Федеральное государственное бюджетное образовательное учреждение высшего образования «Оренбургский государственный медицинский университет» Министерства здравоохранения Российской Федерации

ФГБОУ ВО ОРГМУ Минздрава России

Учебное пособие

Вирусология

Оренбург, 2020

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Кафедра микробиологии, вирусологии, иммунологии

ВИРУСОЛОГИЯ

учебное пособие предназначено: для студентов факультета иностранных студентов
(31.05.01 «лечебное дело»)

Оренбург, 2020

УДК 579(075.8)
ББК 28.4я7
М76

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Учебное пособие «Вирусология» предназначено для изучения разделов «Общая вирусология», «Частная вирусология», по дисциплине «Микробиология, вирусология» при освоении иностранными студентами основной образовательной программы по специальности 31.05.01 «Лечебное дело». Пособие содержит материал, необходимый для самостоятельной теоретической подготовки и овладения практическими навыками на занятиях.

Учебному пособию присвоен гриф редакционно-издательского совета (_____дата и № протокола ученого совета) и рекомендовано для внутривузовского использования.

УДК 579(075.8)
ББК 28.4я7

Federal state budgetary educational institution of higher education "the Orenburg state medical University" The Ministry of health of the Russian Federation

FSBEI OrSMU Ministry of health of Russia

Textbook

VIROLOGY

Orenburg, 2020

Federal state budgetary educational institution of higher education "the Orenburg state medical University" The Ministry of health of the Russian Federation

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The Department of Microbiology, Virology, Immunology

VIROLOGY

Textbook

31.05.01 «Medical care»

For students of the faculty of foreign students

Orenburg, 2020

УДК 579(075.8)
ББК 28.4я7
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М76 Mikhailova, E. A., Virology: textbook/ E. A. Mikhailova, I. E. Lyashenko, O.O. Zherebyatyeva, L. M. Aznabaeva, M. V. Fomina, A.O. Mironchev, S. B. Kirgizova, I.F.Karimov; Orenburg state medical. University of Minzdrav of Russia – Orenburg: OrSMU, 2020. – 122p.

Textbook «Virology» is developed for studying the module «Virology», discipline «Microbiology, Virology» in the development by foreign students of the main educational program by specialty 31.05.01 "Medical care". The manual contains the material necessary for self-theoretical preparation and mastering practical skills within the classroom.

УДК 579(075.8)
ББК 28.4я7

OrSMU, 2020

CONTENTS

PREAMBLE

Fundamental knowledge about the structure and functioning of viruses, the main regularities of interaction of viruses with the cells and with the human body are important areas of formation of biological and medical knowledge. They are the basis for understanding and solving the main tasks of medical Microbiology and Virology – diagnotic, prevention and therapy of infectious diseases. The use of virusological techniques to detect and identify the pathogen and deliver the etiological diagnosis. Knowledge of the features of immunity and main viral diseases epidemioligycal process is aimed at the decision and epidemic prevention tasks. Understanding the features of the mechanisms and regularities of the infectious process caused by viruses required for the formation of clinical thinking and implementation of effective treatment of infectious diseases.

The purpose of textbook – to form a conception about the viruses structure, physiology, ecology and interaction with macroorganism in the infectious process framework.

The studying purpose of this area is the formation of professional competences stated in the Federal State Educational Standart for the specialty "General medicine":

CPC 7 - ready to use main physical-chemical, mathematical and other science concepts and methods in solving professional tasks.

CPC 8 - willingness for the medical use of drugs and other substances and their combinations in the solution of professional tasks.

PC 1 – ability and willingness to implement the complex of measures aimed at the preservation and promotion of health and includes the promotion of healthy lifestyles, prevention and (or) distribution of diseases, their early diagnosis, the identification of the causes and conditions of their emergence and development, as well as to eliminate the harmful effects on human health of environmental factors of its habitat.

Topic 1. MEDICAL VIROLOGY. POXVIRUSES.

I. Home work

1) Reading and learning of "Textbook of Microbiology" by Prof. C.P. Baveja.

The questions for study:

1. Features of viruses.
2. Morphology of viruses.
3. Replication of viruses.
4. Nomenclature of viruses. Viroids. Prions.
5. Virus-host interaction.
6. Pathogenesis of viral infections.
7. Host responses to virus infections.
8. Laboratory diagnosis of viral infections.
9. Poxviruses. Morphology. Antigenic structure. Cultivation. Viruses causing human infections. Prophylaxis.

2) Draw this table in your workbook before class and fill in all empty fields:

Features of viruses

Base necessary components of virus.	
Additional component of virus.	
Classification of viruses by their symmetry.	
Classification of viruses by their shape.	
Classification of viruses by their genome.	
Classification of viruses by their envelope.	
Stages of viral replication.	
Taxa of viruses by taxonomic levels.	

II. Class work

I) Entry test

Write answer for the questions of the entry test in the separate piece of paper and give it to your teacher.

II) Theoretical part

The questions for discussing

1. Features of viruses.
2. Morphology of viruses.
3. Replication of viruses.
4. Nomenclature of viruses. Viroids. Prions.

5. Virus-host interaction.
6. Pathogenesis of viral infections.
7. Host responses to virus infections.
8. Laboratory diagnosis of viral infections.
9. Poxviruses. Morphology. Antigenic structure. Cultivation. Viruses causing human infections. Prophylaxis.

THE THEORETICAL REFERENCE

Features of viruses

Viruses are the smallest obligate intracellular infective agents containing only one type of nucleic acid (DNA or RNA) as their genome. They have no metabolic activity outside the living cells. They do not possess a cellular organization and lack the enzymes necessary for protein and nucleic acid synthesis. Viral genome (nucleic acid) diverts the host's metabolism to synthesize a number of virus specific macromolecules required for the production of virus progeny. They multiply by a complex process and not by binary fission. They do not grow in inanimate media. They are resistant to antibiotics.

Morphology of viruses

Size. Viruses are much smaller than other organisms. The extracellular infectious virus particle is called the *virion.* The size of viruses ranges from 20 to 300 nm in diameter. The largest virus is the smallpox virus (300 nm) and the smallest is the parvovirus (20 nm). In earlier days the virus particles were measured by passing them through the collodion membrane filters of different pore sizes (gradocol membranes). With the development of ultracentrifuge, the virus size could be calculated from the rate of sedimentation of virus in the ultracentrifuge. The latest and the most direct method for measuring virus size is electron microscopy. By this method, both size and the shape of viruses can be made out.

Structure. The virion consists of a nucleic acid core (genome) surrounded by a protein coat, the capsid. The capsid together with the enclosed nucleic acid is known as the nucleocapsid. The capsid is composed of a large number of protein subunits (polypeptides) which are known as capsomers.Two major functions of capsid are, forming an impenetrable shell around the nucleic acid core and to introduce viral genome into the host cells by adsorbing readily to cell surfaces. Certain viruses also contain envelope that surrounds the nucleic acid. The envelope is acquired by the progeny virus during release by budding through the host cell membrane. It is lipoprotein by its nature. The lipid is largely of host cell origin while the protein is virus coded. Protein subunits are exposed as projectile spikes on the surface of the envelope. These structures are called peplomers from peplos meaning envelope. Enveloped viruses are susceptible to the action of lipid solvents like ether and chloroform. Envelopes confer antigenic, biological and chemical properties on viruses.

Symmetry. Three types of symmetry are determined by the arrangement of capsid around the nucleic acid core.

1) Icosahedral (cubical) symmetry: An icosahedron is a polygon with 12 vertices or corners and 20 facets in the shape of equilateral triangular faces. Icosahedral symmetry has a rigid structure. This type of symmetry is found in papova, picorna, adenoviruses (all naked or non-enveloped) and herpes, togaviruses (enveloped).

2) Helical symmetry: The nucleic acid and the capsomers are wound together to form a helical or spiral tube. Most of the helical viruses are enveloped and all are RNA viruses.

3) Complex symmetry: Some viruses do not show either icosahedral or helical symmetry due to the complexity of their structures. These are referred to have complex symmetry e.g. poxvirus.

Shape. The overall shape of virus particles varies in different groups. Poxvirus is brick-shaped, rabies virus is bullet-shaped and tobacco mosaic virus is rod-shaped. Some are irregular and pleomorphic in shape.

Replication of viruses

Due to lack of biosynthetic enzymes, viruses replicate by taking over the biochemical machinery of the host cell to synthesise virus specific macromolecules required for the production of virus progeny. The genetic information necessary for viral replication is contained in the viral nucleic acid. The replicative cycle can be divided into six sequential phases: adsorption, penetration, uncoating, biosynthesis, maturation, release.

1. **Adsorption or Attachment.** The viruses come in contact with the cells by random collision but adsorption or attachment is mediated by the binding of virus surface structures, known as ligands, to the receptors on cell surface. In case of influenza virus, the haemagglutinin (a surface glycoprotein) binds specifically to sialic acid residue of glycoprotein receptor sites on the surface of respiratory epithelium. With the human Immunodeficiency virus (HIV), attachment is between the viral surface glycoprotein gp 120 and the CD4 receptor on host cells.
2. **Penetration.** After attachment, the virus particles may be engulfed by a mechanism resembling phagocytosis, a process known as viropexis. Alternatively, in case of the enveloped viruses, the envelope may fuse with the plasma membrane of the host cell releasing the nucleocapsid into the cytoplasm.
3. **Uncoating**. This is the process of stripping the virus of its outer layers and capsid to release the nucleic acid into the cell. With most viruses, uncoating is affected by the action of lysosomal enzymes of the host cells.
4. **Biosynthesis**. After uncoating, the viral genome directs the biosynthetic machinery of the host cell to shut down the normal cellular metabolism and direct the sequential production of viral components. In general, the nucleic acid genome of most DNA viruses is synthesised in the host cell nucleus. Nucleic acid genome of most RNA viruses is synthesised in the cytoplasm. Viral protein is synthesised only in the cytoplasm. Biosynthesis consists of the

following steps:

1) Transcription of messenger RNA (mRNA) from viral nucleic acid.
2) Translation of the mRNA into 'early proteins' or 'nonstructural proteins'. These are enzymes which initiate and maintain synthesis of virus components. They may also induce shutdown of host protein and nucleic acid synthesis.
3) Replication of viral nucleic acid.
4) Synthesis of 'late proteins' or 'structural proteins' which constitute daughter virion capsids.

Retroviruses exhibits a unique replicative cycle. Virus genome (single stranded RNA) is converted into an RNA : DNA hybrid by the viral enzyme, RNA directed DNA polymerase (reverse transcriptase). Double stranded DNA is synthesised from the hybrid (RNA : DNA). The double stranded DNA form of the virus (provirus) integrates into the host cell genome. The provirus acts as the template for the synthesis of progeny viral RNA. The integration of the provirus into the host cell genome may lead to transformation of the cell and development of neoplasia.

5. **Maturation.** The viral nucleic acid and capsid polypeptide assemble together to form the daughter virions. The assembly takes place in either the nucleus (herpes and adenoviruses) or cytoplasm (picorna and pox viruses). In case of enveloped viruses, the envelope is derived from the nuclear membrane (herpes virus) and from plasma membrane when the assembly occurs in the cytoplasm of host cell (orthomyxoviruses and paramyxoviruses).
6. **Release.** Enveloped viruses are released by a process of budding from the cell membrane over a period of time. The host cell is usually not affected but there are exceptions e.g. polioviruses not only damage host cell but may also be released by the lysis of the host cell. In case of bacterial viruses (e.g. bacteriophages), they are usually released by lysis of the infected bacterium.

Nomenclature of viruses

Viruses are classified on the basis of biological, physical and chemical properties. They are divided into families based on nucleic acid of the genome, size, shape, structure and replicative cycle. Families are subdivided into genera on the basis of physiochemical or serological differences. Each genus has got a number of species. Sometimes family has also got subfamily. The families, subfamilies and genera of viruses are named with suffix *viridae*, *virinae* and *virus* respectively. Viruses are broadly classified into DNA and RNA viruses and then further divided into families, subfamilies, genera and species (Table 1).Viruses can also be differentiated according to their genomes and presence or absence of envelope (Table 2).

Viroids are single-stranded circular RNA molecules that lack a protein coat. They are plant pathogens.

Prions are infectious proteins without any detectable nucleic acid. They are highly resistant to physical and chemical agents. They are resistant to heat (90°C for three minutes), UV rays and nucleases and sensitive to proteases. They produce

slow infections with long incubation period (in years). Diseases caused by them include scrapie of sheep and goats, mink encephalopathy, bovine spongiform encephalopathy, Kuru and Creutzfeldt-Jakob disease.

Table 1 – Taxonomy of DNA Viruses

Family	Subfamily	Genus	Species
Poxviridae	*Chordopoxvirinae*	*Orthopoxvirus*	Variola, Vaccinia, Cowpox, Monkeypox
		Parapoxvirus	Orf virus, milker's node virus
		Molluscipox virus	Molluscum contagiosum virus
		Yatapox virus	Tanapox, Yabapox virus
Herpesviridae	*Alphaherpesvirinae*	*Simplexvirus*	Herpes simplex virus type 1 and 2
		Varicellovirus	Varicella-zoster virus
	Betaherpesvirinae	*Cytomegalovirus*	Human cytomegalovirus
		Roseolovirus	Human herpesvirus type 6
			Human herpesvirus type 7
	Gammaherpesvirinae	*Lymphocryptovirus*	Epstein-Barr Virus
		Rhadinovirus	Human herpesvirus type 8
Adenoviridae	—	*Mastadenovirus*	Adenovirus

Table 2 – Viruses according to their genomes and envelopes

Genome	Envelope	Viruses
ds DNA	Yes	Herpes simplex viruses (type 1 and 2), Varicella-zoster virus, Cytomegalovirus (human herpesvirus 5), Human herpevirus 4, Vaccina, Variola, Molluscum contagiosum virus, Hepatitis B virus (HBV)
	No	Human adenovirus, Human papillomavirus, JC virus, BK virus
ss DNA	No	Parvovirus B 19
ds RNA	No	Human reovirus, Human rotavirus Colarado tick fever virus
ss RNA	Yes	Yellow fever virus, Dengue virus, Japanese encephalitis, Hepatitis C virus, Human corona virus, Sindbis virus, Semliki forest virus, Ross River virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Rubella virus, HTLV, HIV type 1, HIV type 2, Simian immunodeficiency virus, Influenza A virus, Influenza B virus, Influenza C virus, Ebola virus, Marburg virus, Rabies virus, Lassa virus, Hantaan virus
	No	Norwalk virus, Hepatitis E virus, Human poliovirus, Coxsackie A virus, Coxsackie B virus, Echovirus, Enterovirus, Human rhinovirus, Hepatitis A virus

Virus-host interaction

Virus may cause different effects, ranging from no apparent cellular damage to rapid cell destruction. Some viruses (e.g. poliovirus) cause cell death (cytocidal infection). Others may cause cellular proliferation or malignant transformation (oncogenic viruses). In some instances, viruses remain as latent infections (herpes simplex virus) whereas others produce some morphological change in cells to form inclusion bodies (rabies virus).

Cytocidal Infection. Viruses like enteroviruses and reoviruses kill host cells by inhibition of protein, RNA and DNA synthesis.

Cell Transformation. Infection with hepatitis B virus (HBV), hepatitis C virus (HCV), Epstein-Barr virus (EB virus) and several papilloma viruses does not result in cell death, but leads to cell transformation. The transformed cells divide unrestrictedly leading to tumour production and these cells express certain antigens called tumour-associated antigens.

Latent Infections are of different types. Herpes simplex and varicella-zoster viruses remain latent in the nerve root ganglia, to be reactivated periodically in some individuals causing recurrent lesions. Some viruses like hepatitis B virus (HBV) may cause chronic infections which may remain inapparent for many years. However, it may lead to serious consequences, such as cirrhosis or hepatocellular carcinoma. Another type of latent infection is slow progressive infection. An important example of this type of infection is subacute sclerosing panencephalitis (SSPE). This disease develops between 1-10 years after recovery from measles virus infection. Other slowly progressive infections in man are due to human immunodeficiency virus (HIV), Kuru agent and Creutzfeldt-Jakob agent. Yet another class of latent infections is infection by oncogenic viruses.

Inclusion Bodies are virus-specific intracellular globular masses which are produced during replication of virus in host cells. They can be demonstrated in virus infected cells under the light microscope after fixation and staining. They may be present in the cytoplasm (e.g. rabies virus), nucleus (e.g. herpesviruses) or both (e.g. measles virus). In general, viruses that are assembled in the nucleus (usually DNA viruses) produce intranuclear inclusions, whereas assembly in the cytoplasm (mainly RNA viruses) yields cytoplasmic inclusions. Intracytoplasmic inclusions are found in cells infected with rabies virus (Negri bodies), vaccinia (Guarnieri bodies), molluscum contagiosum (molluscum bodies) and fowlpox (Bollinger bodies). They are generally acidophilic and can be seen as pink coloured bodies when stained with Giemsa or eosin-methylene blue stains. However, inclusions of some viruses (e.g. adenovirus) are basophilic (stained by haematoxylin). Demonstration of inclusion bodies helps in the diagnosis of some viral infections. Intracytoplasmic eosinophilic inclusions (Negri bodies) in the brain cells of animals are useful for presumptive diagnosis of rabies.

Alteration in Infected Cells. Respiratory syncytial virus (RSV) causes fusion of adjacent cell membranes leading to syncytium formation. Sometimes

virus coded antigens may appear on the surface of infected cells. These antigens may confer new properties on the host cells. For example, viral haemagglutinin appears on the cells infected with influenza virus and leads to adsorption of erythrocytes to the cell surface (haemadsorption).

Pathogenesis of viral infections

1. Respiratory Tract. The respiratory tract offers the most important portal of entry for viruses. Viruses enter the body through droplets expelled from the nose or mouth of infected persons during talking, coughing or sneezing. Some viruses, such as influenza and rhinoviruses are restricted to the respiratory tract where they multiply and produce local disease. These are known as respiratory viruses. Other viruses, such as measles, rubella, varicella-zoster and cytomegalovirus (CMV) multiply locally to initiate a silent local infection which is followed by lymphatic or haematogenous spread to other sites where more extensive multiplication takes place before producing generalised disease.

2. Alimentary Tract. Next to the respiratory tract, the alimentary tract is the most important route of entry of viruses. The viruses that initiate infection via the alimentary tract are many enteroviruses (e.g. poliovirus, hepatitis A virus), adenoviruses and viruses causing gastroenteritis such as rotavirus, Norwalk virus. Some of these (e.g. gastroenteritis viruses) remain localised to the gut while the others (e.g. poliovirus) are transported to other sites for further multiplication and subsequent spread to the target organs.

3. Skin. Of the viruses that enter through the skin, only a few produce local lesions. Viruses may enter the skin, through abrasions (e.g. papillomaviruses, molluscum contagiosum), insect bites (e.g. arboviruses), animal bites (e.g. rabies virus) or injections (e.g. hepatitis B virus, human immunodeficiency virus). Some viruses such as papillomaviruses, vaccinia, cowpox, molluscum contagiosum produce only local lesions in the skin at the site of entry. Systemic spread of viruses occurs through lymphatic or blood. However, rabies virus travels along the nerves to the spinal cord or brain.

4. Genital Tract. Papillomaviruses and herpes simplex viruses are sexually transmitted and produce local lesions on the genitalia and perineum. HIV, HBV and HCV are also sexually transmitted but do not produce local lesions.

5. Conjunctiva. It may also act as a portal of entry for some adenoviruses (local disease) and a few enteroviruses.

6. Congenital. Congenital infections may occur at any stage from the development of the ovum up to birth. In acute systemic infections, these usually lead to foetal death and abortion. Rubella and cytomegalovirus may produce maldevelopment or severe neonatal disease.

Spread of virus

After entry of the virus, it multiplies initially and proceeds along the lymphatics to the local lymph nodes. After multiplication in the nodes, the virus enters the bloodstream (primary viraemia). It is then transported to the spleen and liver which act as the 'central foci' for viral multiplication. After extensive

multiplication in the spleen and liver, there occurs a massive spillover of the virus into the blood stream (secondary viraemia). This heralds the onset of clinical symptoms. The virus reaches the target organ through the bloodstream. Multiplication in the target sites lead to distinctive lesions.

Incubation period

The incubation period is the time taken for the virus to spread from the site of entry to the target organs for the production of lesions. Its duration is therefore influenced by the relation between the site of entry, multiplication and lesion. When the site of entry and site of lesion are the same (e.g. respiratory viral infections), the incubation period is short – one to three days. In systemic diseases where the virus enters through the alimentary tract and produces lesions in remote target site (e.g. poliomyelitis), the incubation period is longer – 10-20 days.

Host responses to virus infections

The outcome of a virus infection depends on the virulence of the infecting strain and resistance offered by the host. The mechanism of host resistance may be non-specific or immunological.

A. Non-specific responses

l. Age. Most of the viral infections tend to be most serious at extremes of life. Rotaviruses cause severe disease only in infants.

2. Hormones. Corticosteroids administration enhances most viral infections. Injudicious use of steroids in the treatment of herpetoconjunctivitis may cause blindness. The deleterious effect of cortisone may be due to its depression of the immune response and inhibition of interferon synthesis.

3. Malnutrition. Malnutrition interferes with the humoral and cell-mediated immune responses, therefore, it can exacerbate viral infections.

4. Body Temperature. Most of the viral infections are accompanied by fever. Fever may act as a natural defence mechanism against viral infections as most viruses are inhibited by temperature above 39°C.

5. Phagocytosis. Macrophages phagocytose viruses and are important in clearing viruses from blood-stream. Polymorphonuclear leucocytes do not play any significant role.

6. Interferons. Interferons (IFNs) are a family of glycoproteins produced by cells on induction by viral or non-viral microorganisms. These interferons have antiviral activity by inhibiting protein synthesis. There is little or no viral specificity, hence, IFN induced by one virus can confer protection against infection by the same or unrelated viruses. However, some IFNs have a certain degree of host species specificity e.g. mouse interferons are ineffective in humans and vice versa.

Types of interferons**.** They are classified into three types:

1) IFN-α: It is induced by virus infection and produced by leucocytes. It has antiviral activity.
2) IFN-β: It is also induced by virus infection but produced by fibroblasts and epithelial cells. It has antiviral effect.

3) IFN-γ: It is produced by T-lymphocytes and NK cells, on stimulation by antigens or mitogens. It is a lymphokine with immunoregulatory functions. It enhances MHC antigens and activates cytotoxic T-lymphocytes, macrophages and NK cells.

Mechanism of action. Interferons α and β are potent antiviral agents. These are synthesised when a cell becomes infected with a virus. IFN α and β induce the production of three enzymes namely synthetase, RNaseL and protein kinase. This leads to inhibition of viral protein synthesis but does not affect host protein synthesis.

B. Immunological responses

Viruses in general are good antigens and induce both antibody mediated and cell-mediated immunity (CMI). In some viral infections such as poxviruses, measles, herpes simplex virus and CMV infections, CMI appears to be the main immunospecific defence, for others, such as entero and arbovirus infections, antibody production appears to play a major role.

