarily, but there is no evidence for a virus-induced cytopathic effect on the liver cells. The cytopathic effect is immune mediated by cytotoxic T cells.

Clinical findings are similar to HBV (fever, anorexia, nausea, vomiting, and jaundice, dark urine, pale faeces). HCV can ensure chronic liver disease, cirrhosis and hepatocellular carcinoma. The incubation period is long 15-160 days with a mean 50 days. The acute illness is usually mild or anicteric. Overt jaundice is seen in about 5% of patients only. The important part in type C hepatitis is the chronic illness. About 50-80% of patients progress tom chronic hepatitis, with some developing cirrhosis and hepatocellular carcinoma.

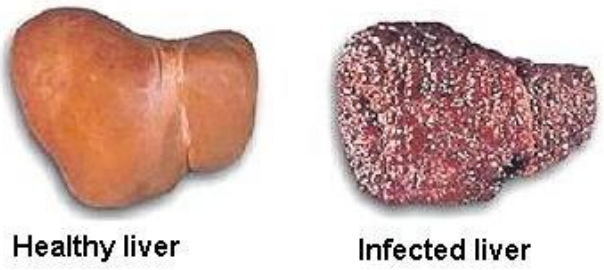
Prophylaxis: Only general prophylaxis, such as blood screening, is possible. No specific active or passive immunising agent available. There is no vaccine.

Treatment: Prolonged treatment with interferon alpha, either alone or in combination with antiviral agents like ribavirin has been reported to be useful in some cases.

Laboratory diagnosis: HCV infection is diagnosed by detecting antibodies to HCV by ELISA.

Hepatitis E virus (HEV)

HEV is spherical, non-enveloped virus. The genome is single stranded RNA positive polarity. The size is 32-34 nm in diameter. They are in Calciviridae family. HEV enters into organism alimentary (transmitted enterically) like HAV. Clinically the disease resembles hepatitis A, with the exception of a high mortality rate in pregnant women. Chronic liver disease does not occur, and there is no prolonged carrier state. There is no antiviral treatment and vaccine. The test for HEV antibody is not available. The diagnosis is typically made by excluding HAV and other causes.



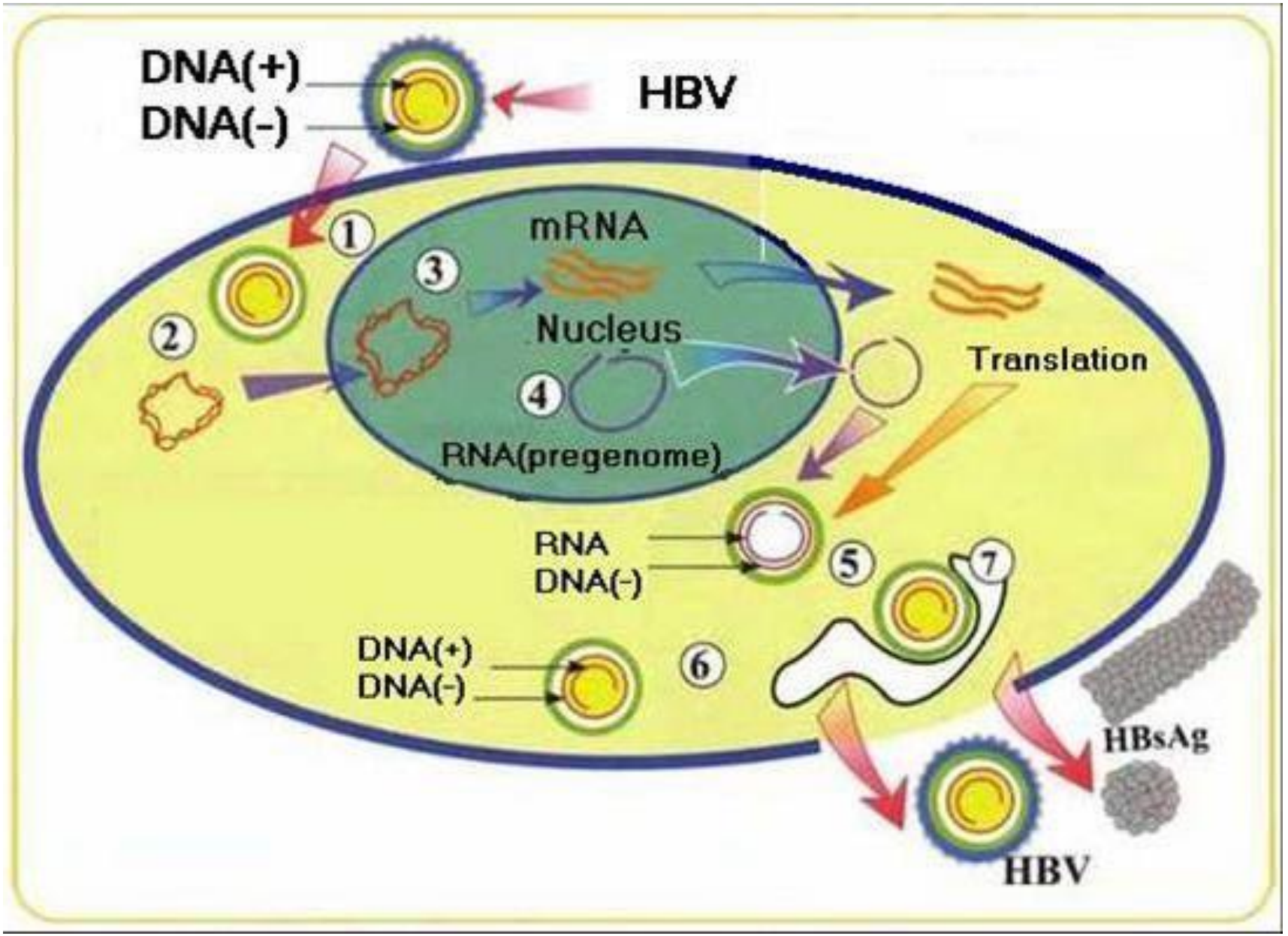
Healthy liver Infected liver

Hepatitis G virus (HGV)

In 1996, HGV was isolated, from patients with post-transfusion hepatitis. HGV is in the Flavivirus family. The role of HGV in the causation of liver disease has yet to be established.

Table1 Human Hepatitis viruses and their comparative properties

Type	Family, genus	Sizes (nm)	Genome	Presence of supercapsid and geometry	Oncogenicity	Reproduction on cell cultures	Routes of transmission
HAV	Picornaviridae, Enterovirus	27-32	single stranded +RNA	**icosahedral**	-	+	fecal-oral
HBV	Hepadnaviridae, Hepadnavirus	42-52	double stranded defective DNA	**icosahedral**	+	-	parenteral, vertical, sexual
HCV	Flaviviridae, Hepacivirus	50-70	single stranded +RNA	**icosahedral**	+	+ -	parenteral, vertical, sexual
HDV	Deltavirus	35-40	single stranded circular RNA	**?**	**?**	-	parenteral, transfusion
HEV	Calicivirus	32-35	single stranded +RNA	**icosahedral**	-	+ -	fecal-oral


DNA(+)
DNA(-)
HBV
mRNA
1
3
2
Nucleus
4
Translation
RNA(pregenome)
RNA
DNA(-)
5
7
DNA(+)
DNA(-)
6
HBsAg
HBV