1. Antibody-Mediated Immunity**.** IgG, IgM and IgA antibodies are produced in response to virus infection. IgG and IgM play a major role in blood and tissue spaces while IgA is more important in mucosal surfaces. IgA is important in resistance to infection of the respiratory, intestinal and urogenital tracts. Antibodies may act in the following ways:

1) Neutralisation of virus which prevents attachment, penetration or subsequent events.
2) Antibody may attach to viral antigens on the surface of infected cells, rendering these cells prone to lysis by complement or destruction by phagocytes or killer (K) lymphocytes.
3) Immune opsonisation of virus for phagocytosis and destruction of virus by macrophages.

2. Cell-Mediated Immunity (CMI). CMI prevents infection of target organs and promotes recovery from disease by destroying virus and virus-infected cells. The different mechanisms involved for virus destruction are as follows:

1) Cytolysis by cytotoxic T-cells and Natural-killer (NK) cells.
2) Antibody-dependent cell-mediated cytotoxicity (ADCC).
3) Antibody-complement-mediated cytotoxicity.

Laboratory diagnosis of viral infections

Following are the indications for laboratory diagnosis of viral infections:

1. For proper management of certain diseases, for example:
   If rubella is diagnosed in the first trimester of pregnancy, abortion is recommended.
   If a baby is borne of an HBsAg positive mother, immunisation at birth is mandatory.
2. Diagnosis of diseases caused by viruses for which antiviral chemotherapy is available (herpes viruses).
3. Screening of blood donors for HIV and hepatitis B virus helps to prevent spread

of these viruses.

4. Early detection of epidemics like influenza, poliomyelitis, encephalitis etc. to initiate appropriate control measures.

In the laboratory, the following methods are commonly employed:

A. Rapid tests. Direct demonstration of virus and its components.
B. Cultural method. Isolation and identification of virus.
C. Serological method. Detection of the specific antibodies

Rapid tests. Direct demonstration of virus and its components.

Microscopy methods:

1. Electron Microscopy. The detection of virus by electron microscopy (EM) is being used increasingly especially for viruses that are difficult to culture. Clinical applications of electron microscopy include detection of rotavirus and hepatitis A virus in faecal specimens. Many other viruses can also be detected in different specimens by electron microscopy.

2. Immunoelectron Microscopy. The sensitivity of electron microscopy can be increased by adding specific antibody to the specimen to aggregate the virus particles. These aggregates can be observed under electron microscopy.

3. Fluorescent Microscopy. Direct or indirect fluorescent antibody technique can be used to detect viruses or viral antigens in vesicle fluid, in cell cultures or in frozen tissue sections. Fluorescent microscopy is a very useful method for diagnosis of rabies in brain of animals suspected to be rabid. The method is also useful for rapid diagnosis of respiratory infections caused by paramyxoviruses, orthomyxoviruses, adenoviruses and herpesviruses.

4. Light Microscopy. Inclusion bodies in tissue sections may be detected by light microscopy. Demonstration of inclusion bodies helps in diagnosis of some viral infections. Negri bodies (inclusion bodies) demonstration in the brain cells of animals is a useful method for presumptive diagnosis of rabies.

Immunological rapid tests – detection of Viral Antigens.

These may be detected by enzyme linked immunosorbent assay (ELISA), radioimmunoassay and latex agglutination.

Genetic rapid tests – detection of Viral nucleic acid fragments or genes.

1. Nucleic Acid Probes. Enzyme-labelled or radiolabeled nucleic acid sequence complementary to unique regions in nucleic acid sequence of a virus is known as nucleic acid probe. Such nucleic acid probes for most viruses are now manufactured commercially.

Two strands of the target DNA molecule in the clinical specimens are first separated and then allowed to hybridise with a labelled single stranded DNA or RNA probe. After hybridisation, this hybridised labelled probe can be detected by different methods depending on the type of label attached to the probe. For example, hybridised enzyme labelled probes can be detected by colour detection using appropriate substrate. Cytomegalovirus, papillomavirus and Epstein-Barr

virus have been identified by use of nucleic acid probes.

2. Polymerase Chain Reaction (PCR). With a PCR technique, a target DNA sequence can be amplified to the point where it can readily be identified using labelled probes in a hybridisation assay. Thus viral DNA extracted from a very small number of virions or infected cells can be detected. The technique can be used for the diagnosis of infections caused by HIV-1, HTV-2, human papillomaviruses, herpes simplex virus, hepatitis B virus, hepatitis C virus, enteroviruses, coxsackieviruses, rotavirus, rubella virus and Epstein-Barr virus.

Cultural method. Isolation and identification of virus.

This is the commonest method used in the diagnosis of virus infections. The specimen should be collected properly and transported with minimal delay to the laboratory. Most viruses are heat labile, therefore, refrigeration is essential during transport. The methods used for isolation depend on the virus sought. In general, the viruses can be grown by inoculation into animals, eggs or cell cultures, after the specimen is processed to remove bacterial contaminants.

The isolates are identified by neutralization test or other suitable serological techniques. Since many viruses (e.g. adenoviruses, herpesviruses) are frequently found in normal individuals, therefore, the results of isolation should always be correlated with clinical data.

Cultivation of viruses. As viruses multiply only in living cells, they cannot be grown on any of the inanimate culture medium. Three methods are employed for the cultivation of viruses: animal inoculation, embryonated egg inoculation, tissue culture.

Animal Inoculation is used for:

1. Primary isolation of certain viruses
2. To study pathogenesis of viral diseases
3. To study viral oncogenesis

Infant (suckling) mice are used in the isolation of arboviruses and coxsackie viruses, many of which do not grow in any other system. Animals may be inoculated by several routes – intracerebral, subcutaneous, intraperitoneal or intranasal. After inoculation, animals are observed for signs of disease or death. Later on, they are sacrificed and tissues are tested for the presence of virus. Besides mice, other animals such as guinea pigs, rabbits and ferrets are also used in some situations.

Embryonated Egg Inoculation. Goodpasture (1931) first used embryonated hen's egg for cultivation of viruses. Embryonated hen's eggs (7 to 12 days old) are inoculated by one of the several routes such as chorioallantoic membrane (CAM), allantoic cavity, amniotic sac and yolk sac..After inoculation, eggs are incubated for 2-9 days. Chorioallantoic Membrane (CAM) CAM is inoculated mainly for growing poxviruses. It produces visible lesions (pocks). Each pock is derived from a single virion. Pocks produced by different viruses have different morphology.

Tissue culture. Three types of tissue cultures are available:

1. Organ culture. Small bits of organs are maintained in tissue culture growth

medium.

2. Explant culture. Fragments of minced tissue can be grown as 'explants'. This method is rarely done nowadays.
3. Cell culture.

This is the type of culture routinely employed for diagnostic virology. Tissues are dissociated into the component cells by the action of proteolytic enzymes such as trypsin. The dissociated cells are washed, counted and suspended in a growth medium. The cell suspension is distributed in glass or plastic bottles, tubes or petri dishes. On incubation, the cells adhere to glass or plastic surface (wall of test tube) and divide to form a confluent monolayer sheet of cells within a period of one week.

Cell cultures are classified into three different types on the basis of their origin, chromosomal characters and the number of generations through which they can be maintained:

1) Primary cell cultures. These are normal cells freshly taken from the organs of animal or human being and cultured. They are capable of very limited growth in culture perhaps 5-10 divisions at the most. Common examples of primary cell culture include monkey kidney cell, human amnion cell and chick embryo cell cultures.

2) Diploid cell strains. These are cells of a single type that contain the same number of chromosomes as the parent cells and are diploid. They can be subcultured for a limited number. After about 50 serial subcultures they undergo 'senescence' and the cell strain is lost. Diploid cells developed from human fibroblasts are susceptible to a number of human viruses. They are useful for the isolation of some fastidious pathogens.

3) Continuous cell lines. These are cells of a single type that are capable of indefinite growth in vitro. They are usually derived from cancerous tissue. These cells grow faster and their chromosomes are haploid. They can be serially cultivated indefinitely, therefore, they are termed continuous cell lines. These cell lines may be maintained by serial subcultures or stored in the cold (-70°C) for use when necessary. HeLa, HEp-2 and KB cell lines have been used in the virus laboratories throughout the world for many years. Some cell lines are now being used for vaccine manufacture, for example Vero cell line for rabies vaccine.

Detection of virus growth in cell cultures

Virus growth in cell cultures can be detected by the following methods:

1. Cytopathic effect. Many viruses cause morphological changes in the cultured cells in which they grow. These morphological changes are known as cytopathic effects (CPE). The viruses causing CPE are called cytopathogenic viruses. CPE induced by viruses are of the following types.

1) Syncytium formation: Some viruses (measles, respiratory syncytial virus) lead to syncytium formation in which infected cells fuse with neighbouring cells to form multinucleated giant cells.

2) Cell necrosis and lysis: Enteroviruses produce rapid CPE with crenation

of cells and degeneration of the entire cell sheet.

3) Cellular clumping: Adenoviruses produce large clumps resembling clusters of grapes.

4) Rounding of cells: Viral replication may lead to nuclear pyknosis, rounding, reffactility and degeneration. This is seen in picornaviruses.

5) Discrete focal degeneration: Herpes virus produces discrete focal degeneration.

2. Haemagglutination. A large number of viruses contain haemagglutinin spikes (peplomers) on the capsid or envelope which can agglutinate erythrocytes of different species. Haemagglutination of influenza virus has been studied extensively. The viral haemagglutinin (glycoprotein) has special affinity for a different glycoprotein located in the 'receptor areas' on the surface of erythrocyte. When erythrocytes are added to viral suspension, the virus and ertythrocytes collide in the suspension and adhere to each other resulting in haemagglutination. This test provides a simple and rapid method for detection of viruses in egg or tissue culture fluid. The haemagglutination reaction is specifically inhibited by the antibody to the virus. The haemagglutination inhibition test (HI) is routinely used for detecting antiviral antibody in diagnosis and research.

Use of erythrocytes in haemagglutination

1. The influenza, parainfluenza and mumps viruses haemagglutinate erythrocytes of fowl, man and guinea pig and elution occurs at 37°C.
2. Toga, rubella and rabies viruses haemagglutinate goose erythrocytes at 4°C.
3. Enteroviruses and reovirus haemagglutinate human erythrocytes at 37°C.

3. Haemadsorption. When haemagglutinating viruses (such as orthomyxo and paramyxo viruses) grow in cell cultures, their presence can be detected by the addition of guinea pig erythrocytes to the cell cultures. If the viruses are multiplying in the culture, the erythrocytes will adhere to the infected cells. This is known as haemadsorption. Specific antiserum against the virus blocks haemadsorption.

4. Plaque assay. A viral suspension is inoculated into confluent monolayer of cultured cells in a bottle or petri dish. After allowing time for adsorption, the monolayer is covered with agar gel, to ensure that the spread of progeny virions is confined to immediate vicinity of infected cells. Each infectious virus particle gives rise to a localised focus of infected cells, called a plaque. Plaques can be seen with the naked eye. Each plaque indicates an infectious virus, therefore, the plaque titre is the infectivity titre.

5. Immunofluorescence. Viruses can be detected in infected cells by -taining with fluorescent conjugated antiserum and examined under the fluorescent microscope for the presence of virus antigen. This gives positive results earlier than other methods and, therefore, it is used to identify many viral isolates.

6. Electron microscopy. Viruses can be detected by electron microscopy of ultra thin sections of infected cells.

Serological method. Detection of the specific antibodies

The demonstration of a rise in titre of antiviral antibodies during the course of a disease is strong evidence that it is the aetiological agent. For this, paired sera should be collected from the patient, the acute sample collected early in the course of the disease and the convalescent sample collected ten to fourteen days later. Examination of a single sample of serum is meaningful when IgM specific antibodies are detected. The serological techniques employed would depend on the virus, but those in general use are neutralisation, ELISA, haemagglutination inhibition, complement fixation test, immuno-fluorescence and latex agglutination tests.

Poxviruses

Poxviruses belong to the family *Poxviridae* and cause a number of human diseases. The family poxviridae is divided into two subfamilies, *Chordopoxvirinae* (the poxviruses of vertebrates) and *Entomopoxvirinae* (the poxviruses of insects). Chordopoxvirinae have eight genera but members of only four genera are related to human infections (Table 3). They are the largest and the most complex of all viruses. They can be seen under the light microscope. They replicate in the cytoplasm unlike other DNA viruses.

Table 3 – Poxviruses causing human diseases

Genus	Virus	Primary host	Diseases
Orthopoxvirus	Variola	Man	Smallpox (now eradicated)
	Vaccinia	Man	Vaccinia
	Monkey pox	Monkeys	Human generalised infections (rare)
	Cow pox	Cows	Human localised ulcerating lesions (rare)
Parapoxvirus	Milker's node	Cows	Human localised infections (rare)
	Orf	Sheep	
Molluscipoxvirus	Molluscum contagiosum	Man	Benign skin lesions
Yatapoxvirus	Tanapox	Monkeys	Human localised infections (rare)

Morphology. The Poxviruses are brick-shaped. They are the largest animal viruses measuring 300 x 200 x 100 nm in size and can be seen under the light microscope. Nucleocapsids of these viruses do not show any discernible symmetry

and hence are known as *complex viruses.* In vertical section, poxviruses have a biconcave double stranded DNA core, surrounded by a double layered membrane. The envelope is the outermost layer. On either side of the DNA core is a lens shaped structure called the lateral body.

Antigenic structure. All poxviruses share a common nucleoprotein (NP) antigen. Other antigens include LS antigen (a complex of the heat labile L and the heat stable S antigens), agglutinogen and haemagglutinin.

Cultivation. They grow in chorioallantoic membrane (CAM) of chick embryo and in tissue culture. Both variola and vaccinia viruses produce pocks on the CAM in 48-72 hours. Variola pocks are small, shiny, white, convex, non-necrotic and non-haemorrhagic. In contrast, vaccinia pocks are larger, irregular, greyish, flat, necrotic and some of these are haemorrhagic.

Tissue cultures of monkey kidney, HeLa and chick embryo cells can be used to grow these viruses. Cytopathic effects are produced by vaccinia in 24-48 hours but variola takes longer to produce these changes. Eosinophilic inclusion bodies (Guarnieri bodies) can be demonstrated in the stained preparations. Vaccinia can produce plaques in chick embryo tissue cultures but variola virus cannot.

Viruses Causing Human Infections

1.Variola. Variola virus causes smallpox and has a narrow host range i.e. humans and monkeys only. In past, smallpox epidemics appeared in two forms florid (fatal disease) occurred in India and alastrim (nonfatal disease) seen in Latin America. The name variola major was given to the virus causing classical smallpox and variola minor to the other virus causing alastrim. Two viruses are antigenically similar but differ in certain biological properties.

Smallpox has been eradicated and routine vaccination is now stopped. The world's last naturally occurring case of smallpox was recorded in Somalia, in October 1977. On May 8, 1980, the WHO announced the global eradication of small pox. Eradication could be achieved because of

- no subclinical infection or carrier state,
- an effective vaccine (originally discovered by Jenner in 1796),
- no animal reservoir, and
- aggressive surveillance-containment measures.

Although smallpox has been eradicated, two laboratories still hold stocks of variola virus. These two laboratories are the WHO Collaboration Centre in Atlanta, USA and Koltsovo, Russian Federation.

2. Vaccinia. Origin of vaccinia virus is not known. In the past, the vaccinia virus was used for small pox vaccination. It may have evolved from cowpox or smallpox virus. It is an 'artificial virus' and does not occur in nature as such. It causes a localised skin infection and has a broad host range including rabbit and mice. Vaccinia virus is being used as a vector for incorporating genes for protective antigens for several different pathogens. Many genes have been inserted, including those coding for the antigens of HIV, hepatitis B virus and rabies. Vaccinia virus is being employed as a vector for the development of

recombinant vaccines. Vaccinia and variola viruses are similar in their properties. However, vaccinia virus has been studied in greater detail than variola, as it is safer to work with.

3. Cowpox. In cows, it produces ulcers on the teats and udders and human infection is acquired from cows by the process of milking. The lesions appear on the hands of man. These are localised lesions and undergo changes from macules to pustules as in smallpox. Rodents are the reservoir hosts of cowpox virus.

4. Human Monkeypox. Infection is probably acquired by handling infected animals. Human monkeypox resembles mild smallpox.

5. Milker's Node. It is transmitted to humans from teats and udder of cattle. It is similar to that of cowpox, but rarely becomes pustular.

6. Orf. It is a contagious pustular dermatitis of sheep and goats. It is acquired by man after contact with an infected animal. In man, the disease occurs as a single lesion on finger or hand or occasionally on the face.

7. Molluscum Contagiosum. It is a benign epidermal tumour-like lesion, that occurs only in humans. It mainly involves the arms, legs, buttocks and genital area. It is a contagious disease. The virus is also transmitted sexually in adults. Nodules show hyaline acidophilic inclusion bodies (molluscum bodies) within the proliferated epidermal cells. Molluscum contagiosum virus has not yet been cultured.

8. Tanapox. It is probably acquired by insect bite. Reservoirs are some wild animals. It produces scanty vesicular lesions of the skin. Epidemics have been reported in East Africa.

9. Yabapox. It produces large benign tumours in monkeys. Similar lesions have been reported in a laboratory person handling affected monkeys.

Prophylaxis

Both vaccinia and variola viruses can be grown on the CAM of chick embryo. The two viruses differ from each other by only a single antigen. A natural infection of smallpox gives complete protection against reinfection. Vaccination with vaccinia induces protection against smallpox for about five years.

Topic 2. MICROBIOLOGY OF ACUTE RESPIRATORY VIRAL INFECTIONS.

I. Home work

1) Reading and learning of "Textbook of Microbiology" by Prof. C.P. Baveja.

The questions for study:

1. Influenza. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.
2. Adenovirus infection, rhinovirus infection. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.

3. Infections caused by herpes viruses: chickenpox, shingles, genital herpes, neonatal herpes infections, cytomegalovirus infection. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.
4. Measles, parainfluenza, mumps. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.
5. Arbovirus infections – definition.
6. Tick-borne and Japanese encephalitis. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
7. Hemorrhagic fever: Crimean, yellow, Hemorrhagic fever with renal syndrome (HFRS). Etiology, epidemiology, pathogenesis, laboratory diagnosis, therapy and prevention.
8. Rubella. Etiology, epidemiology, pathogenesis, laboratory diagnosis, prevention.

2) Draw this table in your workbook before class and fill in all empty fields:

Epidemiology of ARVI

Disease	Pathogen	Transmission	Source (vector)
Influenza			
Adenovirus infection			
Tick-borne encephalitis			
Japanese encephalitis			
Yellow fever			
Rubella			

II. Class work

I) Entry test

Write answer the questions of the entry test in the separate piece of paper and give it to your teacher.

II) Theoretical part

The questions for discussing

1. Influenza. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.
2. Adenovirus infection, rhinovirus infection. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.
3. Infections caused by herpes viruses: chickenpox, shingles, genital herpes, neonatal herpes infections, cytomegalovirus infection. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.
4. Measles, parainfluenza, mumps. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific prophylaxis and therapy.
5. Arbovirus infections – definition.

6. Tick-borne and Japanese encephalitis. Etiology, epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
7. Hemorrhagic fever: Omsk, Crimean, yellow, HFRS. Etiology, epidemiology, pathogenesis, laboratory diagnosis, therapy and prevention.
8. Rubella. Etiology, epidemiology, pathogenesis, laboratory diagnosis, prevention.

THE THEORETICAL REFERENCE

All these viruses were grouped under myxovirus (*myxa*meaning mucus) due to their affinity to mucins. The influenza, mumps, parainfluenza, and Newcastle disease viruses were initially grouped in this class. However, two separate families were created subsequent to the recognition of clear differences between these viruses. These two families are: Orthomyxoviridae, consisting of influenza viruses, and Paramyxoviridae, consisting of parainfluenza, mumps, measles, respiratory syncytial, and Newcastle disease viruses. Differences between orthomyxoviruses and paramyxoviruses are summarized in table. The genus *Orthomyxovirus* includes influenza viruses, the causative agents of worldwide epidemics of influenza.

Differences between orthomyxoviruses and paramyxoviruses

Properties	Orthomyxoviruses	Paramyxoviruses
Size	80 – 120 nm	100 – 300 nm
Shape	Spherical	Pleomorphic
Genome	Segmented eight pieces of RNA	Single, linear RNA
Gene reassortment	Common	Not reported
Antigenic stability	Variable	Stable
Hemolysis	Absent	Present
Site of synthesis of ribonucleoprotein	Nucleus	Cytoplasm
DNA-dependent RNA synthesis	Required for multiplication of the virus	Not required for the multiplication of the virus

Influenza Viruses

Influenza viruses are classic respiratory viruses. They cause influenza, an acute respiratory disease, with well-defined systemic symptoms. Influenza is an acute infectious disease of the respiratory tract that occurs in sporadic, epidemic, and pandemic forms.

Morphology. Influenza viruses show following features:

- Influenza viruses are spherical or filamentous, enveloped particles 80–120 nm in diameter.

- Influenza virus is composed of a characteristic segmented single-stranded RNA genome, a nucleocapsid, and an envelope.
- The viral genome is a single-stranded antisense RNA. The genome consists of an RNA-dependent RNA polymerase, which transcribes the negative-polarity genome into mRNA. The genome, therefore, is not infectious. The viral RNA has a molecular weight of 5 million daltons and a length of 13,600 nucleotides. Characteristically, it is segmented and consists of seven or eight segments. These segments code for different proteins which are NS1, NS2, NP, M1, M2, M3, HA, and NA.
- The genome is present in a helically symmetric nucleocapsid surrounded by a lipid envelope. The envelope has an inner membrane protein layer and an outer lipid layer. The membrane proteins are known as matrix or M protein and are composed of two components M1 and M2. Two types of spikes or peplomers project from the envelope: (*a*) the triangular *hemagglutinin* (HA) peplomers and (*b*) the mushroom-shaped *neuraminidase* (NA) peplomers.

Viral replication. Influenza virus, hepatitis delta virus, and retroviruses are the only RNA viruses that have an important stage of their replication cycle in the nucleus. Infection of the host cell begins by adsorption of the cell by influenza virus, which is mediated through the HA. HA is first cleaved by an extracellular protease to a modified HA that actually mediates the attachment of the virus to the cell surface. Once inside the cell, the virus uncoats within the endosome. In the nucleus of the host cell, the virion RNA polymerase transcribes the eight-genome segments into eight viral mRNAs. Most of the viral mRNAs, however, move out of the nucleus into the cytoplasm, where they are translated into viral proteins. Some of the viral mRNAs continue to remain in the nucleus and serve as the templates for synthesis of the negative-strand RNA genomes for the progeny virions. In the nucleus also, two proteins, namely, nucleoprotein (NP) and matrix protein are synthesized, which then bind with the RNA genome of the viral progeny and form a complex, which is subsequently transported to the cytoplasm. Matrix protein mediates the interaction of the nucleocapsid with the envelope, and finally, the virion is released from the cell by budding from the cell membrane at the site where the HA and NAs are present.

Antigenic and genomic properties. Influenza viruses have two types of antigens:

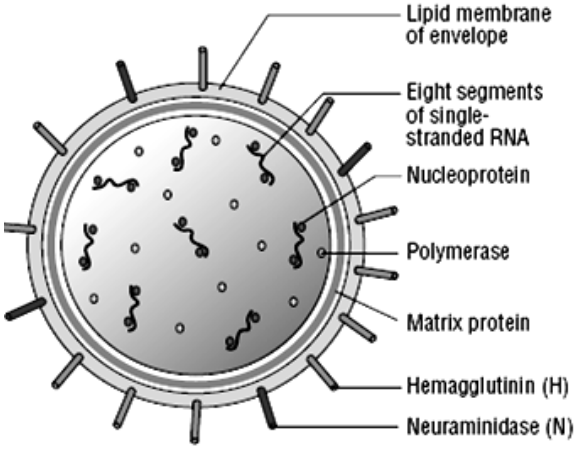
Group-specific antigens - the ribonucleoprotein (RNP) antigen, or the "soluble" antigen, or the internal antigen is the group-specific antigen. Influenza viruses are divided into types A, B, and C on the basis of variation in this nucleoprotein antigen.

Type-specific antigens: The surface antigen, or "viral" antigen, or "V antigen" is composed of two virus-encoded proteins, HA and NA, which are the type-specific antigens.

Hemagglutinin: HA is a trimer and is composed of two polypeptides, HA1 and HA2, responsible for hemadsorption and hemagglutination. The hemagglutinin

consists of 500 spikes, each measuring 12 nm in length. The triangular-shaped HA is inserted into the virus membrane by its tail end. The distal end, which contains five antigenic sites (designated as HA1–HA5), is responsible for binding of virion to host cells. Influenza viruses adsorb many avian and mammalian erythrocytes. Hemagglutinin binds with the neuraminic acid (sialic acid) cell receptor, the cell surface glycoprotein receptor, and initiates the infection in the host cell. Adsorption of erythrocytes occurs at 4°C, but at 37°C there is detachment of the red cells due to destruction of the glycoprotein receptors by the viral enzyme, neuraminidase. The hemagglutinin agglutinates certain red blood cells, which is inhibited by the neutralizing antibodies. This forms the basis of the hemagglutination inhibition test used in the serodiagnosis of influenza. Hemagglutinin has potency to undergo antigenic variations. The nucleotide and amino acid sequences of the polypeptides, HA1 and HA2, undergo radical changes in antigenic shift. In antigenic drift, only minor changes take place in the compositions of HA antigenic sites.

Neuraminidase*:* The NA is a glycoprotein and tetramer. It consists of 100 mushroom-shaped spikes.The mushroomshaped NA is inserted into the virus membrane by its hydrophobic tail end. The distal end contains antigenic as well as enzymatically active sites. The NA causes hydrolysis of *N*-acetyl neuraminic acid or sialic acid residues present on the glycoprotein receptors on red cells, hence causes elution or detachment of the cells adsorbed to virion particles. The function of the neuraminidase is to cleave the neuraminic acid and to release progeny virions from the infected host cells. The neuraminidase also degrades the mucus layer, thereby exposing the epithelial membrane of the respiratory tract for infection by the virus. All the strains of influenza A, B, and C share the common internal proteins, such as nucleoproteins and membrane proteins. They, however, differ in their surface proteins, such as HAs and NAs. The influenza viruses are further subdivided into subtypes, A0, A1, A2, and A3, and these subtypes into strains.



Schematic diagram of structure of an influenza virus.

Antigenic variations. Antigenic variation is a unique feature of influenza virus. The surface antigens HA and NA show variations and are primarily responsible for antigenic variations exhibited by influenza viruses. The internal RNP antigen and M protein are stable, hence do not contribute to the antigenic variations. Antigenic variations are of two types: antigenic shift and antigenic drift.

Antigenic shift: The abrupt, drastic, discontinuous change is called the antigenic shift. This occurs due to major antigenic changes in HA or NA antigens, and is caused by replacement of the gene for HA by one coding for a completely different amino acid sequence. The antigenic shift is characterized by alteration of virtually all the antigenic sites of the HA. This occurs independently in the HA and NA.

Antigenic drift: The gradual, sequential, regular antigenic change in influenza virus is known as antigenic drift. This occurs due to minor antigenic changes in the HA or NA occurring at frequent intervals. This is caused even by a single mutation affecting HA glycoprotein. The antigenic drift is characterized by changes in certain epitopes in the HA, while others are being conserved.

Gene reassortment. Because the influenza virus genome is segmented, genetic reassortment can occur when a host cell is infected simultaneously with viruses of two different parent strains. This process of genetic reassortment accounts for the periodic appearance of the novel types of influenza A strains that cause influenza pandemics. Influenza viruses of animals, such as aquatic birds, chickens, swine, and horses show high host specificity. These animal viruses are the source of the RNA segments that encode the antigenic shift variants that cause epidemics among humans. For example, if a person is infected simultaneously by an avian and human influenza strains, then it is possible that a genetic reassortment could occur in infected cells in humans. The reassortment could lead to emergence of a new human influenza A virus, the progeny of which will contain a mixture of genome segments from the two strains (e.g., a new variant of human influenza A virus bearing the avian virus HA) Many studies have conclusively demonstrated that the aquatic birds (such as water fowl) are a common source of these new genes. The pigs act as mixing vessels, where these virulent genes of water fowl mix with the genome of influenza virus giving rise to new variant of influenza virus.

Designation of influenza viruses. Influenza virus type A can be classified into subtypes based on the variations in their surface antigens. The WHO proposed a new system of classification in 1971 and was later modified, which takes into account the nature of both the surface antigens. According to this, the complete designation of a strain will include the (*a*) type, (*b*) place of origin, (*c*) serial number, and (*d*) year of isolation followed by (*e*) antigenic subtypes of the HA and NA in parentheses. For example: influenza A/Singapore/1/57 (H2N2) indicates that influenza was first originated from Singapore and was isolated for the first time in the year 1957. The HA and NA antigens are H2 and N2 as shown in the parentheses.

Other properties. Influenza viruses are inactivated by ether, formaldehyde, phenol, salts of heavy metals, and common chemical disinfectants. Heating at 50°C for 30 minutes inactivates most strains of the virus. Influenza viruses are resistant to slow drying. They remain viable in dust up to 2 weeks, at 70°C for a longer period, and by freeze-drying indefinitely.

Virus Isolation and Animal Susceptibility. Influenza viruses are isolated from respiratory secretions by growing in chick embryos or tissue cultures (monkey kidney or baboon kidney cell lines). The influenza viruses grow in the allantoic and amniotic cavity of the chick embryos. After an incubation period of 3 days, the fluid is tested for hemagglutination activities of the viruses. Cell lines are widely used for culture of influenza viruses. They can grow in several primary and continuous cell lines. Rhesus Monkey kidney cell lines (LLC-MK2) and Madin–Darby canine kidney (MDCK) are the continuous cell lines frequently used to isolate influenza viruses. Human influenza virus causes experimental infections in a variety of animals. Intracerebral inoculation of mice by neurotrophic strains produces fatal encephalitis. It causes an acute respiratory disease on intranasal inoculation in ferrets.

Epidemiology

Influenza occurs worldwide. Influenza virus is transmitted from person to person primarily in droplets released by sneezing and coughing

Geographical distribution. Influenza viruses cause epidemic, endemic, and pandemic influenza. Influenza A virus causes epidemics and occasionally pandemics; influenza B virus only causes epidemics; and influenza C viruses only cause minor respiratory illness and do not cause any epidemics. Influenza epidemics have been recorded throughout the history. The worst of these was the 1918 pandemic, which caused about 20 million deaths worldwide and about 500,000 deaths in the United States. In temperate climates, the epidemics typically occur in the winter and cause considerable morbidity in all age groups. Influenza occurs throughout India. The worst, severe pandemic in the year 1918–1919 (Spanish flu) caused nearly 10 million deaths in India.

Reservoir, source and transmission of infection. Infected humans are the main reservoir of infections for influenza A virus. Respiratory secretions of infected persons are the important source of infection. The virus is excreted in respiratory secretions immediately before the onset of illness and for 3–4 days thereafter. Wild aquatic birds are known reservoirs of influenza A. They secrete the viruses in their feces, which contaminates ponds and lakes. The virus is spread from person-to-person primarily by air-borne respiratory droplets released during the acts of sneezing and coughing. Influenza B virus only causes epidemics. Infection is from humans-to-humans. No animal reservoir hosts are known. Influenza C virus only causes minor respiratory illness and does not cause any epidemics.

Influenza epidemics and pandemics. Influenza epidemics are of two types. Yearly epidemics are caused by both type A and type B viruses. The rare, severe influenza pandemics are always caused by type A virus. Antigenic shift and antigenic drift are the two different mechanisms responsible for producing the strains that cause these two types of epidemics.

Antigenic shift: A major change in one or both of the surface antigens, a change that yields an antigen showing no serologic relationship with the antigen of the strains prevailing at the time is called antigenic shift. Antigenic shift has been demonstrated in type A influenza virus only. Influenza A virus causes epidemics and occasionally, pandemics. Pandemics are caused by the virus strains undergoing antigenic shift. Antigenic shift variants appear less frequently, about every 10 or 11 years. It is demonstrated that pandemic strains are the recombinant strains, originated from some animal or bird reservoir, either spreading to humans directly by host range mutation or as a result of a recombination between human and nonhuman strains. The pandemic strains also show the capability to spread rapidly among the population. The completely novel antigens that appear during antigenic shift are acquired by genetic reassortment. The donor of the new antigens is probably an animal influenza virus. Type A viruses have been identified in pigs, horses, and birds, and animal influenza viruses possessing antigens closely related to those of human viruses. Fourteen distinct HA and nine NA antigens are known. Since continued surveillance of animal influenza viruses in recent years has failed to discover new antigens, these may represent the full variety of major influenza virus surface antigens (subtypes).

Antigenic drift: Repeated minor antigenic changes, on the other hand, generate strains that retain a degree of serologic relationship with the currently prevailing strain. This is called antigenic drift. The epidemics are caused by influenza A virus undergoing antigenic variations due to antigenic drift resulting from mutations and selections. Antigenic drift variants occur very frequently, virtually every year. This is responsible for emergence of the strains that cause yearly influenza epidemics. When persons are reinfected with drift viruses, the serum antibody responses to the surface antigens that are shared with earlier strains to which the person has been exposed are frequently stronger and of greater avidity than are the responses to the new antigens. This phenomenon, which has been called "*original antigenic sin*" is sometimes useful in serologic diagnosis. Antigenic drift represents selection for naturally occurring variants under the pressure of population immunity. Influenza A is generally more pathogenic than influenza B. Influenza A is a zoonotic infection and more than 100 types of influenza A viruses have been described which infect birds, pigs, horses, dogs, and seals. The 1918 pandemic of influenza that resulted in millions of human deaths worldwide is believed to have originated from a virulent strain of H1N1 from pigs or birds.

H5N1 bird flu

The H5N1 flu, caused by an avian subtype influenza virus, which has been associated with bird flu in the domesticated birds, can be transmitted from birds to humans. The H5N1 was first described in Hong Kong in 1997. Human infections caused by this virus were established in only 18 individuals of which six died. Since then, sporadic cases of H5N1 infection continued to be described in southern China. An epidemic of bird flu occurred in domesticated birds in Southeast Asia, primarily Vietnam, in January 2004. More than 240 human cases have been documented and more than 140 persons have died due to the poultry outbreaks and bird-to-human transmission. Most deaths have been reported in Vietnam and Indonesia. Sporadic outbreaks have continued to occur since the 2004 outbreak even outside Southeast Asia, including Turkey. Till now, no conclusive evidences are available to show the human-to-human transmission of H5N1. However, scientists are concerned that a slight mutation could convert H5N1 to a strain that would be easily transferred from human-to-human. Such a strain, it is believed, could potentially spread rapidly and cause a catastrophic worldwide pandemic. Hence, efforts are currently underway to develop an effective vaccine against H5N1. In addition, the number of drugs that are effective against influenza are being increasingly evaluated. Ribavirin has shown activity when tested in animal models. Another avian subtype, H9N2, was described in two young children in March 1999. However, after that despite concern, no further outbreak of H9N2 infection has been documented. Experts are also concerned that a virulent strain of H9N2 influenza similar to H5N1 flu may mutate to allow human-to-human infection and that such a strain may possess the combination of transmissibility, infectivity, and lethality.

H1N1

A novel H1N1 swine origin influenza virus (SOIV) H1N1 emerged in 2009 to produce the first human influenza pandemic of the twenty-first century. Within 1 year, this virus spread to 214 countries and caused more than 18,000 confirmed deaths worldwide. First described in April 2009, the novel swine origin influenza virus emerged due to a “triple-reassortment” of influenza viruses of avian, swine, and human origin. This led to the popular term “swine flu” being used for this virus. The 2009 pandemic H1N1 virus is composed of (*a*) PB2 and PA segments from North American avian viruses; (*b*) the PB1 segment of the human H3N2 viruses; (*c*) hemagglutinin (HA; of the H1 subtype), nucleoprotein (NP) and NS segments derived from classical swine H1N1 viruses, and the neuraminidase (NA; of the N1 subtype), and (*d*) M segments of Eurasian “avian-like” swine viruses. Unlike previous strains of influenza virus, the novel swine origin influenza virus did not have a predilection for populations more than 60 years of age. Rather, more number of younger people less than 40 years of age were found to be affected. A small percentage of those affected were found to develop pneumonia or acute respiratory distress syndrome. The number of cases decreased greatly by May 2010, and the pandemic was officially declared to be at an end in August 2010. For diagnosis of H1N1 influenza, testing of samples like nasopharyngeal swabs or

oropharyngeal swabs is essential. Real time RT-PCR for viral nucleic acid is the recommended method for its diagnosis, and other rapid tests like antigen detection were not found to be useful. The treatment for severe cases of H1N1 influenza is the neuraminidase inhibitor, oseltamivir. Two types of influenza vaccines have been developed against H1N1 influenza – a trivalent inactivated vaccine and a live attenuated influenza vaccine, which can be administered intranasally.

Pathogenesis of influenza

Inhaled influenza viruses reach lower respiratory tract, tracheobronchial tree, the primary site of the disease. They attach to sialic acid receptors on epithelial cells by HA present on the viral envelope. Relatively few viruses are needed to infect lower respiratory tract than the upper respiratory tract. Neuraminidase of the viral envelope may act on the *N*-acetyl neuraminic acid residues in mucus to produce liquefaction. In concert with mucociliary transport, this liquefied mucus may help spread the virus through the respiratory tract. Infection of mucosal cells results in cellular destruction and desquamation of the superficial mucosa. The resulting edema and mononuclear cell infiltration of the involved areas are accompanied by symptoms including nonproductive cough, sore throat and nasal discharge. Although the cough may be striking, the most prominent symptoms of influenza are systemic: fever, muscle aches and general prostration. The virus remains localized to the respiratory tract, hence viremia does not occur. In an uncomplicated case, virus can be recovered from respiratory secretions for 3–8 days. Peak quantities of 10^4–10^7 infectious units/mL are detected at the time of maximal illness. After 1–4 days of peak shedding, the titer begins to drop, in concert with the progressive abatement of disease. Occasionally, in patients with underlying heart or lung disease, the infection may extensively involve the alveoli, resulting in interstitial pneumonia, sometimes with marked accumulation of edema and lung hemorrhage. Pure viral pneumonia of this type is a severe illness with a high mortality. Virus titers in secretions are high, and viral shedding is prolonged. In most cases, however, pneumonia associated with influenza is caused by bacteria, principally pneumococci, staphylococci and Gramnegative bacteria. These bacteria can invade and cause disease, because the preceding viral infection damages the normal defenses of the lung.

Host immunity

Antibody is the primary defense in immunity to reinfection. IgA antibody, which predominates in upper respiratory secretions, is less persistent than secretory IgG, but contributes to confer immunity. Secretory IgG antibody, which predominates in lower respiratory secretions, appears to be the most important. Antibodies provide long-lasting immunity against the infecting influenza strain. Only antibodies directed against HA is able to prevent infection. Antibodies against HA neutralizes the infectivity of the virus, thereby preventing the disease. Antibodies against NA do not neutralize infectivity but reduce severity of the disease. They act presumably by impairing the action of NA against *N*-acetyl

neuraminic acid residues in the virion envelope and thus promoting virus aggregation. The antibodies against the internal ribonucleoprotein groupspecific antigen do not confer any immunity. Immunity to influenza virus is strain-specific and lasts for many years. Recurrent cases of influenza are caused primarily by antigenically different strains. The role of cell-mediated immunity in conferring protection against influenza is not clear.

Clinical Syndrome

Influenza syndrome. The virus causes classic influenza syndrome.

Incubation period is short (1–3 days). The classic influenza syndrome is a febrile illness of sudden onset, characterized by tracheitis and marked myalgias. Headache, chills, fever, malaise, myalgias, anorexia and sore throat appear suddenly. The body temperature rapidly rises to 101–104°F (38.3–40.0°C) and respiratory symptoms ensue. Nonproductive cough is characteristic. Sneezing, rhinorrhea, and nasal obstruction are common. Patients may also report photophobia, hoarseness, nausea, vomiting, diarrhea, and abdominal pain. They appear acutely ill and are usually coughing. Minimal to moderate nasal obstruction, nasal discharge and pharyngeal inflammation may be present. Lung examination is usually normal. The viruses mostly cause subclinical infections. They do not display the classic syndrome described above. Moreover, the influenza syndrome is uncommon in children and is not seen in infants. A given patient may exhibit symptoms including predominantly sneezing, nasal obstruction, and nasal discharge (common cold); nasal obstruction, discharge, and sore throat (upper respiratory illness); sore throat with erythema (pharyngitis); hoarseness (laryngitis); or cough (tracheobronchitis). Fever may be absent.

Complications

Secondary bacterial infections: Life-threatening influenza is often caused by secondary bacterial infections with staphylococci, pneumococci, and *Haemophilus influenzae*. Pneumonia may develop as a complication and may be fatal, particularly (*a*) in elderly persons above 60 years with underlying chronic disease, (*b*) in people with impaired resistance (chronic cardiorespiratory disease, renal disease, etc.), and (*c*) in pregnant women.

Central nervous system complications: Guillain–Barre syndrome characterized by encephalomyelitis and polyneuritis is a rare complication of influenza virus infection. This condition was documented in the United States in the year 1976, following extensive vaccination with inactivated H3N2 influenza virus.

Other complications: Reye's syndrome is a noted complication of influenza B infection. The condition is seen most commonly in young children and is associated with degenerative changes in the brain, liver and kidney.

Laboratory Diagnostics

During an epidemic of influenza, the clinical diagnosis can be made, but definitive diagnosis depends on the laboratory methods.

Specimens include nasal or throat washings or sputum for viral antigen and viral RNA, throat gargles for isolation of viruses and serum for viral antibodies.

Direct antigen detection. A rapid, specific diagnosis of influenza is made by demonstrating viral antigens directly on cells obtained from the nasopharynx. Immunofluorescence (IF) or enzyme-linked immunosorbent assay using specific monoclonal antibodies are used commercially to detect viral antigen. The results of the rapid tests are useful to start treatment with the NA inhibitors within 48 hours of the onset of symptoms.

Isolation of the virus. Throat gargles are the specimen of choice. The specimen is collected in saline broth or a buffered salt solution and is sent immediately to the laboratory, or if delayed it must be stored at 4°C. The virus is isolated from the specimen by inoculation into embryonated eggs or into certain cell cultures.

Egg inoculation: The specimen is inoculated into the amniotic cavity of the chick embryo. After incubation at 35°C for 3 days, the amniotic fluid and allantoic fluid are harvested and tested for the presence of viral HA. This is carried out by using fowl and guinea pig red cells in parallel and incubating at room temperature and at 4°C. Usually, influenza A viruses agglutinate only guinea pig cells, influenza B both fowl and guinea pig red cells, and influenza C agglutinate only fowl cells at 4°C. If the test is positive, the isolate is then typed by a serological test (e.g., hemagglutination inhibition test) using specific antisera to types A, B, and C.

Cell culture: Influenza virus is usually isolated from respiratory secretions by growing in tissue cultures (monkey kidney or baboon kidney cell lines). The cell cultures are incubated at 33°C in the roller drums in the presence of trypsin, but without serum. Virus growth in tissue cultures is detected by direct demonstration of viral antigen in infected cell cultures by IF or by testing for hemadsorption with human O, fowl and guinea pig red cells. In a positive hemadsorption test, red cells adhere to the virus budding from infected cells. If the culture tests positive, serologic tests with specific antisera may be used to identify the virus.

Serodiagnosis. Demonstration of a rise in serum antibody titer between acute-phase and convalescent-phase sera by a serological test is diagnostic of infection. The acute-phase sera are collected within a few days of illness and the convalescent sera 7–10 days after the illness. Complement fixation tests (CFTs) with RNP antigens of influenza types A, B, and C and also the CFTs with V antigens are employed for demonstration of rising antibody titer in the paired sera samples. Hemagglutination inhibition test, enzyme neutralization test, radial immunodiffusion test, and ELISA are the other tests also used for demonstration of antibodies. However, none of these techniques are useful to identify all infections. Various approaches followed for laboratory diagnosis of influenza are summarized in Table 61-6.

Molecular Diagnosis. Reverse transcriptase-polymerase chain reaction is used recently for the detection of viral nucleic acid in the nasopharyngeal cells for diagnosis of influenza.

Treatment

Amantadine and rimantadine are the specific antiviral agents available for treatment of influenza. These medicines are effective against influenza A virus but not against influenza B virus. These drugs when given within 1–2 days of the onset of illness, reduce severity of the disease and also hasten the disappearance of fever and other symptoms. The drugs are recommended for prevention of influenza in elderly unimmunized population in whom the influenza can cause a life-threatening infection. Emergence of viral resistance can occur during treatment. Zanamivir (*Relenza*) and oseltamivir (*Tamiflu*) are newer drugs for treatment of influenza and are effective against both influenza A and B viruses. These are the NA inhibitors, which act by inhibiting the release of viruses from infected cells. These drugs also prevent the spread of virus from one cell to another. Relenza is used in the form of nasal spray, whereas Tamiflu is given orally.

Prevention and Control

This is based on the following:

1. Immunoprophylaxis by vaccines

2. Chemoprophylaxis

Immunoprophylaxis by vaccines

Influenza A subtypes H1N1 and H3N2 are most common prevailing human influenza viruses. The trivalent vaccine used worldwide contains influenza A strains from H1N1 and H3N2, along with an influenza B strain. Influenza virus vaccines have been used for about 40 years to prevent influenza, primarily influenza A and B. Following types of vaccines are used:

Inactivated vaccines: Initially inactivated vaccines were used. The viruses for these vaccines are grown in chick embryos, inactivated by formalin, purified to some extent, and adjusted to a dosage known to elicit an antibody response in most individuals. The vaccines contain the strains of types A and B viruses that are believed most likely to produce epidemics during the following winter. The vaccine is administered parenterally. One or two doses are required, depending on the immune experience of the population with related antigens. The vaccines are recommended especially for persons at high risk, especially those over 65 years of age and those with chronic cardiopulmonary diseases. Local and systemic reactions to the vaccine are minor and occur in the first day or two after vaccination. In some persons, the vaccine may cause reactions allergic to egg proteins present in the vaccine. The killed vaccines do not induce formation of secretory antibodies in the secretory mucosa, although they elicit production of specific protective antibodies in the serum.

Live attenuated vaccines: Live attenuated vaccines are now being developed as alternatives to inactivated vaccines. These vaccines induce production of specific secretory antibodies in respiratory mucosa. Earlier, the live vaccine used the viruses that were attenuated by repeated egg passage and was given by intranasal instillation. But these vaccines often failed to protect the children from clinical disease. Recently, temperature-sensitive (*ts*) mutant strains have been used in the live attenuated vaccine preparations. These avirulent mutants are able to grow at 32–34°C in the nasopharyngeal secretions, but not at 37°C in the lungs. These vaccines are useful to protect the children from clinical disease.

Recombinant vaccines: Recombinant vaccines using recombinant strains are now being evaluated in influenza. Recombinant strains are produced by hybridization between the *ts* mutants and new antigenic variants of the strains. These strains carry the surface antigens of the new variants and growth properties of the old established strains. The recombinant vaccines are indicated most importantly for immunoprophylaxis of a new strain of influenza virus, threatening to cause pandemics of influenza.

Chemoprophylaxis

Chemoprophylaxis by amantadine and rimantadine hydrochloride has been shown to be more successful. These two drugs effectively prevent infection and illness caused by type A, but not by type B viruses (because they lack M2 components). The persons with high risk can be protected by administering in a dosage of 100 mg/day. The drugs interfere with virus uncoating and transport by blocking the transmembrane M2 ion channel. These antiviral agents prevent about 50% of infections and about 67% of illnesses under natural conditions. When administered for 10 days to household contacts of a person with influenza, these drugs protect up to 80% of the persons from illness. Side effects are greater for amantadine but are limited to the central nervous system.

Arboviruses

Introduction. Arboviruses are RNA viruses that are transmitted by arthropods. The word *arbovirus* is an acronym for arthropod-borne viruses that are transmitted by arthropods, from one vertebrate host to another. Arbovirus is a collective name for a large group of viruses of which about 100 are known human pathogens. Most of the arboviruses causing infections in humans are zoonotic, with humans acting as accidental host. Humans play an important role in the maintenance or transmission of the virus in the cycle. Yellow fever virus and dengue virus are two exceptions, which are not zoonotic. The viruses multiply in tissues of the arthropod host without producing any disease. Some arboviruses are maintained in nature by transovarian transmission in arthropods. Dengue, Japanese encephalitis (JE), yellow fever, Western equine encephalitis, Eastern equine encephalitis, St. Louis encephalitis, West Nile fever, and sandfly fever are some of the major arbovirus diseases distributed worldwide. In India, the most important

arbovirus infections are JE, chikungunya fever, dengue, Kyasanur forest disease (KFD), Chandipura fever, and encephalitis.

Classification. Arboviruses were named initially, according to the disease they cause, such as yellow fever virus, or depending upon the place from where they were first isolated, e.g., West Nile fever and St. Louis encephalitis. Taxonomically, most arboviruses and roboviruses are recently classified into five families, namely, Togaviridae, Flaviviridae, Bunyaviridae, Reoviridae, and Rhabdoviridae. Most viruses of medical importance are included in the family Togaviridae, some belong to family Flaviviridae, and some to families Reoviridae and Rhabdoviridae. Togaviruses, flaviviruses, bunyaviruses, reoviruses, and rhabdoviruses associated with human infections.

Important Properties of Arboviruses

Togaviruses are single-stranded RNA viruses. They are spherical, 17 nm in diameter, and have an icosahedral nucleocapsid/ Arboviruses surrounded by an envelope. They replicate in cytoplasm of the infected host cell, and after assembly in the host cell they show budding through the cell membrane. All viruses are serologically related. Togaviruses are divided into two families: alphaviruses and rubiviruses.

The major togaviruses are chikungunya, Venezuelan and Western equine encephalitis viruses, Eastern encephalitis viruses, Mayaro, O_nyong-nyong, Semliki Forest, and Sindbis viruses. Most of these viruses are transmitted by mosquito arthropods to humans.

Flaviviruses are similar to togaviruses in that they are also single-stranded RNA viruses and have an icosahedral nucleocapsid surrounded by an envelope. They differ from togaviruses in being small, only 40–50 nm in diameter; in contrast, the togaviruses are relatively large and have a diameter of 70 nm. The flaviviruses replicate in the cytoplasm and assembly of viruses occurs within endoplasmic reticulum. All flaviviruses are serologically related.

Bunyaviruses are large, spherical (80–120 nm in diameter) and have a triple-segmented and a single-stranded RNA. They have a helical nucleocapsid surrounded by an envelope. They replicate in the cytoplasm, and the assembly of the virion occurs by budding on smooth membrane of the Golgi system. The members of the family Bunyaviridae are classified into four genera: *Bunyavirus*, *Hantavirus*, *Nairovirus* and *Phlebovirus*.

Reoviruses are spherical viruses, measure 16–18 nm in diameter, and are nonenveloped viruses. The genome consists of a double-stranded RNA of 10–12 segments. Replication and assembly of the virus occurs in the cytoplasm of the host cell. Reoviruses are divided into two genera: *Coltivirus* and *Orbivirus*. Coltiviruses include Colorado tick fever virus, transmitted by ticks. Orbiviruses include African horse sickness virus and blue tongue virus.

Rhabdoviruses include the vesiculoviruses, which are transmitted by either mosquitoes or sandflies. Vesicular stomatitis virus and Chandipura viruses are the common examples.

Life Cycle

The life cycle of the arboviruses is characterized by their ability to multiply in both the vertebrate hosts and blood-sucking arthropods. Nevertheless, for effective transmission to vectors, the virus must be present in sufficiently higher number in the blood stream of the vertebrate hosts, which can be taken up in small volume of blood ingested during bite of the arthropod. After ingestion, the virus replicates in the gut of arthropods and then spreads to other organs including the salivary gland. Only the females of the species serve as the vector of the virus, because female species require blood meal for production of the progeny. Most of these viruses in their life cycle show an *extrinsic incubation period* in the vectors, during which viruses are replicated sufficiently in the vector and are present in sufficient numbers in the saliva to transmit infection to the vertebrate host. The extrinsic period for most of the viruses ranges from 7 to 14 days. Some arboviruses are transmitted by vertical transmission through transovarial passage from a mother tick to baby ticks.

Disease caused by arboviruses occurs primarily in dead-end host, but neither in the arthropod vector nor in the vertebrate animal that act as the natural host. For example, after infection yellow fever virus causes disease in humans, who are a dead-end host, but causes a harmless infection among the jungle monkeys in South Africa, which serve as the natural host for yellow fever virus.

Clinical Syndromes

Arboviruses cause diseases in humans, which may be one of the following clinical syndromes:

1. Fever with or without maculopapular rash.
2. Encephalitis, often with high mortality.
3. Hemorrhagic fever, also frequently severe and fatal.

However, there are some arboviruses that can cause more than one syndrome in the infected human host, e.g., dengue virus. Recovery from the disease usually confers a lifelong immunity.

Epidemiology

Arbovirus infection occurs in distinct geographical distribution and vector patterns. The viruses occur in tropics as well as in temperate countries. They show a tendency to cause sudden outbreak of disease that usually occurs in the adjoining areas between human dwellings in jungle or forest areas.

Control methods essentially include vector controls and vaccination. The vaccination has been effective only in yellow fever and a few other diseases but not in most of arboviral diseases. Hence, vector control continues to be a mainstay in prevention of arboviral diseases.

Rubella virus

Rubella virus causes rubella, a mild viral disease affecting the skin, lymph nodes and less commonly, the joint. It also causes congenital rubella syndrome. It is an RNA virus classified as a rubivirus in the family Togaviridae.

Properties of the Virus

Morphology. Rubella virus shows following features:

1. It is a pleomorphic, roughly spherical virus measuring 50–70 nm in diameter.
2. It consists of a single-stranded RNA genome, an icosahedral nucleocapsid, and lipoprotein envelope.
3. The virus unlike measles and mumps viruses has a p ositivestrand RNA, therefore does not contain any virion polymerase.
4. The envelope contains hemagglutinin peplomers.

Viral replication. Rubella virus penetrates the cell and uncoats, and the positivestrand RNA genome is translated into several structural and nonstructural proteins. RNA-dependent RNA polymerase is an important nonstructural protein, which replicates the genome first by making progeny. The virion acquires its envelope from the outer membrane of cell as the virion comes out of the cell. Both replication and assembly of the virion occur in the cytoplasm of the cell.

Antigenic and genomic properties. Rubella has only one serotype.

Other properties. The virus agglutinates erythrocytes of human, chick, goose, and pigeon at 4°C. The virus is heat sensitive. It is readily inactivated by heating at 56°C, but survives for several years at 60°C. The virus is inactivated by chemicals, such as betapropiolactone, formaldehyde, chloroform, and ether.

Virus Isolation. The virus is grown with difficulty in the cell lines. The virus is not cytolytic but produces limited cytopathologic effects in certain cell lines, such as Vero and RK-13 cell lines. In cell line, the replication of rubella prevents the replication of superinfectious picornavirus. This phenomenon is known as heterologous interference, which is made use of in detection of rubella virus.

Pathogenesis and Immunity

Pathogenesis of rubella. Initially rubella virus infects the nasopharynx of the upper respiratory tract and then spreads to local lymph nodes. From there, the virus spreads by the blood throughout the body to the internal organs and skin. The occurrence of mild rash is characteristic. The exact cause of pathogenesis of rash is not known, but may be due to antigen–antibody mediated vasculitis. During prodromal period and for nearly 2 weeks after the onset of rash, the infected persons continue to secrete virus in the respiratory droplets.

Host immunity. Rubella infection is characterized by development of circulating antibodies, which are produced after the phase of viremia and their development correlates with the appearance of the rash. The circulating antibodies limit the spread of the virus in the blood. These antibodies also cross the placenta and protect the newborn from an attack of rubella. Natural rubella infection usually confers lifelong immunity. Reinfection may occur occasionally after the natural disease or vaccination or exposure to the virus. The formation of immune

complexes is suggested to contribute to the development of rash and arthralgia associated with rubella infection.

Clinical Syndromes.

Rubella virus causes rubella and congenital rubella syndrome. Incubation period varies from 14 to 21 days. Rubella is a milder and more subtle disease than measles. A three-day maculopapular or macular rash, which starts on the face and progresses downward to involve the extremities, is the characteristic presentation in symptomatic cases. The rash typically lasts 3 days. Tender lymphadenopathy that affects all the nodes but most commonly affects suboccipital, postauricular, anterior, and posterior cervical nodes is the hallmark of rubella. In adults, rubella produces a more severe disease with manifestations of arthralgia and polyarthritis, and rarely thrombocytopenia or postinfectious encephalopathy.

Congenital rubella syndrome. Congenital rubella syndrome is the most severe and important complication of rubella, which occurs in the fetus of pregnant women without immunity to the virus. The fetus is at major risk until fifth month of pregnancy. Maternal immunity to the virus due to prior exposure or vaccination prevents spread of the virus to the fetus. In the first trimester, 80% of the infants would be affected, and severity of the disease depends on how early the infection occurs. Cataract, mental retardation, and deafness are the most common manifestations of congenital rubella infection. The congenital rubella results in congenital anomalies or even death of the fetus. In addition, the infants infected *in utero* continue to excrete rubella virus for up to 1 year. These children constitute a public health hazard because they are considered as an exposure threat to nonimmune pregnant women. The virus can be transmitted to pregnant women from children.

Epidemiology

Humans are the natural host. The disease is prevalent worldwide. In countries where vaccination is not routinely used, epidemics of rubella occur every 6–9 years. Respiratory droplets are the source of infection. The virus is transmitted from person-to- person by inhalation of respiratory droplets. The infection is usually acquired during childhood. Patients are most contagious during the time of appearance of the rash. The viruses are excreted in the pharynx and in the respiratory droplets from 7 days before until 7 days after the rash. The infection is usually acquired during childhood. The infection can also be transmitted transplacentally from mother to fetus, resulting in congenital infection.

Laboratory Diagnosis

Specimens. Throat swab in the adult and urine, cerebrospinal fluid (CSF), or throat swab in infants with congenital rubella are the frequently used specimens.

Isolation of the virus. Viruses can be isolated in rabbit kidney (RK-13), rabbit cornea (SIRC), and Vero cells. The virus produces little cytopathic effects.

Serodiagnosis. The diagnosis of rubella is usually confirmed by demonstration. of rubella antibodies in the serum. Demonstration of specific IgM rubella antibodies in a single acute phase serum sample is diagnostic of rubella.

Diagnosis can also be made by demonstration of a fourfold or a greater rise in IgG antibody titer between acute phase and convalescent phase sera by using hemagglutination inhibition test or enzyme-linked immunosorbent assay (ELISA). In pregnant women, demonstration of IgM rubella antibodies indicates recent infection. Demonstration of 1:8 or greater titer of IgG antibody in serum indicates immunity and consequent protection of the fetus. Demonstration of rubella virus in amniotic fluid collected by amniocentesis indicates a definite rubella infection in the fetus. The presence of IgM antibodies in the serum of the infant indicates recent infection because IgM does not cross the placenta from the mother as does IgG. Confirmation of diagnosis of congenital rubella syndrome in an infant after 1 year with serology alone is very difficult.

Treatment and Prevention

There is no antiviral therapy. Preventive measures include vaccination and administration of immunoglobulins.

Vaccination. Prevention of rubella is best carried out by immunization with live attenuated vaccine. The main objective of rubella vaccination program is to prevent congenital infection by decreasing the number of susceptible people, especially children. Vaccination causes a significant reduction of the likelihood of exposure of the pregnant women to the virus (refer the box Vaccine). Immunoglobulins can be given to pregnant women in the first trimester of pregnancy who have been exposed to a known case of rubella and for whom termination of pregnancy is not an option. Nevertheless, cases of congenital rubella syndrome have occurred in infants born to mothers who received immunoglobulins shortly after exposure. No adequate treatment is available for pregnant women exposed to rubella.

Vaccines. Live rubella vaccine contains live, cold-adapted RA-27/3 vaccine strain grown in human diploid cells. The virus is usually administered as measles, mumps, and rubella vaccine (MMR vaccine). The vaccine is very effective and induces long-lasting humoral and cellular immunity. It causes few side effects, such as transient arthralgia in some women. The vaccine also induces production of respiratory IgA, thereby interrupting the spread of virus by nasal droplets. The use of vaccine has resulted in significantly reduced incidence of rubella and congenital rubella syndrome worldwide.

- This vaccine is usually given subcutaneously to children 15 months of age.
- It is also recommended for unimmunized young adult women if they are not pregnant and for those women who will not conceive for the next 3 months.
- It is contraindicated for use in pregnant women and in immunocompromised patients.

Yellow Fever Virus

Yellow fever is a mosquito-borne acute febrile illness that occurs in Africa and South America. The disease is not reported in India. Yellow fever virus is the type-specific virus of the family Flaviviridae. It is a single-stranded, enveloped,

RNA virus. The envelope consists of a lipid bilayer containing an envelope glycoprotein and a matrix protein. The single RNA is complexed with a capsid protein. Yellow fever is transmitted by the bite of Ades mosquito. During blood meal, the mosquito deposits the saliva containing the virus into a bite wound. The virus replicates locally and in regional lymph nodes draining the wound. Subsequently, the virus spreads by blood to the bone marrow, liver, myocardium, spleen where further replication of virus occurs. The condition is associated with hemorrhagic manifestations occurring as a result of disseminated intravascular coagulation. This occurs due to bleeding from the gastrointestinal mucosa and from abdominal and pleural serous layers. This is associated with reduction in the synthesis of coagulation factor and altered platelet function. The condition progresses to shock and finally death due to multiple organ failure involving liver, kidney, brain, and heart. Host immunity is characterized by the presence of viral neutralizing antibodies by the end of the first week during which the virus is rapidly cleared from the circulation. The role of immunity in the pathogenesis of the disease is not known. An attack of yellow fever confers a lifelong immunity. Incubation period is short and varies from 3 to 6 days. No prodromal symptoms occur. As the name suggests, yellow fever is characterized by jaundice and fever. This is an illness characterized by an acute onset of fever followed by jaundice within 2 weeks of onset of symptoms. Typically, symptoms begin suddenly with fever, chill, malaise, prostration, headache, giddiness, myalgia, anorexia, nausea, and vomiting. This may be associated with bleeding from the nose, gums, gastrointestinal tract, or skin that usually occurs within a few weeks of illness. Hemorrhagic diathesis progressing to disseminated intravascular coagulation is the most important complication of the disease. Multiple organ failure involving liver, kidney, and heart is responsible for death of the patient. Yellow fever is a severe and lifethreatening disease. The yellow fever is endemic in South America and in the Caribbean enzootic countries of Africa. Although yellow fever can be transmitted in Asian countries, till now no documented transmission of yellow fever has been reported from Asian countries including India. It has been suggested that previous infection with another flavivirus, such as dengue virus, may confer protection from yellow fever virus. This is the reason suggested for the absence of yellow fever in Asian countries including India. Yellow fever shows following transmission cycles:

Jungle (sylvatic) cycle: In this cycle, the mosquito transmits the virus to wild, nonhuman primates; from these hosts, it is transmitted to another mosquito. Humans are the incidental hosts in the cycle. This sylvatic cycle is present in rain forests, and the humans, such as men clearing the trees in the forest are bitten by the mosquito. *Aedes* species are the main vectors in Africa. Wild *Aedes* species are the vectors in South America.

Urban cycle: This cycle is confined to urban areas in which the mosquitoes transmit the virus to a human host and then it is transmitted to another mosquito. *A. aegypti*, a domestic mosquito, is the primary vector.

Intermediate cycle: This cycle is confined to Savanna forest area of Africa and is the most common cycle responsible for outbreaks of yellow fever in Africa. In this cycle, the mosquito transmits the virus to wild nonhuman primates and human hosts and then it is transmitted to another mosquito. The yellow fever is transmitted from infected humans to mosquitoes, which are diurnal feeders. Humans suffering from the disease during initial 3–4 weeks of illness are the source of infection. The extrinsic incubation period is 12–21 days. Vertical transmission of yellow fever takes place from female mosquitoes to the female progeny in 1% of cases. This is the important reason for the survival of the virus in dry season. Approximately, 3–10 variants are necessary to infect a mosquito. Seasonal transmission of yellow fever occurs during midrainy season and early dry season in Africa. In South America, this occurs from January to March. An estimated 200,000 cases of yellow fever occur in Africa and South America, causing 20,000–30,000 deaths annually. Laboratory diagnosis of yellow fever can be made by:

- Isolation of yellow fever virus from the clinical specimens.
- Demonstration of IgM-specific antibodies in the serum or demonstration of fourfold or more rise in serum IgG.
- Detection of yellow fever antigen in tissues by immuno histochemistry
- Detection of viral genomes by PCR, and
- Elevated transaminase and bilirubin levels, which are demonstrated during the toxic stage of illness.

Vaccination is the most widely used preventive measure against yellow fever (refer the box Vaccines). Other preventive measures include mosquito eradication program and personal protective measures. The latter include the use of proper clothings, insect repellants, etc., which avoid being bitten by mosquitoes and thereby exposure to yellow fever virus.

Vaccines. The 17 D strain of yellow fever virus is a widely used attenuated live virus vaccine. This avirulent 17 D strain vaccine was recovered during the serial passage of a pantropic strain of yellow fever virus through tissue culture. The strain that has lost its capacity to induce viscerotropic or neurotropic disease has been used as a vaccine for more than 40 years. The vaccine, which is prepared in eggs and is available as a dried powder, is maintained in cold chain for effective immunization. The vaccine is highly effective. A single dose of vaccine produces neutralizing antibodies – indicator of protection in more than 99% of vaccinated people by 30 days. The vaccine provides immunity mainly for 30–35 years and probably for whole life after a single dose. The vaccine is highly safe, associated with very few side effects. The vaccine is indicated for (*a*) all the individuals residing in countries endemic for yellow fever and (*b*) travelers visiting to the countries South America and Africa. The vaccine is contraindicated for (*a*) infants below 9 months, (*b*) pregnant mothers, and (*c*) individuals with immune deficiency diseases, such as HIV or persons receiving organ transplantation, etc.

Topic 3. MICROBIOLOGY OF VIRAL HEPATITIS

I. Home work

1) Reading and learning of "Textbook of Microbiology" by Prof. C.P. Baveja.

The questions for study:

1. Enteral viral hepatitis A, E: morphology of pathogens, especially the epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
2. Parenteral viral hepatitis b, C, D, G, TTV: etiology, epidemiology, pathogenesis, laboratory diagnosis, prevention.

2) Draw this table in your workbook before class and fill in all empty fields:

Epidemiology of viral hepatitis

Disease	Pathogen	Transmission	Source (vector)
hepatitis A			
hepatitis E			
viral hepatitis B			
viral hepatitis C			
viral hepatitis D			
viral hepatitis G			
TTV			

II. Class work

I) Entry test

Write answer the questions of the entry test in the separate piece of paper and give it to your teacher.

II) Theoretical part

The questions for discussing

1. Enteral viral hepatitis A, E: morphology of pathogens, especially the epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
2. Parenteral viral hepatitis B, D, C, G, TTV: etiology, epidemiology, pathogenesis, laboratory diagnosis, prevention.

THE THEORETICAL REFERENCE

Hepatitis is a clinical syndrome caused by many pathogens including viruses. There are six medically important viruses that are called hepatitis viruses because their main site of infection is liver. These viruses are hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), and newly described G virus (HGV). Although these viruses infect the liver as common target organ, they however, differ greatly in their morphology, replication pattern, and course of infection.

These viruses infect the liver and cause distinct clinical pathology by producing characteristic symptoms of jaundice and production and release of liver

enzymes in the serum. Most of these diseases spread very fast because infected individuals are contagious not only during stage of manifestation of the disease but also during the phase of incubation.

Hepatitis A virus

Hepatitis A virus (HAV) is a picornavirus that is most commonly transmitted by fecal–oral route. It has a relatively short incubation period of 3–4 weeks after which jaundice starts suddenly. It is unique in that it does not cause chronic disease or fatal disease.

Properties of the Virus

Morphology. Hepatitis A virus shows following morphological features:

- It is a typical enterovirus in the family Picornaviridae.
- It is a small, nonenveloped virus measuring 27 nm in diameter.
- It has a single-stranded positive-sense RNA genome. The HAV was originally designated as enterovirus, but it is recognized as a prototype of *Hepatovirus*. The naked capsid is more stable than other picornaviruses to acid and other treatment.

Viral replication. Hepatitis A virus replicates in the cytoplasm of the infected cell. It has a replicative cycle similar to that of other picornaviruses. Briefly, it combines specifically with a receptor expressed on liver cells and few other cells. However, unlike other picornaviruses, it is not cytolytic and is released by exocytosis.

Antigenic and genomic properties. There is only one serotype in HAV. The virus does not show any antigenic relationship with hepatitis B or other hepatitis viruses.

Other properties. Hepatitis A virus is highly resistant to environmental factors. It is stable at 60°C for 1 hour, 56°C for 30 minutes, and 4°C for weeks. It is stable to acidic pH, at pH 1. It is resistant to inactivation by lipid solvents, such as ether and chloroform, to action of detergents, and to drying. The virus is inactivated by formalin (0.35%) at 37°C during a period of 24 hours and by treatment with peracetic acid (2%) for 4 hours and beta-propiolactone (0.25%) for 1 hour. It is also inactivated by exposure to ultraviolet radiation (2 _W/cm2/minute). The virus is inactivated by routine chlorine treatment of drinking water.

Virus Isolation and Animal Susceptibility. Hepatitis A is the only virus that can be grown in human and simian cell culture. However, it is difficult to grow routinely from feces of infected patients.

Pathogenesis and Immunity

Hepatitis A virus infection is transmitted by the fecal–oral route and is associated with hepatic injury; however, the pathogenesis of HAV infection is not completely understood.

Pathogenesis of hepatitis A virus infection. The virus appears to replicate first in the gastrointestinal tract and then spreads to the liver. The viruses in the liver infect hepatocytes and cause damage to hepatocytes. But the mechanism by

which HAV causes cytopathic effect is not known. Cytotoxic T cells appear to cause damage to hepatocytes; hence once the infection is cleared, the cell damage is repaired and no chronic infection occurs. The classic findings in the hepatocytes include mononuclear infiltrate, ballooning, degeneration and acidophilic (*Councilman-like*) bodies. The liver pathology caused by HAV cannot be distinguished histologically from that caused by other hepatitis viruses.

Host immunity is mediated primarily by circulating antibodies. Acute infection is characterized by the appearance of IgM anti-HAV, which is detectable at the time of appearance of jaundice in the initial stage of infection. But the IgM antibody disappears several months after jaundice. The IgG antibody appears 1–3 weeks after appearance of IgM antibodies. IgG antibody appears to provide lifelong immunity against recurrent HAV infection.

Clinical Syndrome

Hepatitis A virus causes acute hepatitis A, the symptoms of which are similar to those caused by Hepatitis B virus (HBV) infection.

Acute hepatitis A. The incubation period of HAV is 15–45 days, with an average of 4 weeks. It is relatively short compared to long incubation period of HBV infection. Fatigue, nausea, vomiting, fever, hepatomegaly, jaundice, anorexia, and rash are the most common signs and symptoms of the disease. The condition is also associated with passing of dark-colored urine, pale feces, and elevated serum transaminase levels. Hepatitis A virus infection is usually a self-limiting mild disease and in most cases resolves spontaneously in 2–4 weeks. Hepatitis A virus infection confers lifelong immunity to HAV. Chronic hepatitis or chronic carrier state does not occur with HAV infection. Hepatitis A virus also never causes hepatocellular carcinoma. Acute hepatitis A is relatively more serious and has high mortality in adults than in children. The exact cause for this is unknown. Acute liver failure and cholestatic hepatitis are some of the rare complications. The mortality caused by HAV is very low, approximately 0.01%.

Epidemiology

Nearly two-thirds of acute cases of hepatitis are caused by HAV.

Geographical distribution. Hepatitis A virus infection is common in the developing countries of Asia, Africa, and Central and South America. High prevalence of HAV infection has been documented in the Middle East.

Reservoir, source, and transmission of infection. Humans are the reservoirs for HAV. Humans infected with HAV are the important source of infection. The virus is excreted in the stool during the first 2 weeks of infection, prior to the onset of symptoms; hence the quarantine of patients is not useful. The infected children and adults appear to be noninfectious after the appearance of jaundice. Contaminated food or water is the main source of infection. Wide outbreak can occur from a single contaminated source, such as uncooked vegetables, infected shellfish, and contaminated food and water.

Infection with HAV occurs throughout the world but is more common in developing countries, in the areas of low socioeconomic status and poor sanitation.

International travelers to areas endemic for HAV, drug addicts using injectable drugs, and homosexual men are at high risk for HAV infection. Close contacts of infected individuals are also at high risk. Infection is not transmitted from infected mother to neonates. Secondary infection occurs at a high rate in household contacts of acute HAV (20%).

Laboratory Diagnosis

Specimens. These include (*a*) serum for antibody detection test and (*b*) liver, bile, stool, blood for HAV antigen and genome.

Direct antigen detection. Hepatitis A virus is present in stools during 2 weeks prior to the onset of jaundice and up to 2 weeks after the onset of jaundice. The virus can be demonstrated in the stool during this period by using immunoelectron microscopy.

Isolation of the virus. Although the virus has been grown in human and simian cell culture, its isolation by culture of feces in the cell line is not routinely done, because the facilities for growing the virus are not widely available.

Serodiagnosis of HAV infection depends on the demonstration of specific antibodies in the serum. Serological tests demonstrating these anti-HAV antibodies in the serum are the most widely used to confirm the diagnosis of HAV infection. Enzyme-linked immunosorbent assay (ELISA) is the method of choice for detection of IgM and IgG antibodies in the serum. IgM antibody is the first antibody to appear at the onset of symptoms and continues to persist at a high level for 1–2 months. It usually disappears by 4–6 months but occasionally persists longer. Hence, demonstration of IgM antibody is diagnostic of a recent infection. IgG antibody appears in the serum shortly after the appearance of IgM antibodies and usually increases as the IgM level decreases. A fourfold rise in IgG antibody titers is also diagnostic of infection.

Molecular Diagnosis. DNA probes and polymerase chain reaction (PCR) are used to demonstrate HAV genome in stool as well as in the serum of infected patient.

Other tests. Liver function tests are highly useful for supplementing the diagnosis of HAV infection. Hepatitis A virus infection is associated with a consistent increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Increase in ALT and AST levels is nearly 4–100 times more than the normal levels. Increase in serum levels of ALT and AST are usually seen 1 week before. The ALT and AST remain at peak level within 3–10 days after the onset of clinical illness. Serum bilirubin level is also increased, and it increases with the appearance of jaundice. Decrease in serum albumin level and prolongation of prothrombin time indicate a severe hepatocellular infection.

Treatment, Prevention and Control

No antiviral therapy is available against HAV infection. Treatment of the condition is always supportive. Prevention of HAV infection depends on: (*a*) vaccines, (*b*) prophylaxis with immune serum globulin, and (*c*) measures to prevent feco-oral spread of infection.

Vaccines. Active immunization with vaccines containing formalininactivated HAV is available. The vaccine is effective and safe. It is given in two doses, an initial dose followed by a booster dose after 6–12 months. No booster dose is given. Vaccine is recommended for:

(a) Use in children over 2 years of age,
(b) Travelers to developing countries,
(c) Patients with chronic liver disease, and
(d**)** Homosexual men.

Another vaccine that contains inactivated HAV and recombinant vaccine is used to immunize against both HAV and HBV.

Immunoglobulin. Postexposure prophylaxis consists of administration of hepatitis A immunoglobulin to contacts within 2 weeks of exposure. The immunoglobulin is given in a dose of 0.02 mL administered intramuscularly in a single dose. Postexposure prophylaxis is recommended for household contact of infected patients and contact in child-care center during outbreaks.

Prevention of fecal–oral spread of infection. General prevention measures consist of hand washing, drinking of safe drinking water, and good personal hygiene and sanitation. All these contribute to prevention of fecal–oral spread of infection of HAV.

Hepatitis E virus

Hepatitis E virus (HEV) is the primary cause of enterically transmitted non-A non-B hepatitis virus (NANBH), most commonly seen in developing countries including India. The virus has many similarities with HAV. The virus was first observed during the electron microscopy of feces contaminated with enteric NANBH. Hepatitis E virus is currently classified in the family Calciviridae. It resembles calciviruses, such as Norwalk virus. However, the HEV genome is different from genome of other calciviruses, and analysis of the genome sequence suggests that it is more similar to rubella virus. Therefore, HEV still remains to be classified. It is a nonenveloped, spherical virus measuring 32–34 nm in diameter. The surface of the virion shows indentations and spikes. It is icosahedral. The virus contains a positive-sense singlestranded RNA, approximately 7.6 kb in size. The viral genome contains three open reading frames (ORFs). ORF1 is the largest, which codes for nonstructural protein responsible for viral replication. ORF2 encodes for the capsule. The function of ORF3 is not known. The virus is heat stable. The cloning of viral genome showed HEV to consist of only one serotype. In HEV infection, the IgM antibody titer appears first, which falls rapidly after infection, becoming virtually undetectable within 6 months. Anti-HEV IgG, however, persist longer for more than 6 months. The IgG antibody appears to offer protection against reinfection by HEV. Hepatitis E virus usually causes an acute, self-limiting disease similar to HAV. Earlier it was mistaken for HAV due to clinical and epidemiological similarity. Hepatitis E virus infection, now, has been recognized as a distinct clinical entity, different from the infection caused by HAV. Clinically, HEV infection differs from HAV infection by causing acute disease and

by the occurrence of symptoms much later than those of HAV disease. The incubation period of HEV infection varies from 2 to 9 weeks with an average of 35 days. Hepatitis E virus causes a serious infection in pregnant women. It causes fulminant disease in pregnant women, especially in last trimester of pregnancy and has a high fatality rate of 15–20%. Encephalopathy and disseminated intravascular coagulation are the important causes of death. The rate of fulminant hepatic failure in infected pregnant women is very high. Hepatitis E virus infection does not appear to cause chronic liver diseases. Infiltration of portal tract by lymphocytes and polymorphonuclear leukocytes, balloon hepatocytes, formation of acidophilic bodies, and intralobular necrosis of hepatocytes is the classic pathological finding of HEV infection. Hepatitis E virus is distributed worldwide. It is most commonly found in developing countries. The epidemics of HEV have been recorded in India, Pakistan, Nepal, China, Burma, North Africa, and Mexico. During 1986–1988, one such large outbreak was reported in north-east China affecting nearly 10,000 people. In India, the largest epidemic of HEV occurred in Delhi during the winter of 1955–1956, affecting more than 30,000 persons. Anti-HEV antibodies are observed in serum of as many as 60% of children in India below 5 years of age. Tropical climate, poor sanitation, and poor personal hygiene all contribute to the epidemic of the disease in developing countries. Hepatitis E virus is transmitted primarily by fecal–oral route due to fecal contamination of water in endemic areas. Fecal contaminated water is the important source of infection. The reservoir of HEV is unknown, but it may be transmitted by animals.

Treatment of HEV infection is mainly supportive. No vaccine is available now for prevention of HEV. Administration of immunoglobulin does not prevent development of clinical disease. Hepatitis E virus infections respond poorly to treatment with serum IgG.

Hepatitis B virus

Hepatitis B virus is a major cause of infectious hepatitis worldwide. It is a hepadnavirus, which shows restricted host range and limited tissue tropism. The virus usually causes chronic disease and is associated with hepatocellular carcinoma. The term serum hepatitis was used after an outbreak of hepatitis among American soldiers in 1942. The cause of outbreak was linked to yellow fever vaccine that was given to the soldiers which was contaminated by human serum. Blumberg and his colleagues in 1965 described the Australian antigen, which was later called hepatitis B surface antigen (HBsAg). DS Dane in 1970 was first to describe hepatitis B viral particle in human serum by electron microscopy.

Properties of the Virus

Morphology. Hepatitis B virus shows following features:

It is a small (3.2 kb), enveloped DNA virus.

The genome is a small, circular, partially double-stranded DNA.

It is partially double stranded, because its positive strand is incomplete. The complete negative strand possesses four genes: genes S, C, P, and X. The gene S codes for HBsAg and also for HBeAg (hepatitis Be antigen).

Although HBV is a DNA virus, the gene P codes for DNA polymerase that has reverse transcriptase activity. Gene X codes for the X protein that has transcription-regulating activity.

The virion is a double-walled, spherical structure and measures 42 nm in diameter. It was first demonstrated by Dane in 1970 and so is known as Dane particle. By electron microscopy three types of particles can be seen in the serum from patients with hepatitis B. These are (*i*) spherical particles measuring 22 nm in diameter, (*ii*) filamentous or tubular particles with a diameter of 22 nm and of varying length, and (*iii*) double-walled, spherical structures measuring 42 nm in diameter. The former two particles are antigenically identical and are known as *hepatitis B surface antigen,* or HBsAg. The latter particle is the complete hepatitis viral particle known as *Dane particle*.

The HBV consists of nucleocapsid which surrounds HBV DNA and DNA polymerase with reverse transcriptase and ribonuclease activity. The nucleocapsid also encloses a protein attached to genome, which is surrounded by hepatitis B core antigen (HBcAg). The envelope which encloses the virus consists of HBsAg and also HBeAg.

***HBsAg*:** This antigen consists of three glycoproteins, namely S, L, and M. These glycoproteins are all encoded by same gene. The S glycoprotein is the major component of HBsAg, which self-associates into 22-nm spherical particles that are released from the cells. The filamentous particles of HBsAg found in the serum contain large amount of S and only small amount of L and M glycoprotein, and other proteins and lipids. The glycoprotein L is essential for virion assembly and for formation of filamentous particles and retention of the structures in the cell.

Viral replication. Hepatitis B virus replication shows many unique features.

- First, the virus shows a well-defined tropism for replication in the liver.
- Second, although a DNA virus, it encodes reverse transcriptase and replicates though RNA intermediates. The virus replicates in the nucleus of the cell. The virus infects hepatocytes, the attachment of which is mediated by HBsAg glycoprotein. The infection is initiated by the binding of HBsAg to serum albumin and other serum proteins, which subsequently causes the virus to infect the liver. Inside hepatocytes, the partial DNA strand of the genome is converted to covalently closed circular double-stranded DNA. Later on, this genome is transported to the nucleus of the cell. The DNA acts as a template for all viral transcription including 3.5 kb pregenomic RNA. Subsequently, the genome is enclosed by virus capsular antigen. Within the core, both negative- and positive-strand DNA viruses are synthesized. Finally, the core buds from the membrane, acquiring HBsAgcontaining envelope and is released from the infected cell.

Antigenic and genomic properties. HBsAg is antigenically complex. The HBsAg glycoprotein contains a group-specific antigen termed *a* and type-specific antigens termed *d* or *y* and *w* or *r*. Combination of these antigens results in four major subtypes of HBV (*adw*, *adr*, *ayw* and *ayr*).

These subtypes show distinct geographic distributions:

- Subtype *ayw* is common in Asia, Middle East, and western and northern India.
- Subtype *adr* is common in South and East India and in Far East countries.
- Subtype *adw* is prevalent in Europe, Australia, and the Americas.
- Subtype *ayr* is very rare. A total of eight genotypic variants (genotypes A, B, C, D, E, F, G, and H) of HBV have also been described. The prevalence of different genotypes varies in different countries. The infection caused by the genotype C is associated with rapid progression of the disease and poor response to antiviral treatment than caused by genotype D.

Other properties

Hepatitis B virus is an extremely resistant virus capable of withstanding extreme temperature and humidity. It is stable when stored at 20°C for 15 years, at 80°C for 24 months, at 44°C for 7 days, at 37°C for 60 minutes, and at room temperature for 6 months. HBV, however, is sensitive to higher temperature and is killed rapidly after heating at 100°C for 1 minute and at 60°C for 10 hours. HBsAg is a stable antigen. It is stable at pH 2.4 for up to 6 hours, but is associated with loss of infectivity of the virus. HBsAg is also destroyed by treatment with 0.5% sodium hypochlorite within 3 minutes. The HBsAg is, however, not destroyed by ultraviolet irradiation of plasma or other blood products, thereby retaining the infectivity of the virus.

Pathogenesis and Immunity

The pathogenesis and clinical manifestations of hepatitis B infection are primarily due to the interaction of the viruses and host immune system.

Pathogenesis of HBV infection. Hepatitis B virus, after entering the blood, infects the hepatocytes in the liver with the expression of viral antigen on the surface of infected cells. Cytotoxic T cells, such as activated CD4 and CD8 lymphocytes, recognize various HBV-derived proteins present on the surface of hepatocytes resulting in an immunological reaction.

1. The virus by itself does not cause any cytopathic effect in the infected liver cells.
2. The injury or cytopathic effects most probably occur as a result of cell-mediated injury.
3. The formation of antigen–antibody complexes is responsible for some of the symptoms, such as arthralgia, arthritis seen during early stage of the disease.
4. The immune complex is also responsible for some of the complications associated with chronic hepatitis, such as immune complex glomerulonephritis, cryoglobulinemia, and vasculitis.
5. A restricted T-cell-mediated lymphocyte response occurs against the HBV-infected hepatitis.

A chronic carrier stage with HBV infection is an important event in the pathogenesis of HBV infection. A person with chronic carrier stage has HBsAg persisting in the blood for at least 6 months. This stage is caused by a persistent infection of the hepatocytes that leads to the presence of HBV and HBsAg in the

blood. This chronic carrier stage occurs in about 5% of patients with HBV infection in contrast to no chronic carrier stage in patients with HAV infection.

arrier state or will be free of infection depends on the cytotoxic T-cell response. If the cytotoxic T-cell response is strong, the infection is cleared in the person but if the response is inadequate, the person becomes a chronic carrier. During the chronic stage, the HBV DNA is present in episome in the cytoplasm of persistently infected cells, and in some cells the viral DNA is integrated with cellular DNA. Chronic carrier state is more likely to occur when infection occurs in a newborn than in adult. It has been observed that approximately 90% of the infected neonates become chronic carriers. Approximately 20% of HBsAg carriers, nearly 1% of all adult patients infected with HBV, and high percentage of neonates infected with the virus progress to develop hepatocellular carcinoma or cirrhosis. The hepatocellular carcinoma appears to be the result of persistent cellular regeneration that tends to replace the dead hepatocytes. Also it is suggested that the integration of HBV DNA with hepatocytes DNA could activate a cellular oncogene, resulting in loss of control of the growth of hepatocytes. However, the HBV genome has no oncogene which can be responsible directly for causing hepatocellular carcinoma.

Host immunity. Hepatitis B virus natural infection induces a lifelong immunity. The immunity is primarily mediated by humoral antibodies against HBsAg. Antibodies to HBsAg are protective. These antibodies bind to surface antigens or with the virus and prevent it from interaction with receptors on the hepatocytes. These antibodies appear to neutralize the infectivity of HBV. But the antibodies against core antigen HBcAg are not protective because the antibodies cannot act with HBcAg present inside the cells.

Clinical Syndromes

Hepatitis B virus is one of the most important causes of acute and chronic hepatitis. The clinical manifestations vary from subclinical hepatitis to symptomatic and icteric hepatitis. The incubation period varies from 6 weeks to 6 months. The clinical manifestations of HBV infection depend on (*a*) age of infection, (*b*) immune status of the host, and (*c*) the level of HBV.

Acute hepatitis B virus infection. The prodromal or preicteric phase is characterized by gradual onset of anorexia, malaise, and fatigue. During the icteric phase, the liver becomes tender with development of jaundice. Nausea, vomiting and pruritus with passing of dark-colored urine, are the symptoms noted in this stage. Clinical manifestations of acute hepatitis B are similar to that of hepatitis A but with the difference that the symptoms tend to be more severe and life-threatening with HBV infection. The clinical disease associated with acute HBV infection may range from mild disease to a disease as severe as fulminant hepatitis occurring in less than 1% of the patients.

Chronic hepatitis B virus infection. Chronic HBV infection is one of the major complications of HBV infection. The risk of chronic infection is also higher

in those infected at birth (90%) and in patients who are immunocompromised. Only 5–10% of older children or adults progress to develop chronic infection.

Complications of Hepatitis B virus infection: Cirrhosis and hepatocellular carcinoma are the long-term but rare complications of hepatitis B. Perinatal transmission or infection in children is associated with few or no symptoms, but infection has a high risk of becoming chronic. Fulminant hepatic failure is another major complication of HBV infection. This condition occurs in approximately 0.5–1% of HBV-infected patients. The condition progresses to fulminant hepatic failure with coagulopathy, encephalopathy, and cerebral edema. The case fatality rate of these patients is very high nearing 80%. Patients with chronic HBV infection have a very high risk of developing hepatocellular carcinoma. The cancer appears to be due to repeated episodes of chronic inflammation and cellular regeneration. The cancer that develops an average of 25–30 years after initial infection is the leading cause of cancer-related deaths in areas where HBV is endemic. Glomerulonephritis, polyarteritis nodosa, varieties of skin manifestations, cardiopulmonary manifestations, and joint and neurologic manifestations are other important complications of HBV infection.

Epidemiology

Hepatitis B is a major cause of infectious hepatitis worldwide.

Geographical distribution. Hepatitis B virus is the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma worldwide. Nearly, onethird of the world population is believed to be infected with HBV. More than 10% of people living in sub-Saharan Africa and in East Asia are infected with HBV. Approximately, 5–10 lakh persons die annually from HBV-related liver disease. An estimated 250,000 persons die from chronic HBV infection every year in the world. Estimates suggest that 400–500 million people worldwide are HBV carriers. The HBV carrier rate varies from 1% to 20% worldwide. This variation is due to difference in the age of infection and in the mode of transmission.

Reservoir, source and transmission of infection. Individuals with chronic HBV infection are the major reservoir of HBV infections. These people with HBeAg in their serum tend to have high viral titers and thus greater infectivity. Hepatitis B virus is present at a high level in serum. The virions are also present at very low levels in semen, vaginal mucosa, saliva, and tears, and all are infectious. The virus is not detected in urine, stool, or sweat; hence these specimens are not infectious. The hepatitis B virus can be transmitted in the following ways:

Perinatal transmission: This is the major route of transmission of the virus worldwide. The transmission occurs from infected mother to child due to contact with mother's infected blood during the time of delivery as opposed to transplacental passage of the virus. Although HBV is found in breast milk, the role of breast-feeding in transmission is unclear.

Parenteral transmission: This transmission occurs due to transfusion of HBV-infected blood and blood products. This was one of the important modes of transmission before 1970s, but with the starting of screening of blood donors for

HBsAg, the rate of blood transfusion associated HBV infection has reduced considerably in India and other parts of the world. Patients with hemophilia, renal dialysis, and those receiving organ transplantation and intravenous drug users remain at increased risk of infection. The risk of acquiring HBV among health workers after needle stick injury from infected individuals is estimated to be as high as 5%.

Sexual transmission of HBV: Hepatitis B virus is transmitted sexually more easily than Hepatitis C virus (HCV) or Hepatitis D virus (HDV). The infection is associated with vaginal intercourse, genital rectal intercourse, and nongenital intercourse. However, the HBV is not transmitted by hugging and kissing or by sharing towels, eating utensils, or food. Health workers with exposure to infected blood or body fluids, heterosexual persons with multiple partners, household contact, or sexual partners of HBV carriers are other groups at risk.

Laboratory Diagnosis

Laboratory diagnosis plays an important role to confirm the HBV etiology of hepatitis.

Specimen. Serum is an important specimen because definitive diagnosis of HBV depends on serological testing for HBV infections.

Serodiagnosis. Diagnosis of acute infection is made by demonstration of HBsAg as well as HBeAg in the serum. Both HBsAg and HBeAg are the important serum markers of acute HBV. They indicate viral replication. When viral replication slows, HBeAg disappears and anti-HBeAg is detected. Hepatitis B surface antibody (HBsAb) produced may persist for many years. This is followed by demonstration of IgM antibodies against hepatitis B core antigen (HBcAb).

HBsAg. The antigen appears in blood during incubation period and is detectable in most patients during prodrome and acute phase of the disease.

Persistent presence of HBsAg in blood for at least 6 months indicates the carrier state and also indicates the risk of chronic hepatitis and hepatic carcinoma. It is not detectable in the serum during convalescent stage. The presence of HBsAg alone does not necessarily indicate replication of complete virion, and the patients may not have symptoms of liver damage.

HBsAb. HBsAb is a protective antibody that neutralizes the virus and is usually not detectable during the acute disease since it forms immune complex with HBsAg because it is bound to the large amount of HBsAg present in blood. It is also not detectable in the chronic carrier stage.

HBcAb. Demonstration of HBcAb is useful to confirm the diagnosis of HBV infection. Total HBcAb including IgM and IgG antibodies indicates exposure to the virus and viral replication. The HBcAb appears shortly after HBsAg in acute disease and persists for life. Detection of IgM HBcAb is diagnostic of acute HBV infection during window phase. The HBcAb are present in individuals with acute infection, chronic infections, and also in those who have recovered from acute infection. Therefore, the presence of HBcAb IgG does not differentiate between

acute and chronic infection. HBcAg is not detectable in the serum, but can be demonstrated in the liver cells by immunofluorescence.

HBeAg. HBeAg is present in the blood during the incubation and also during the prodrome and early acute disease. This is also present in certain chronic carriers. The presence of HBeAg indicates a high likelihood of infectivity and transmissibility. Chronic replication of HBV is characterized by the presence of circulating HBsAg, HBeAg usually with HBcAg. Both HBsAg and HBeAg are not present in serum during convalescence. Serum IgG antibodies to HBsAg, HBcAg, and HBeAg appear during the stage of convalescence. In essence, hepatitis B serology is useful to describe the course and nature of the disease. Acute and chronic HBV infection can be differentiated by the presence of HBsAg and HBeAg in the serum and distribution pattern of the antibodies to the indivi duals' HBV antigen. Interpretation of common serological markers in HBV infection is summarized in Table 4.

Table 4 – Interpretation of common serological markers in HBV infection

Serologic reactivity	Pre-symptoms	Early Acute	Acute	Chronic	Late acute	Resolved	Vacci-nated
Anti-HBc	-	-	-	+	+/-	+	-
Anti-HBe	-	-	-	-	+/-	+/-	-
Anti-HBs	-	-	-	-	-	+	+
HBeAg	-	+	+	+	-	-	-
HBsAg	+	+	+	+	+	-	-
Infectious virus	+	+	+	+	+	-	-

Molecular Diagnosis. Hepatitis B virus DNA and DNA polymerase activity are detectable during the incubation period and early in the disease.

HBV DNA levels are typically low or absent in inactive carriers.

HBV DNA levels are higher in patients with chronic hepatitis and are associated with increased infectivity.

The detection of viral DNA in the serum indicates acute infection. HBV PCR for demonstration of HBV DNA is highly valuable to monitor the treatment of chronic HBV infection with antiviral therapy. This is also useful to identify HBV as the cause of liver infection in HBsAg-negative patients.

Other tests. These tests include elevation of ALT and AST. High levels are found in acute hepatitis (1000–2000 IU/mL). Estimation of serum bilirubin indicates the intensity of jaundice.

Treatment

No specific antiviral treatment is available for patients with acute HBV infection. Supportive and symptomatic care continues to be the mainstay of therapy for most of the patients. Therapy is recommended for patients with chronic hepatitis B infection. Interferon and nucleoside analogs, such as lamivudine, adefovir, and telbivudine are the antiviral drugs used widely. These antiviral drugs

achieve viral suppression as demonstrated by loss of HBeAg in serum and suppression of HBV DNA.

Interferons: Interferon-alpha (IFN-α) has been the mainstay in treatment of chronic hepatitis B since its introduction in mid-1980s. Interferon acts by immunomodulation and prevents progression of acute hepatitis to chronic stage. It also promotes more rapid resolution of viremia and normalization of serum aminotransferase levels.

Nucleoside analogs: These block the replication of viruses by directly blocking the replication of HBV. These nucleoside analogs are highly effective against HBV, and are bioavailable and extremely well targeted. However, neither interferon nor nucleoside analogs cure HBV infection. The goal of antiviral therapy is only to reduce morbidity due to HBV and to prevent complications.

Prevention and Control

Hepatitis B infection can be prevented by the use of either vaccine or hyperimmunoglobulin or both.

Vaccines. Hepatitis B infection is one of the major diseases of humans that can be prevented with vaccination. Plasma-derived and recombinant DNA HBV vaccines are the two types of vaccines that use HBsAg to stimulate the production of anti-HBs in noninfected individuals. The vaccines are highly effective with more than 95% of seroconversion. The vaccine for adults is recommended at 0,1 and 6 months and for infants at the time of birth, at 1–2 months and at 6–18 months.

The vaccine is indicated for all infants and for all people who are at high risk of infection.

The high-risk group includes the people who are frequently exposed to blood and blood products, patients receiving multiple transfusion or dialysis, patients suffering from sexually transmitted disease, and intravenous drug users.

Hepatitis B immunoglobulin (HBIg) is used for passive immunization of patients after or just before the exposure. This immunoglobulin is derived from human plasma and contains high titer of HBsAb. It is prepared from plasma from patients who have recovered from hepatitis B infection. Passive immunization with HBIg is recommended for: people who have a history of recent exposure to patient(s) infected with HBV, a household contact with acutely infected patient, sexual contact with an acutely infected patient and infants born to HBsAg-positive mother.

Screening of blood and blood products for HBsAg is important to prevent transfusion-related HBsAg.

Hepatitis C virus

Hepatitis C virus is a flavivirus with an RNA genome and is the most important cause of parenteral non-A, non-B hepatitis (NANBH) worldwide. Prior to identification of the virus, it was known as NANBH to differentiate it from viral

causes of nonalcoholic hepatitis. Most patients infected with HCV have chronic liver disease, which progresses to cirrhosis and hepatocellular carcinoma.

Properties of the Virus

Morphology. Hepatitis C virus is the only member of the genus *Hepacivirus* in the family Flaviviridae of RNA-containing viruses. HCV appears to be closely related to hepatitis D and dengue and yellow fever virus. It resembles flavivirus in structure and organization, and hence has been classified as a new genus *Hepacivirus* in the family Flaviviridae. Hepatitis C virus shows following morphological feartures:

It is a spherical, enveloped, 9.4 kb, single-stranded RNA virus with a diameter of 55 nm.

The genome is approximately 9500 base pairs that encode 10 structural and regulatory proteins. Structural proteins include the core and two envelope proteins, namely, E1 and E2. These two envelope proteins undergo variation during infection due to hypervariable regions within their genes.

The viruses are ether sensitive and acid sensitive.

Viral replication. These viruses like other flaviviruses replicate in the endoplasmic reticulum of hepatocytes.

Antigenic and genomic properties

Hepatitis C virus shows a considerable degree of genomic variations. There are six major genotypes (genotypes 1–6) and numerous subtypes which differ in their worldwide distribution. Molecular differences between these genotypes are relatively large with as little as 55% genetic sequence homology and more than 80 subtypes are described. This genetic variability is the main stumbling block against the effort to develop an anti-HCV vaccine.

Genotype 1 is the main HCV genotype prevalent worldwide and accounts for 40–80% of all isolates. It is associated with more severe liver diseases and high risk of hepatocellular carcinoma. HCV genotype 1, particularly 1b as well as genotypes 2 and 3, are usually less responsive to interferon therapy than other HCV genotypes.

Genotypes 2 and 3 are also found globally, but to a lesser extent.

Pathogenesis and Immunity

The ability of HCV to remain cell associated and prevent host cell death is the main determinant of viral pathogenicity, which causes persistent infection in the liver. Presence of closely related but heterogeneous population of virus genome is one of the important factors responsible for persistence of HCV infection in the liver.

Pathogenesis of hepatitis C virus infections. Hepatocytes and possibly B lymphocytes are the natural targets of HCV. Results of recent studies show that at least 50% of hepatocytes may be infected with HCV in patients with chronic hepatitis. In most infected people, viremia persists and is associated with a variable degree of hepatic inflammation and fibrosis. Chronics hepatitis is characterized by lymphocyte infiltration either within the portal tract or in the liver lobule and portal

and periportal fibrosis. Portal inflammation, interphase hepatitis, and lobular necrosis are the main histopathological features of chronic hepatitis caused by HCV.

Host immunity. Immunity to HCV may not be lifelong, and serum antibodies to HCV are usually protective. Cell-mediated immunity, mainly cytotoxic T lymphocytes, contributes primarily to liver inflammation and ultimately to tissue necrosis.

Clinical Syndromes

Hepatitis C virus can cause: (*a*) acute HCV infection, (*b*) chronic HCV infection, and (*c*) cirrhosis and other complications induced by hepatitis. The incubation period of hepatitis C varies from 15 to 60 days with an average period of approximately 8 weeks.

Acute HCV infection. Most patients with acute HCV infections are symptomatic and do not develop any jaundice. The symptoms of acute HCV infection tend to be mild and may appear similar to those of HBV infections. In symptomatic cases, jaundice occurs in less than 25% of acutely infected patients, whereas hepatomegaly is seen in one-third of cases. But most of the cases (80%) are asymptomatic and do not develop any jaundice.

Chronic HCV infection. Hepatitis C virus is a major cause of chronic hepatitis worldwide. Most patients with chronic hepatitis are asymptomatic and may have nonspecific symptoms, such as fatigue or malaise in the absence of hepatic synthesis dysfunction.

Cirrhosis and other complications induced by hepatitis C. Hepatitis C virus is now a leading cause of hepatitis and cirrhosis. An estimated 20% of patients with chronic hepatitis progress to cirrhosis. This process may take an average of 20 years after initial infection worldwide. Patients with this condition have a secondary risk of liver failure, portal hyper tension, and other complications. Hepatocellular carcinoma is one of the most important complications in 1–5% of patients with underlying cirrhosis. This condition usually develops after 30 years in patients who are chronically infected and have cirrhosis. The increased total number of deaths due to HCV-related complications, such as cirrhosis and hepatocellular carcinoma, has been reported from many countries.

Epidemiology

Hepatitis C is prevalent worldwide.

Geographical distribution. More than 3% of world's population is infected with HCV. Worldwide 170 million people are estimated to be infected with HCV. It is the most important cause of parenteral NANBH worldwide. The prevalence rates are reported to be as high as 22% in Egypt due to use of parenteral antischistosomal therapy. The prevalence rates in healthy blood donors are also equally high. Hepatitis C virus is highly prevalent in central Europe, Middle East, Spain, Italy and Japan.

Reservoir, source and transmission of infection. Hepatitis C is exclusively a human disease. Patients who are infected with the virus are the important reservoir of infection. Blood or blood products and also organs of infected patients are the major sources of infection. Hepatitis C can be transmitted by following methods:

Blood transfusion: Blood transfusion is the most important route of transmission of HCV. The current risk of transfusion derived HCV is estimated to be one case in every 100,000 units transfused.

Parenteral transmission: HCV is transmitted parenterally (*a*) through transfusion of infected blood or blood products, (*b*) transplantation of organs from infected donors, and (*c*) also by sharing of contaminated needles among intravenous drug users. The use of intravenous drugs is most important risk factor responsible for around 50% of both acute and chronic infections.

Sexual transmission: Sexual transmission is believed to be responsible for approximately 20% of cases of hepatitis C. The presence of coexisting sexually transmitted disease, such as HIV, appears to increase the risk of transmission.

Perinatal transmission: Perinatal transmission is possible and is observed in fewer than 5% of children born to HCV-infected mothers. The risk of perinatal transmission of HCV is higher in children born to mothers who are coinfected with HCV and HIV.

Other methods of transmission: Hemodialysis, tattooing, body piercing, and acupuncture with unsterile equipments are other, but less frequent, means of transmission of HCV. Needle stick injury among healthcare workers who are exposed to infected blood accounts for nearly 4% of new infections. The possibility of acquiring HCV after needle stick injury involving an infected patient appears to range from 0% to 7%.

Laboratory Diagnosis

Laboratory diagnosis is most important to establish the specific diagnosis of hepatitis caused by HCV.

Serodiagnosis. Hepatitis C infection can be confirmed by employing serological tests to detect antibodies to HCV. Antibodies are directed against core envelope and NS3 and NS4 proteins and tend to be relatively low in titer. Acute HCV antibodies are usually demonstrated in acute infections 6–8 weeks after initial infection. Then antibodies that are produced persist throughout life in chronic infection. ELISAs, including second- and third-generation ELISAs, are useful for screening of serum for anti-HCV antibodies. These assays are highly specific but cannot differentiate acute infection from chronic infection. The ELISA which employs antibodies against core proteins and nonstructural proteins 3, 4, and 5 is the most recent third-generation enzyme immunoassay (EIA). The test can detect antibodies in the serum 8 weeks after the onset of infection. Recombinant immunoblot assay using recombinant HCV antigen is a highly specific test to detect HCV infection. This test, which is more specific than the ELISA, has been used to confirm positive ELISA results.

Molecular Diagnosis. PCR and branched DNA assays are being used to detect HCV RNA in the serum. HCV RNA testing is the most specific test for HCV infection and useful in diagnosing acute HCV infections before antibodies are developed. This is also helpful to (*a*) assess the HCV genotype, (*b*) to confirm false-positive cases, such as autoimmune hepatitis, and (*c*) to predict the response to interferon therapy. Hepatitis C virus genotyping is a recent method, which is frequently helpful for predicting the likelihood of response and duration of treatment. The genotyping is performed either by direct sequence analysis or restriction fragment length polymorphism.

Liver biopsy is the most accurate method to evaluate the extent of HCV-related liver disease. It is usually recommended for all patients before the start of antiviral therapy. However, it is generally not used to diagnose HCV.

Treatment, Prevention and Control

A combination therapy of pegylated interferon and antiviral agent ribavirin is the current option of treatment for patients with chronic HCV infections. Other therapeutic options include the use of protease inhibitors, ribozymes, and viral vaccines.

No vaccine against HCV is available. Immunoglobulin is not useful in preventing transmission and, in fact, administration of immunoglobulin has been associated with HCV. Transmission of HCV can be prevented by screening and preventing donation of blood, organs, or semen from HCV-positive donors.

Hepatitis D virus

Hepatitis D virus is the smallest of known human pathogens that causes infections in humans. It is an RNA virus, which is structurally unrelated to hepatitis A, B, or C virus. Hepatitis D virus is unique in being an incomplete virus and requires the presence of HBV to replicate and infect other hepatocytes. Hence, HDV infection occurs only in those patients who suffer from HBV infection. Hepatitis D virus was first reported by Rizzetho and colleagues in Italy in 1977. They demonstrated the viral antigen in nuclei of hepatocytes of patients infected with HBV and suggested it to be a new hepatotropic virus delta, or HDV. Hepatitis D virus is a spherical, enveloped virus measuring 85 nm in diameter. It contains a single-stranded negative-sense 1.7 kb RNA. In blood, HDV or delta agent contains delta Ag (HDAg) surrounded by HBsAg envelope. HBsAg is required for HDV replication, but it may be suppressed to undetectable levels with active HDV replication. The single-stranded RNA is circular and is surrounded by delta-antigen core, which in turn is surrounded by an envelope which contains HBsAg. Delta antigen may occur in two sizes: small (24 kDa) or large (26 kDa). Delta antigen is the only protein coded for HDV RNA, and it is distinct from antigenic determinants of HBV.

Patient may acquire HDV infection in two ways: by coinfection or superinfection.

Since both HDV and HBV are transmitted by the same route, a person can be coinfected with HBV and delta agent at the same time.

A patient with chronic HBV infection may also be superinfected with delta agent.

Hepatitis D virus causes a more rapid and severe disease with rapid progression in HBV carriers superinfected with delta and HBV. Delta agent replicates in the liver, causing liver damage and cytotoxicity. Chronic HBV carriers superinfected with HDV usually also develop chronic HDV infection. Chronic coinfection often leads to a rapidly progressive subacute or chronic hepatitis, resulting in more rapid progression to cirrhosis. Delta agent causes damage to liver cells as a result of direct cytopathic effect in combination with underlying immunopathology of HBV disease. Hepatitis D virus causes an acute and chronic inflammatory disease of liver. Although HDV can replicate independently within the hepatocytes, it requires HBsAg for its propagation. Death of hepatocytes in the liver may occur as a result of direct cytotoxic effect of HDV or through a host-mediated immune response. Hepatitis D virus is distributed worldwide. It is believed to infect approximately 15 million (5%) of world's 300 million HBsAg carriers. The highest prevalence of HDV has been reported in Italy, North Africa, Middle East, West Africa, and central Asia including China, Japan, Taiwan, and Myanmar. The infection is most common among adults and children. It is observed more commonly among patients with history of intravenous drug users. Hepatitis D virus, like hepatitis B virus, is a blood pathogen and is transmitted mostly by blood and vaginal secretions. It is most commonly transmitted by nonpercutaneous routes, especially by close intimate contact in endemic areas of Mediterranean countries. Infection appears to be more commonly transmitted through contaminated blood and blood products in nonendemic areas of northern Europe and North America. Sharing of contaminated needles in intravenous drug users is believed to be the most common method of transmitting HDV. The sexual and perinatal transmission of HDV is also described. Intravenous drug use and multiple blood transfusions are the important risk factors for parenteral transmission of the disease. The incubation period varies from 21 to 45 days but may be shorter in cases of superinfection. The clinical course of disease caused by HDV is varied and ranges from acute self-limited infection to acute fulminant liver failure. Clinically, HDV infection is indistinguishable from other forms of viral hepatitis. Patients coinfected with HBV and HDV show a more severe course of the disease than those infected with HBV alone. Complete clinical recovery and clearance of HBV and HDV coinfection is the most common outcome. Nearly 1% of the patients with coinfections progress to develop fulminant hepatitis resulting in more rapid progression to cirrhosis. Laboratory diagnosis of HDV infection is usually carried out by serological as well as molecular tests:

1. ELISA for HDV antigen is usually positive in 20% of patients.

2. IgM ELISA for demonstration of anti-HDV IgM is positive in early stage of infection, whereas IgG ELISA for anti-HDV IgG is positive during later course of infection.
3. Serum antibodies against HDAg are almost exclusively associated with chronic HDV infection.
4. Reverse transcriptase-PCR (RT-PCR) is the most sensitive method (90%) for detection of HDV RNA in blood in the stage of coinfection.
5. In superinfections, high level of both IgM and IgG antibodies as well as high level of HDAg and HDV RNA is demonstrated.

No specific therapy is available for treatment of HDV infection of liver. Lamivudine and ribavirin appear to be ineffective against HBV and HDV coinfection. Antiviral therapy with interferon is also ineffective in patients with chronic infections. Vaccination with HBV vaccine protects against subsequent HDV infection. HDV virus is prevented best in the patients already infected with HBV by avoiding the use of HDVcontaminated blood or blood products.

Hepatitis G virus

Hepatitis G virus (HGV) is similar to viruses of Flaviviridae family, which includes HCV. The flavivirus-like isolates were first demonstrated in Tamarin monkeys inoculated with blood from a surgeon with acute hepatitis in 1995. A similar virus was isolated from another patient during the same year. These viral isolates were designated GB viruses A, B, and C. A similar virus resembling GBVC (GB virus C) was isolated from a patient with chronic hepatitis in 1996. Now the virus has been designated as HGV. Hepatitis G virus is an RNA virus and its genome codes for 2900 amino acids. The virus shows 95% homology at the amino acid level with GB virus and GBVC, a previously described virus. HGV has 20% homology with HCV. Hepatitis G virus is a blood-borne virus, which is transmitted by transfusion of contaminated blood or blood products. HGV coinfection is observed in 6% of chronic HBV infection and in 10% of chronic HCV infection. Although HGV RNA has been demonstrated in patients with acute, chronic, and fulminant hepatitis, patients with multiple transfusions and hemodialysis, blood donors, and intravenous drug addicts, its role in the pathogenesis of hepatitis is yet to be elucidated. Therefore, whether or not HGV is actually a pathogen in humans, still remains to be clarified.

Topic 4. MICROBIOLOGY OF THE INTESTINAL VIRAL INFECTIONS

I. Home work

1) Reading and learning of “Textbook of Microbiology” by Prof. C.P. Baveja.
The questions for study:

1. Polio. Morphology of causative agent, epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
2. Enterovirus infection Coxsackie and ECHO. Morphology of causative agent, epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
3. Rotavirus infection. The morphology of pathogens, especially the epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.

2) Draw this table in your workbook before class and fill in all empty fields:

Epidemiology of the intestinal viral infections

Disease	Pathogen	Transmission	Source (vector)
Poliovirus			
Infection Coxsackie			
ECHO viruses			
Rotavirus infection			

II.Class work

I) Entry test

Write answer the questions of the entry test in the separate piece of paper and give it to your teacher.

II) Theoretical part.

The questions for discussing

1. Polio. Morphology of causative agent, epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
2. Enterovirus infection Coxsackie and echo. Morphology of causative agent, epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.
3. Rotavirus infection. The morphology of pathogens, especially the epidemiology, pathogenesis, laboratory diagnosis, specific therapy and prevention.

THE THEORETICAL REFERENCE

The family Picornaviridae is one of the largest families of viruses and includes a large number of very small (*pico*: measuring small; *rna: RNA* virus) RNA viruses. They are nonenveloped viruses measuring 27–30 nm in size. The capsid is a naked icosahedral made up of 60 protein subunits. The genome consists of a single linear molecule of single-stranded RNA. The genome RNA is unusual, because it has a protein at the 5_ end that serves as a primer for transcription by RNA polymerase.

Classification

The family Picornaviridae consists of more than 230 members and is divided into five genera: *Enterovirus*, *Rhinovirus*, *Hepatovirus*, *Arthrovirus*, and *Cardiovirus*. The first three genera (*Enterovirus*, *Rhinovirus*, and *Hepatovirus*) are

the important viruses, which cause disease in humans. Two other genera (*Arthrovirus* and *Cardiovirus*) are of veterinary importance and cause foot-and-mouth disease in cattle and meningoencephalomyelitis in mice.

Enteroviruses. Human enteroviruses consist of at least 72 serotypes, which include poliovirus types 1–3, coxsackieviruses A types 1–24, coxsackieviruses B types 1–6, echoviruses types 1–34 and enteroviruses 68–71. Originally hepatitis A virus (enterovirus 72) was included in this group but has been reclassified as a hepatovirus in the genus *Hepatovirus* . The capsids of the enteroviruses are very resistant to environmental conditions and the conditions in the gastrointestinal tracts. They can remain viable for several weeks in feces at room temperature. They also remain viable for a year at – 20 to – 70°C and for months at 4°C. The virus may survive heat at 60°C in food stuffs and also holder method of pasteurization. The enteroviruses are also rapidly inactivated by 0.3% formaldehyde, 0.1 M hydrochloric acid, drying, or ultraviolet light. The enteroviruses are readily killed by moist heat at 50–55°C, and they are also inactivated on exposure to ether, chloroform, and deoxycholate. A higher concentration of chlorine is necessary in water to inactivate the virus in the presence of organic matter, because the latter diminishes the activities of residual chlorine. Most enteroviruses are host specific; they infect only one or a few related species.

Poliovirus

Poliomyelitis is an enteric infection caused by polioviruses transmitted by the fecal–oral route. The worldwide prevalence of poliomyelitis has decreased by more than 99% due to improved socioeconomic conditions and availability of vaccines.

Properties of the Virus

Morphology. Polioviruses show the following features:

- Poliovirus was the first animal virus to be purified and obtained in crystalline form.
- The viruses are spherical particles about 27 nm in diameter
- The virion is composed of 60 subunits, each consisting of four viral proteins (VP1–VP4).
- The viral protein VP1 contains the major antigenic site for combination with type-specific neutralizing antibodies.
- The viral genome is a single-stranded positive-sense RNA, which can be directly translated by host ribosomes to form a polyprotein, which is divided into 11 different proteins.

Viral replication. Polioviruses are cytolytic; they replicate in cytoplasm of the host cell. Polioviruses show high host specificity restricted to primates, which include both humans and nonhuman primates, such as monkeys and apes. This is due to presence of a specific receptor that is present only on primate cell membrane. However, purified viral RNA without the capsid protein can enter and replicate in many nonprimate cells by avoiding the cell membrane receptors. The

virion causes infection first by binding to the specific receptor on the cell membrane and enters the cell. Once inside the cell, the virion uncoats by removing the capsid and releases the RNA genome. The RNA serves as the mRNA and is translated into a very large polypeptide known as noncapsid viral protein. Subsequently, the viral protein is utilized by the viral enzyme protease to form capsid proteins of the progeny virions as well as several noncapsid proteins including the RNA polymerase. RNA polymerase initiates the synthesis of progeny RNA genomes. This is followed by assembly of the progeny virions by coating of the genome RNA with capsid proteins in the cell cytoplasm. The release of progeny virions occurs by the lysis and death of the cells, but not by budding from the cell membrane.

Antigenic properties. The polioviruses have been classified into three serotypes (types 1, 2, and 3) on the basis of neutralization test. The p rototype strains for type 1 poliovirus are Brunhilde and Mahoney strains, for type 2 poliovirus are Lansing and MEFI strains, and for type 3 poliovirus are Leon and Saukett strains. Type 1 is the most common poliovirus and causes most epidemics of poliomyelitis; type 2 strain is usually associated with endemic infections, whereas type 3 strains occasionally cause epidemics. Two antigens, C (coreless or capsid) and D (dense) are recognized by enzyme-linked immunosorbent assay or precipitation test. The C antigen, also known as heated or H antigen is associated with the empty, noninfectious virus. It is less specific and reacts with heterotypic sera. The D antigen, also known as the nature or N antigen is associated with the whole virion. It is more specific and is type specific. Antibodies against D antigen are protective; hence the potency of injectable polio vaccine is measured in terms of D antigen unit.

Other properties. Poliovirus is sensitive to heat and is readily inactivated at 55°C for 30 minutes. It is also sensitive to formaldehyde and oxidizing disinfectants. The viruses are readily destroyed by chlorination in water, but the presence of organic matter delays inactivation. Polioviruses are resistant to lipid-soluble agents (ether, chloroform and bile), proteolytic enzymes of the intestine, and detergents. They survive in feces for months at 4°C and for years at – 20°C. They survive for days to several weeks in feces at room temperature depending on the environmental conditions, such as temperature, moisture, acidity, etc. Polioviruses are killed by lyophilization.

Virus Isolation and Animal Susceptibility

Cell culture. Polioviruses grow readily in cell lines of primate origin. They can be cultured on monkey kidney cell lines, human amnion, HeLa, Hep-2, MRC-5, and other cell lines. The cytopathic effects consist of cell retraction, increased refractivity, cytoplasmic granularity, and nuclear pyknosis and are observed within 48 hours of inoculation. Eosinophilic intranuclear inclusion bodies may be demonstrated in stained preparation of the infected cell lines. Well-formed plaques develop in infected monolayers with agar overlay.

Laboratory animals. Cynomolgus and rhesus monkeys are commonly used animals. They can be experimentally infected by intracerebral or intraspinal inoculation. Cynomolgus monkeys and chimpanzees can also be infected orally. Laboratory-maintained strains may grow in rodents and chick embryos. But fresh isolates of poliovirus cannot grow in these hosts.

Pathogenesis and Immunity

Poliovirus is transmitted by the fecal oral route on ingestion of contaminated water. The viral particles initially multiply in the nasopharynx and the gastrointestinal mucosa. The virions are resistant to acidity of stomach and to lytic activities of the protease and other enzymes of the intestinal tract and bile.

Pathogenesis of poliomyelitis. On entering the body, the virus infects and multiplies in the tonsils and Peyer's patch of the ileum. It then spreads to regional lymph nodes and enters the blood, causing a primary viremia. On continued infection and multiplication of virus in the reticuloendothelial cells, it invades the blood stream again and causes secondary viremia. During this period of viremia, the poliovirus crosses the blood–brain barrier or gains access to the brain by infecting skeletal muscle and traveling up on the nerves to the brain as in rabies virus. Poliovirus shows tissue tropism by specifically combining with neural cells. It recognizes a receptor present on the anterior horn cells of (*a*) the spinal cord, (*b*) dorsal root ganglia, and (*c*) motor neurons. On combination at these sites, the poliovirus causes destruction of the motor neurons, anterior horn, and brain stem. The destruction of motor neurons leads to paralysis of the muscles innervated by those neurons. Paralysis is not caused by the virus infecting the muscles. The poliovirus also infects the brain stem, causing bulbar poliomyelitis associated with respiratory paralysis. Poliovirus causing the pathological changes in the central nervous system (CNS) is usually responsible for causing symptoms of poliomyelitis. Immune mechanisms do not play any role in pathogenesis of the disease.

Host immunity in poliomyelitis is mostly dependent on the humoral antibodies. Both the serum and secretory antibodies play an important role in conferring protection against poliomyelitis.

Serum IgM antibodies appear within a week of infection and persist for nearly 6 months. IgG antibodies, which develop subsequently, persist lifelong. The neutralizing antibodies in the blood protect against disease by the same serotype of poliovirus. These antibodies, however, do not prevent infection of the intestinal neuron and excretion of virus in the feces.

Secretary IgA antibodies provide mucosal immunity against the virus (*a*) by preventing intestinal infections and (*b*) by preventing shedding of virus. The IgA antibody in the breast milk protects the infants from infection. Therefore, the poliomyelitis tends to be more severe and shedding of virus is more prolonged in the infected human host with altered humoral immune response. Cell-mediated immunity plays little or very insignificant role in the immunity against poliovirus. Host immunity is type specific. However, there is a significant amount of

crossprotection between type 1 and 2 and type 2 and 3 polioviruses. There is little or no cross-protection between type 1 and 3 strains.

Clinical Syndromes

Poliovirus causes a wide spectrum of illness in unvaccinated people, such as (*a*) asymptomatic illness, (*b*) abortive poliomyelitis, (*c*) nonparalytic poliomyelitis, (*d*) paralytic polio, and (*e*) postpoliomyelitis syndrome. Incubation period is usually 10 days but may vary from 4 days to 4 weeks.

Asymptomatic illness. Most patients (90–95%) infected with poliovirus develop inapparent infection and are asymptomatic. This asymptomatic illness is caused as a result of viral infection confined to the oropharynx and the intestine.

Abortive poliomyelitis is the minor illness occurring in approximately 5% of infected people. This is a febrile illness characterized by fever, headache, sore throat, loss of appetite, vomiting, and abdominal pain. Neurological symptoms are typically absent. The duration of this illness is usually less than 5 days.

Nonparalytic poliomyelitis also known as aseptic meningitis is caused by invasion of virus into the CNS. The symptoms of nonparalytic poliomyelitis are similar to those of abortive poliomyelitis but are more intense. Stiffness of the posterior muscles of the neck, trunk, and limbs is present in addition to the symptoms of the minor illness. This condition occurs in 1–2% of infected patients.

Paralytic polio. This is the major illness, which occurs in 0.1–2% of individuals infected with poliovirus. This is the most severe manifestation of poliomyelitis and appears 3–4 days after the abortive poliomyelitis has subsided. This condition is caused due to invasion of virus from blood to the anterior horn cells of the spinal cord and the motor cortex of the brain. Depending on the various tissues or organs affected and the intensity of neuronal infection, poliomyelitis may be of two types, as follows:

Paralytic poliomyelitis: Paralytic poliomyelitis or spinal paralysis is characterized by spinal paralysis involving one or more limbs. Asymmetrical flaccid paralysis with no sensory loss is the typical manifestation. This condition is caused mostly by poliovirus type 1. This condition is seen in some of the vaccinated individuals following vaccination. This occurs due to reversion of the attenuated vaccine virus types 2 and 3 to virulence types. In this condition, there may be paralysis of only one limb, such as one leg or there may be complete flaccid paralysis of both the legs and hands. The condition may progress to death or may recover completely with residual paralysis.

Bulbar poliomyelitis: This is caused due to involvement of the cranial nerves, most commonly 9th, 10th, and 12th. This condition tends to be more severe with involvement of the muscles of the pharynx, vocal cords, and respiration. The condition may cause death in 75% of the patients.

Postpoliomyelitis syndrome. This condition is sequelae of poliomyelitis, which may develop 20–40 years after infection with poliovirus. This condition is seen in 20–80% of patients who have recovered from poliomyelitis. Recurrence of

weakness or fatigue is observed in this condition, and it usually involves the muscles that were initially affected by the poliovirus.

Epidemiology

Poliovirus mainly affects children. However, individuals of any age may also develop the disease.

Geographical distribution. Polio continues to remain a major public health problem in developing countries. Six countries have been identified as endemic for poliomyelitis in the year 2003. These include Afghanistan, Egypt, Niger, Nigeria, Pakistan, and India. Significant progress has been made in reducing the prevalence of poliomyelitis in India. Efforts have been recently maximized to eliminate poliovirus infection in the very near future.

Reservoir, source, and transmission of infection. Natural infection occurs only in humans. Infected humans excreting poliovirus in their stool are the major reservoir of infection. Infected stool containing poliovirus is the major source. The viruses secreted in throat secretions during early stage of the illness may also be another source of infection. The poliovirus is transmitted: primarily by the fecal–oral route by ingestion of food and water contaminated with human feces, by inhalation or through fomites contaminated with respiratory secretions.

Poor sanitation, low socioeconomic status, and crowded living conditions facilitate transmission of infection. Immunocompromised patients, such as those with HIV infection, B cell dysfunction, and IgA deficiency are particularly at high risk of developing poliomyelitis when exposed to poliovirus.

Laboratory Diagnosis

Specimens. Stool, throat swab, and cerebrospinal fluid (CSF) are the specimens used for isolation of the viruses. Viruses can be isolated from feces for more than 30 days during the illness and from the throat swab during the first few days of the illness. The virus is isolated rarely from the CSF specimens.

Microscopy. Microscopy of the CSF shows a predominantly lymphocytic pleocytosis with the presence of 25–500 cells/mm3. The virus is rarely demonstrated in the CSF.

Isolation of the virus. Isolation of viruses from clinical specimen by tissue culture is the most specific method in the diagnosis of poliomyelitis. **Cell culture:** Virus isolation from feces and throat swab is carried out by cultivation on monkey kidney, human amnion, HeLa, Hep-2, Buffalo green monkey (BGM), MRC-5, and other cell cultures. The cytopathic effects produced by the virus are observed within 48 hours. These consist of cell retraction, increased refractivity, cytoplasmic granularity, and nuclear pyknosis. Identification of serotype is carried out by performing neutralization test. Differentiation of a wild virulent virus strain from that of an attenuated vaccine strain can be done by virulence test in the monkeys and by performing polymerase chain reaction (PCR). Isolation of poliovirus from feces does not indicate a diagnosis of poliomyelitis, as large numbers of asymptomatic illnesses are seen. Hence, isolation of poliomyelitis virus from feces needs to be interpreted carefully along with clinical presentation of the disease.

Serodiagnosis is based on demonstration of a fourfold increase in the antibody titer of the serum collected at the time of acute illness and the period of convalescence. Neutralization or complement fixation test is carried out to demonstrate antibodies. Neutralizing antibodies appear early and are present throughout life. However, serodiagnosis is less frequently used for diagnosis of poliomyelitis.

Molecular Diagnosis. PCR is a highly sensitive and specific test for diagnosis of poliomyelitis. The test, however, is not widely available in the developing countries.

Treatment, Prevention and Control

No antivirals are available for the treatment of poliomyelitis. Poliovirus vaccines are the key component in prevention of polio and have played an important role in the effort to eradicate polio worldwide.

Vaccines. Two types of vaccines used are as follows:

Inactivated polio vaccine: Inactivated polio vaccine or Salk vaccine was developed by Jonas Salk in 1956 for immunization against poliomyelitis. The vaccine contains formalininactivated strains of the three serotypes of the poliovirus grown in monkey kidney cell culture. The vaccine is given to children by deep subcutaneous or intramuscular injections in three doses. The vaccine is given at 2 months, 4 months, and at school entry, which is usually at 4 years. It does not cause development of vaccine-associated poliomyelitis (VAP). The injection of the vaccine elicits the production of higher IgG antibody titers in the serum, but it does not stimulate the production of detectable level of secretory IgA in the gut, hence does not prevent alimentary tract infection. The main advantage of this vaccine is that since it uses killed viruses, it can be safely used for immunization in immunocompromised hosts. The disadvantages of this vaccine are that (*a*) it is not as immunogenic as oral polio vaccine, (*b*) it does not induce local mucosal immunity in the gut, and (*c*) it needs to be administered parenterally, which shows poor compliance.

Live attenuated oral polio vaccine: Live attenuated oral polio vaccine or Sabin vaccine was developed by Albert Sabin in the year 1962. It contains live attenuated strains of the three serotypes of poliovirus cultured in monkey kidney cells or human diploid cells. The vaccine is given orally, which causes natural infection. It elicits production of both local secretory IgA antibodies in the pharynx and alimentary tract, and humoral IgG antibodies in the serum. The virus lacks the capability to multiply in the CNS, hence is non-neurovirulent. The vaccine virus is excreted for several weeks in the feces during which the vaccine virus spread may occur to close contacts, inducing or boosting immunity in them. The first dose of vaccine is given to infants at age of 1S months along with DPT. Second, third, and fourth doses of vaccine are given when children are raised to 2S, 3S, and 16–24 months, respectively. Oral polio vaccine has been used since the 1960s. This vaccine has been mainly responsible for significant decrease in prevalence of disease throughout the world. The advantages of this vaccine are many: first, it

induces local mucosal immunity; second, it provides appropriate herd immunity, and finally, it is cost-effective, especially in developing countries.

The major disadvantage of this vaccine is the loss of efficacy of the vaccine due to improper cold chain. Association of the vaccine with VAP is another major disadvantage. Although the virus content in this vaccine is attenuated, it may become neurotropic and may produce disease similar to wild type virus. The risk of VAP has been estimated at between 0.5 and 3.4 cases per million. Advantages and disadvantages of Sabin and Salk vaccines are summarized in Table 60-3. Recently, a new monovalent oral poliovirus type I vaccine (MOPVI) has been introduced. This is used to eliminate some of the last poliovirus reservoirs in the endemic countries. The vaccine is contraindicated in children who are immunocompromised and also in those whose caretakers are immunocompromised.

Coxsackie viruses

Coxsackie viruses are so named, because the viruses were first isolated in Coxsackie village in New York, United States, by Dandruff and Sickel in the year 1985. These viruses based on the pathological changes produced in suckling mice are classified into two groups: coxsackieviruses A and coxsackieviruses B.

The structure and morphology of the coxsackieviruses and the RNA genome are similar to those of poliovirus. But unlike poliovirus, they can infect mammals other than primates. Replication cycle is similar to that of poliovirus. Coxsackie A viruses consist of 24 serotypes and are associated with diseases involving vesicular lesions, such as herpangina. Coxsackie B viruses consist of six serotypes and are most frequently associated with myocarditis and pleurodynia. Coxsackie A viruses show tropism for skin and mucous membranes, whereas coxsackie B viruses show predilection for visceral organs, such as liver, heart, pancreas, and pleura. Both the viruses infect anterior horn cells of the motor neurons and meninges and cause paralysis. They replicate first in the oropharynx and gastrointestinal tract from where they spread by the blood circulation. Host immunity is IgG-antibody mediated and is type specific. Coxsackievirus infection occurs worldwide. Humans are the only natural hosts. The viruses are transmitted primarily by fecal–oral route.

Diseases caused by Coxsackie A

Diseases specifically caused by coxsackie A viruses include the following:

Herpangina: This condition is caused by coxsackie A virus serotypes 2, 4, 5, 6, 8, and 10. The infection is most commonly seen in children between 1 and 7 years. The symptoms include sudden onset of fever, sore throat, and difficulty in swallowing. The classical finding is a painful vesicular eruption of the oral mucosa around the soft palate and uvula. Less commonly, these lesions may be found on the hard palate. The condition is self-limiting. The viruses are isolated from the lesions or from the feces.

Hand-foot-and-mouth disease: This is a vesicular exanthema usually caused by coxsackievirus serotypes 5 and 16. This is mainly a disease of children,

seen most commonly in patients younger than 10 years. After an incubation period of 3–6 days, the infection begins with prodromal symptoms, such as fever, cough, sore throat, malaise, and anorexia. After 12–36 hours, vesicular eruptions appear on the hands, feet, and oral cavity. This is a self-limiting condition and the illness subsides in a few days.

Diseases Caused by Coxsackie B. Diseases specifically caused by coxsackie B viruses include the following:

Pleurodynia: This condition, also known as epidemic myalgia or Bornholm disease, is caused by coxsackie B virus serotypes 3 and 5. This condition is an acute illness, which manifests with a sudden onset of fever accompanied by muscular pain and pain in the chest and abdomen. The pain is spasmodic in nature. The condition usually lasts for 4 days but may relapse again after the condition has been asymptomatic for several days.

Myocarditis: This is a serious condition caused by coxsackie B virus, mostly in newborn infants. Shortness of breath, dull or sharp chest pain, and fever are the common manifestations of the condition. This is a life-threatening condition mostly in newborns. In adults, the condition presents with myocardial infection with fever. The condition is associated with a high morbidity.

Diseases Caused by Coxsackie A and B. Diseases caused by both coxsackie A and B viruses include aseptic meningitis.

Aseptic meningitis: The condition is caused by coxsackie A serotypes 2, 4, 7 and 9, and by coxsackie B viruses. The laboratory diagnosis of coxsackievirus infection is made by isolation of the virus from the feces or from lesions by intracerebral or intraperitoneal inoculation into a 1-day-old mouse. Identification of the virus is carried out by histopathology study of the infected mouse after sacrifice. Some of the coxsackieviruses can also be isolated by culture in human diploid embryonic lung fibroblasts, BGM, and HeLa cell lines. Cytopathic effects resemble those of polioviruses but develop more slowly, beginning as foci of rounded refractile cells, which die and fall off the glass.

ECHO viruses

ECHOviruses were originally isolated from the feces of an individual who had no clinical illness and caused a cytopathic effect in the cell culture. The prefix ECHO is an acronym for *e*nteric *c*ytopathogenic *h*uman *o*rphan viruses (ECHO viruses). They were earlier called “orphans”, because these viruses were not associated earlier with any disease but now they are known to cause a variety of human illnesses. The echoviruses resemble other enteroviruses in their properties. They are classified into 32 serotypes (1–34 except 10 and 28) on the basis of their type-specific neutralization capsid antigen. Some serotypes of echoviruses (types 3, 6, 7, 11, 12, 13, 19, 20, 21, 24, 29, 30 and 33) cause hemagglutination of human red cells. Agglutination of red cells is followed by elution, making the red cells inagglutinable by echoviruses or coxsackieviruses but not by myxoviruses. All echoviruses grow in human and monkey kidney cell lines, producing cytopathic effects. Humans are the natural hosts for these viruses. Echoviruses are found in

the intestinal tract of the infected humans. The infection like other enterovirus is transmitted by fecal–oral route.

Most of the echoviruses cause asymptomatic infections in humans, but some of them have been associated with many clinical syndromes. Nonspecific febrile illness associated with rash, headache, and common-cold-like symptoms is caused by many serotypes of echoviruses. Aseptic meningitis is also caused by echoviruses. Some strains of echovirus have been associated with gastroenteritis (serotype 8) and respiratory diseases in children (serotypes 1, 11, 19, 20 and 22). Laboratory diagnosis of echovirus infection is made by isolation of viruses from feces, throat swab, or CSF. They are cultured using human diploid embryonic lung fibroblast and human rhabdomyosarcoma cell lines. The growth of the virus is detected by cytopathic changes, which is similar to that of other coxsackieviruses.

Other Enteroviruses. Four new enteroviruses have been described that include enteroviruses 68, 69, 70, and 71. Of these, enterovirus 69 does not cause any human diseases, but rest of the three enteroviruses cause diseases in humans. Type 68 causes pneumonia and bronchitis, type 70 causes acute hemorrhagic conjunctivitis (AHC), and type 71 causes meningoencephalitis and paralysis.

Enterovirus 70. Enterovirus 70 is the causative agent of acute hemorrhagic conjunctivitis (AHC). The condition was first recognized in 1969 in Ghana and Indonesia. Subsequently, the condition spread widely, involving several parts of Japan, England, Europe, Africa, Middle East, and Southeast Asia including India. Coxsackievirus A 24 has also been known to cause the similar disease. AHC is a highly contagious ocular infection, which can cause large-scale epidemics. Transmission is facilitated by overcrowding and nonsanitary conditions. The infection is transmitted directly from finger or fomite to eye. The condition is most prevalent in adults between 20 and 50 years. The incubation period is about 24 hours, and the onset is abrupt. The most common symptoms include pain in the eyes, burning sensation, swelling of the eyelids, and foreign body sensation in the eye. Photophobia and watery discharge may also be found in some cases. The other eye becomes infected a few hours after infection of the first eye. Fever, malaise, and headache are the nonspecific symptoms. AHC is a self- limiting condition. The symptoms typically improve by second or third day of infection, and the recovery is complete within 7–10 days. Isolation of the virus is made by culturing in human diploid embryonic kidney and HeLa cell lines.

Topic 5. MICROBIOLOGY OF SLOW VIRUS INFECTIONS

I. Home work

1) Reading and learning of “Textbook of Microbiology” by Prof. C.P. Baveja.

The questions for study:

1. The definition of the term "Slow infection."

2. HIV infection: morphology of the pathogen, epidemiology, pathogenesis, laboratory diagnosis, specific prevention.
3. Rabies: morphology of the pathogen, epidemiology, pathogenesis, immunity, laboratory diagnostics, specific prophylaxis.
4. Subacute sclerosing panencephalitis: morphology of the causative agent, pathogenesis, laboratory diagnosis.
5. Diseases caused by prions (Kuru, a disease of Creutzfeldt-Jakob, etc.). Features of causative agents, pathogenesis, laboratory diagnosis.

2) Draw this tab le in your workbook before class and fill in all empty fields:

Epidemiology of slow viral infections

Disease	Pathogen	Transmission	Source (vector)
HIV infection			
Rabies			
Kuru			

II. Class work

I) Entry test

Write answer the questions of the entry test in the separate piece of paper and give it to your teacher.

II) Theoretical part

Answer the questions of teacher and demonstrate your knowledge of the theme.
The questions for discussing

1. The definition of the term "Slow infection."
2. HIV infection: morphology of the pathogen, epidemiology, pathogenesis, laboratory diagnosis, specific prevention.
3. Rabies: morphology of the pathogen, epidemiology, pathogenesis, immunity, laboratory diagnostics, specific prophylaxis.
4. Subacute sclerosing panencephalitis: morphology of the causative agent, pathogenesis, laboratory diagnosis.
5. Diseases caused by prions (Kuru, a disease of Creutzfeldt-Jakob, etc.). Features of causative agents, pathogenesis, laboratory diagnosis.

THE THEORETICAL REFERENCE

Rabies Virus

Rabies virus causes rabies, a viral infection of the central and peripheral nervous systems that causes encephalitis with or without paralysis. It is mostly fatal. Rabies virus is the most important member of the rhabdoviridae family, which causes disease in humans. It causes rabies, a recognized zoonotic disease worldwide. Rabies is the most fatal infection in humans. No specific antirabies agents are useful, once clinical signs or symptoms develop.

Classification

The family Rhabdoviridae is classified into two genera: *Lyssavirus* and *Vesiculovirus*. The genus *Lyssavirus* consists of more than 80 viruses and includes a rabies serogroup, which consists of 10 viruses including the classic rabies virus.

The rabies virus is the prototypical human Lyssavirus pathogen. Other viruses included in this group are Mokola virus, Duvenhage virus, Obodhiang virus, Kotonkan virus, Rochambeau virus, European bat Lyssavirus types 1 and 2, and Australian bat Lyssavirus. These viruses rarely cause human disease. The genus *Vesiculovirus* includes vesicular stomatitis virus and other viruses infecting vertebrates and invertebrates.

Properties of the Virus

Morphology. Rabies virus shows following features:

- It is a bullet-shaped virus with one end rounded or conical and the other end planar or concave.
- It is a negative-sense, nonsegmented, single-stranded RNA virus measuring approximately 60 _ 180 nm.
- It is composed of an internal protein core or nucleocapsid, which contains the nucleic acid. It also consists of an outer envelope, a lipid-containing bilayer covered with transmembrane glycoprotein spikes. The nucleocapsid shows helical symmetry, containing a linear negative-sense RNA with an RNA-dependent RNA transcriptase. The virus genome is unsegmented.

The genome encodes for production of five proteins associated with either the ribonucleoprotein (RNP) complex or the viral envelope. The L (transcriptase), N (nucleoprotein), and NS (transcriptase-associated) proteins comprise the RNP complex, together with the viral RNA. These proteins are aggregated together in the cytoplasm of virus-infected neurons and constitute Negri bodies, the characteristic histopathological finding of rabies virus infection. The M (matrix) and G (glycoprotein) proteins are associated with the lipid envelope. The G protein forms the protrusions that cover the outer surface of the virion envelope and is the only rabies virus protein known to induce virus-neutralizing antibody.

Viral replication. Rabies virus replicates in the cytoplasm. It causes infection by combining with the acetylcholine receptor of the cell surface. The virus then enters the cell, uncoats, and the viral RNA polymerase synthesizes five mRNAs that encode viral proteins. The virusencoded RNA polymerase facilitates the replication of the viral genomic RNA, which is followed by assembly of the genomic RNA with virion proteins to form the nucleocapsid. Finally, the virions bud from plasma membrane by budding through the cell membrane, during which they acquire their envelopes.

Antigenic and genomic properties. Rabies viruses of humans and animals are of single antigenic type. Rabies virus contains the following antigens:

G protein: The glycoprotein or G protein is present on the surface spikes present on the outer lipoprotein envelope of the virion. It mediates the attachment of virus to the acetylcholine receptors of neural tissues. The G protein is important in pathogenesis and virulence of the virus. It is strongly antigenic and elicits the production of neutralizing antibodies, which are protective. It also induces hemagglutination-inhibiting antibodies and stimulates cell-mediated immunity. G protein is a serotype-specific antigen.

N protein: Nucleoprotein or N protein is a group-specific antigen. It shows cross-reaction with some rabies-related viruses. It is antigenic and produces antibodies, which are not protective but are of diagnostic value. These antibodies can be demonstrated by serological tests, such as immunofluorescence (IF) test for the purpose of diagnosis.

Other antigens: These include membrane proteins, glycolipid, and RNA-dependent RNA polymerase.

Typing of strains. Rabies viruses may be typed as fixed or street viruses. The *fixed viruses* are the viruses that by several intracerebral passages in animals or cell culture undergo certain changes, such as they show a fixed incubation period of 6–7 days. The *street viruses* are those viruses that are isolated from natural human and animal infections and show a variable incubation period of 1–12 weeks. The monoclonal antibodies and genetic sequencing are now being used to differentiate and type street rabies viruses. Typing of street viruses has been found to be helpful in identifying viral variants originating in major host reservoirs throughout the world. This is also found to be useful to suggest the likely sources of human exposure when a history of definitive animal bite was otherwise missing from a patient's case history.

Street virus	Fixed virus
Virus is isolated from natural human or animal infection	Virus is isolated after several serial intracerebral passages in rabbits
Can cause fatal encephalitis in laboratory animals after a long incubation period of 1–12 weeks	Neurotropic but much less infective. Can cause fatal encephalitis in laboratory animals after a short and fixed incubation period of 6–7 days
Negri bodies can be demonstrated in the brain of infected animals	Negri bodies cannot be demonstrated in the brain of infected animals
Cannot be used for vaccine production	Can be used for vaccine production

Other properties. Rabies virus possesses hemagglutinating property. The virus agglutinates goose erythrocytes at 0–4°C and at pH 6.2. Rabies virus is sensitive to ethanol, iodine, soap, quaternary ammonium compounds, detergents, ether, chloroform, acetone, etc. It is inactivated by formalin, phenol, beta-propiolactone, ultraviolet radiation, and sunlight. It can be inactivated at 50°C for 1 hour or at 60°C for 5 minutes. It can also be inactivated on exposure to CO2. The virus is resistant to drying and cold. It survives at 4°C for weeks; hence viruses can be preserved by storing at – 70°C or by lyophilization.

Virus Isolation and Animal Susceptibility

Embryonated egg. The rabies virus grows in the yolk sac of the chick embryo. The rabies vaccine strains, such as Flury and Kelev strains, are grown in the yolk sac.

Cell culture. Rabies virus can grow in several primary and continuous cell cultures. These are WI-38, BHK (baby hamster kidney)-21, and chick embryo-related (CER) chick embryo fibroblast, and porcine, mouse, or hamster kidney cell lines. The fixed virus strains are adapted to grow in vero and human diploid cell lines. The cytopathic effect produced by the virus in the cell lines is minimal.

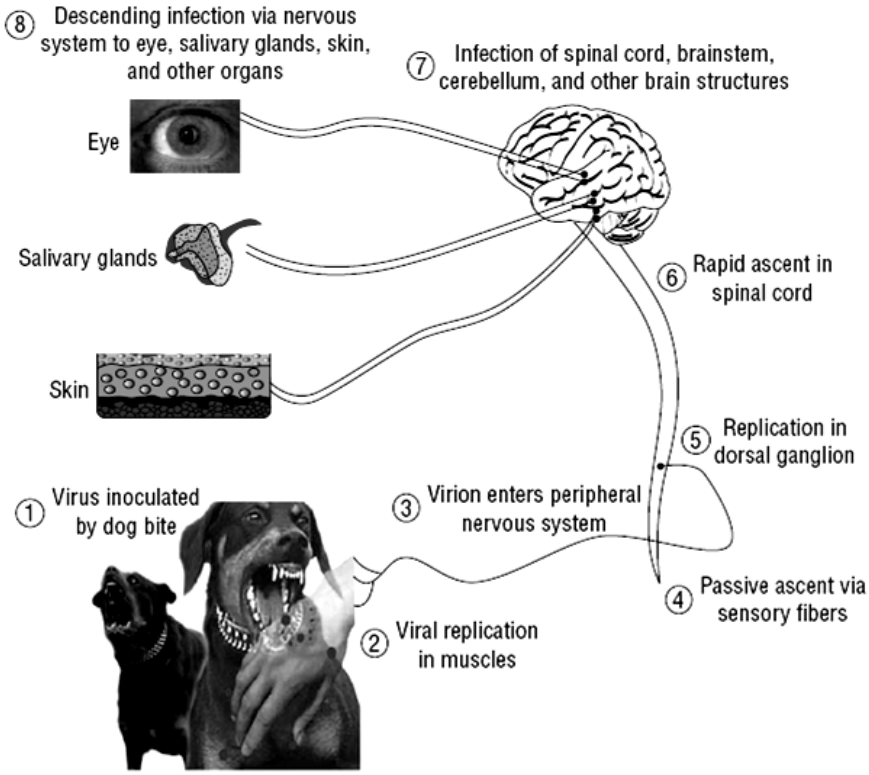
Laboratory animals. Mouse is the animal of choice. Intracerebral route is the frequently used route of infection. Intracerebral inoculation causes encephalitis, and the inoculated mouse die within 5–30 days.

Pathogenesis and Immunity

Rabies virus has a broad host range. The virus can infect all mammals, although certain mammals (such as dogs, foxes, wolves, and bats) are important for transmission of infection.

Pathogenesis of rabies. The virus may enter the peripheral nervous system directly at the site of bite. In some cases, however, it may replicate in muscle tissue after entering the host, remaining at or near the site of introduction for most of the incubation period. However, the precise sites of viral sequestration remain unknown, since neither the antigen nor the virus can usually be found in any organ during this phase. The virus infects the sensory neurons and moves rapidly by axonal transport centripetally to the central nervous system (CNS) for replication. During its transport within the neurons, it is protected from the host immune system. The virus travels along the axons at a rate of 12–24 mm in a day to enter the spinal ganglion. Its multiplication in the ganglion is indicated by the onset of pain or paresthesia at the site of the inoculum, which are the first clinical symptom and a hallmark finding. From here, the virus spreads quickly, at a rate of 200–400 mm/day, into the CNS, and the spread is marked by rapidly progressive encephalitis. Thereafter, the virus spreads to the periphery and salivary glands.

During the course of infection, encephalitis develops, associated with the death of neurons and demyelination. Acidophilic intracytoplasmic neuronal inclusion bodies are found in infected neurons, which is important for laboratory diagnosis.



Host immunity. Production of cytokines (such as interferon), induced during rabies virus infection or vaccination, has been reported to abort the disease if it occurs shortly after viral infection. Recently, it has been demonstrated that animals immunized with purified RNP complexes or recombinant nucleoprotein vaccines resisted lethal challenge with rabies virus. However, the role of N protein in protection, illness, or recovery is unclear.

Clinical Syndrome

Rabies virus causes rabies, the most fatal infection in humans. No specific antirabies agents are useful, once clinical signs or symptoms develop.

In general, four stages of rabies are recognized in humans. These are (*i*) incubation, (*ii*) prodromal period, (*iii*) acute neurologic period, and (*iv*) coma; which subsequently lead to death.

Incubation period: The average period of incubation is 20–90 days. Rarely, incubation lasts as long as 19 years. In more than 90% of cases, incubation is less than 1 year. During the incubation period, the virus travels from peripheral areas to the CNS. The patients remain asymptomatic during the period. The incubation period is less than 50 days if the patient is bitten on the head or neck or if a heavy inoculum is transferred through multiple bites, deep wounds, or large wounds. A person with a scratch on the hand may take longer to develop symptoms of rabies than a person who receives a bite on the head. The rabies virus is protected from the immune system during this period and no antibody response is observed.

Prodromal period: The virus enters the CNS during the prodromal period. The duration of this period is 2–10 days. The period is characterized by

nonspecific symptoms and sign Paresthesia or pain develops at the inoculation site and is pathognomonic for rabies. Paresthesia occurs in 50% of cases during this phase and may be the patient's only presenting sign. Other symptoms may include malaise, anorexia, headache, fever, chills, pharyngitis, nausea, emesis, diarrhea, anxiety, agitation, insomnia, and depression.

Acute neurologic period: This period is associated with objective signs of developing CNS disease. The duration is 2–7 days. Furthermore, it presents with the following conditions:

***Furious rabies*:** Patients develop agitation, hyperactivity, restlessness, thrashing, biting, confusion, or hallucinations. After several hours to days, this becomes episodic and interspersed with calm, cooperative, lucid periods. Furious episodes last for less than 5 minutes. Episodes may be triggered by visual, auditory, or tactile stimuli, or they may be spontaneous. Seizures may occur.

Hydrophobia and aerophobia are pathognomonic for rabies and occur in 50% of patients. Attempting to drink or having air blown in the face produces severe laryngeal or diaphragmatic spasms and a sensation of choking.

This may be related to a violent response of the airway irritant mechanisms. Even the suggestion of drinking may induce hydrophobic spasm.

Autonomic instability is observed, including fever, tachycardia, hypertension, hyperventilation, drooling, anisocoria, mydriasis, lacrimation, salivation, perspiration, and postural hypotension. Other neurologic signs include cranial nerve involvement with diplopia, facial palsy, and optic neuritis. This phase may either end in cardiorespiratory arrest or progress to paralysis.

***Paralytic rabies*:** It is also known as dumb rabies or apathetic rabies, because the patient is relatively quiet compared to a person with the furious form. Paralysis develops from the outset. Fever, headache, and nuchal rigidity are prominent. Paralysis is symmetric and may be either generalized or ascending and may be mistaken for Guillain–Barre syndrome. Calmness and clarity gradually deteriorates to delirium, stupor, and then coma.

Coma: The patient may go into coma within 10 days of onset; but duration is variable. Coma leads to respiratory failure within a week of neurologic symptoms. Hypoventilation and metabolic acidosis predominate. Acute respiratory distress syndrome is common. Without intensive supportive care, respiratory depression, arrest, and death occur shortly after coma. Most cases result in death within 14 days because of complications, despite intensive supportive care. Recovery is very unlikely. A few reports indicate that those patients who survived had pre-exposure or postexposure prophylaxis supported by most advanced life-support system.

Epidemiology

Rabies is a recognized zoonotic disease worldwide.

Geographical distribution. Rabies has been recognized for over 4000 years. Today it is found in most countries, except many Australian islands, Great Britain, Japan, Hawaii and most of the Caribbean islands. The risk of rabies is

highest in countries with hyperendemic canine rabies, including most of Asia, Africa, and Latin America. Rabies is endemic in India. It has been estimated that more than 30,000 people die of rabies in India every year. More than 70,000 people in India receive antirabies vaccination per year. With the dog population of over 16 million, the problem of rabies is huge. The virus is usually transmitted from a rabid animal to humans mostly by bites or other forms of traumatic contacts.

Reservoir, source, and transmission of infection. Rabies is a zoonotic disease. Dogs are the important reservoir of infection. Other animal reservoirs include silver-haired bats, eastern pipistrella, raccoons, skunks, foxes, or cats, ferrets, cattle, opossums, fowl, etc. Foxes are more infectious than dogs and other animals, because larger amounts of virus (up to 106 infectious doses/mL) are present in their saliva. The virus is excreted in saliva of infected dogs, foxes, wolves, jackals, vampire bats, raccoons, and skunks. The virus is found in the salivary gland of these infected animals. Infected saliva or infected CNS tissue, including corneal transplants in humans, are the sources of infection. The virus is transmitted to humans by following ways:

Bite of a rabid dog or other infected animals is the main route of transmission of infection. Contact of saliva with broken skin or with mucous membranes, exposure to aerosolized secretions from an infected animal, and contact with unpasteurized milk from dairies are very uncommon modes of transmission.

Corneal transplants: The only documented cases of rabies caused by human-to-human transmission occurred in eight recipients of transplanted corneas. Currently, donated corneas are not accepted if the donor died from encephalitis that may be consistent with rabies. In India, few such cases have also been documented. With the exception of corneal transplants, man-to-man infection is rare.

Laboratory Diagnosis in Humans

Specimens. Saliva, serum, cerebrospinal fluid (CSF), blood, urine, and skin and brain biopsy are the frequently used specimens for diagnosis of rabies.

Microscopy. Demonstration of Negri bodies by microscopy is the characteristic histopathological feature of rabies. Impression smears of the human brain tissue collected at postmortem are stained by Seller's technique for demonstration of Negri bodies. The stain contains methylene blue alcohol as fixative and basic fuchsin as staining reagent.

Negri bodies are demonstrated in 80% of human cases of rabies. Therefore, failure to demonstrate Negri bodies in neural tissue does not rule out the diagnosis of rabies.

Negri bodies are made up of a finely fibrillar matrix and rabies virus particles. They appear as intracytoplasmic, round or oval, purplish pink structures with characteristic basophilic inner granules on staining by Seller's technique. They measure 1–27 mkm in size. They are found in the neural tissues and are

found more in the cerebellum and hippocampus. They are not found in non-neural tissues, such as corneal specimen, saliva, or skin.

Direct Antigen Detection. Viral antigens can be demonstrated in the corneal smear, skin biopsy collected from the face or neck, and saliva (antemortem) or in the brain tissue (postmortem) for diagnosis of rabies. Direct fluorescent antibody (DFA) test using specific monoclonal antibodies is a specific method to demonstrate rabies antigen in clinical specimens.

Isolation of the virus. Viruses can be isolated from brain tissue, CSF, saliva, and urine by culture in cell lines or in animals.

Culture: Isolation of virus in cell lines (WI-38, BHK-21, and CER) is a sensitive method. The cytopathic effect produced by the virus is minimal; hence, the presence of virus in the inoculated cell cultures is made by demonstration of viral antigen within 2–4 days. The DFA test using monoclonal rabies antibodies tagged with fluorescein isothiocyanate is a frequently used specific method to demonstrate the antigen.

Animal inoculation: Mouse is the animal of choice. CSF, saliva, and urine are inoculated intracerebrally. The inoculated mice are observed for the signs of clinical illness. After death of the mice or after 28 days of inoculation, the brain tissues are examined for the presence of Negri bodies by microscopy or for viral antigen by the DFA test.

Serodiagnosis. Rabies antibodies are found in the serum as well as in the CSF of human cases. A high titer of antibodies is found in the CSF. Antibodies in the serum are demonstrated by a rapid fluorescent focus inhibition test titer in which results are positive in 50% of cases. In the CSF, positive antibody titers (2–25% of serum titer) are found after the first week of illness.

Molecular Diagnosis. Polymerase chain reaction is being increasingly evaluated for the diagnosis of rabies. The nucleic acid sequence-based amplification (NASBA) on saliva and CSF can be used for rapid diagnosis as early as 2 days after symptom onset. The NASBA technique on urine samples may be used in the future.

Other tests. WBC count ranges from normal to elevated, with 6–8% atypical monocytes. Albuminuria and sterile pyuria may be observed. Respiratory alkalosis due to hyperventilation develops in the prodromal and early acute neurologic phases, which is followed by respiratory acidosis as respiratory depression progresses. In the CSF, after the first week of illness, 80% monocytosis is observed. Protein and glucose test results are normal.

Antemortem diagnosis of human rabies

Until recently, most of the cases of rabies were fatal and patients used to die of the rabies. But with intensive support care and better management of patients, few cases have survived. Hence in such situations, laboratory methods for antemortem diagnosis of rabies in humans are proving to be more useful.

Corneal smears, skin biopsy from nape of the neck or face, and saliva are the usual specimens.

Diagnosis is made by demonstration of rabies virus antigen by IF or the viral DNA by molecular methods.

Nucleic acid sequence based amplification (NASBA) on saliva and CSF can be used for rapid diagnosis as early as 2 days after symptom onset.

Laboratory Diagnosis in Animals of rabies in dogs and other animals is useful to assess the risk of infection and to monitor postexposure prophylaxis in humans bitten by the animals.

Specimens. The brain of the dead animal is the specimen of choice. The brain is collected carefully from the dead animal. Part of the specimen is collected in 50% glycerol saline (preservative) for isolation of virus. The other part of the brain including the hippocampus and cerebellum (abundant in Negri bodies) is collected in Zenkers' fixative for demonstration of Negri bodies.

Microscopy. Impression smears of the brain tissue are stained by Seller's technique for demonstration of Negri bodies. This is still a useful method in the laboratories lacking facilities for cell culture and antigen detection methods.

Direct antigen detection. Demonstration of viral antigens in the infected brain tissue and in saliva of the animal by DFA is a useful method for diagnosis of rabies in animals. Detection of antigen in the saliva shows whether the animal was excreting the virus in the saliva or not.

Isolation of the virus. Viruses can be isolated from animal brain issue and saliva by culture in cell lines or in animals, as described earlier for diagnosis of human rabies.

Treatment, Prevention and Control

No specific antirabies agent is available. Although until recently rabies was considered to be invariably fatal, it has now been demonstrated that complete recovery can occur from established rabies with intensive supportive care and management of complications.

Specific prophylaxis in rabies, depending upon whether given before or after exposure can be discussed as pre-exposure prophylaxis and postexposure prophylaxis.

Pre-exposure prophylaxis is given to persons at high risk, such as dog handlers, other animal handlers, and veterinarians. This is achieved by use of cell culture vaccines, which are more safe. Preexposure prophylaxis requires three doses of cell culture vaccines, given on day 0, 7, 21, or on day 0, 28 and 56. A booster dose is recommended after 1 year, and then one dose every year. Animal control and vaccination strategies now have stronger roles than postexposure prophylaxis in preventing the spread of rabies.

Postexposure prophylaxis is started immediately after exposure to infection. After exposure to possibly infected dog or other rabid animals, immediate preventive actions are taken up, which consist of (*a*) local treatment, (*b*) confirmation whether or not the animal is rabid, (*c*) administration of hyperimmune serum, and (*d*) antirabies vaccine.

Local treatment: This involves prompt cleaning of the wound. Animal bites deposit the virus in the wounds. The wound should be immediately scrubbed with soap and water followed by treatment with quaternary ammonium compounds, such as Cetavlon, tincture, or aqueous solution of iodine or alcohol.

Confirmation whether or not the animal is rabid: This can be made by clinical observation of suspected dog. If dog is still healthy 10 days after biting human, rabies is extremely unlikely. Diagnosis can also be made by demonstration of the Negri bodies in brain tissues at autopsy or viral antigens in the brain tissue or saliva.

Administration of hyperimmune serum: Passive immunization is carried out by administering purified equine rabies immune globulin (ERIG) and human rabies immune globulin (HRIG). Administration of HRIG is promptly made to ensure passive immunization against rabies. The recommended dose of HRIG is 20 IU/kg body weight. Fifty percent of the dose is given into the wound and 50% intramuscularly. It is usually given before or simultaneously with the first dose of the rabies vaccine.

Antirabies vaccine: Rabies is the only disease where postexposure vaccination is employed extensively and successfully. This is due to long incubation period of the disease. The chances of preventing the rabies are more when vaccination is given to humans as early as possible after exposure. Since the time of Pasteur's successful use of a rabies vaccine obtained from desiccated preparation of spinal cord of rabbits in the year 1885, a wide variety of vaccines are being used worldwide to protect humans against rabies even after infection with the virus. Of late, neural-tissue vaccines are gradually being replaced by cell culture vaccines that are grown in human diploid cells and then inactivated with propiolactone (refer the box Vaccines).

Cell culture vaccines: These vaccines include human diploid cell vaccine, purified chick embryo cell vaccine, and purified vero cell vaccines. All these cell culture vaccines are equally effective and safe. The dosage schedule is same for children and adults. These are given intramuscularly or subcutaneously in the deltoid region in adults and on the anterolateral side of the thigh in children. Postexposure prophylaxis requires a course of five to six doses, starting as soon as possible.

These are given on days 0, 3, 7, 14, 30, and optionally 90.

The first dose on the day 0 is combined with an injection of human hyperimmunoglobulin (20 IU/kg). Vaccination with complete dosages gives virtually complete protection, for at least 5 years. During this period of 5 years, if any further exposure occurs, only one or two booster doses (on days 0, 3) may be given. After 5 years, a full course of five injections is given if the patient is again exposed to infection.

Immunoprophylaxis in rabies

(according to the World Health Organization Department of Communicable Diseases Surveillance and Response)

Category I

■ Touching, feeding of animals or licks on intact skin
■ No exposure
■ Therefore, no treatment if history reliable

Category II

■ Minor scratches or abrasions without bleeding or licks on broken skin
■ Nibbling of uncovered skin
■ Use vaccine alone

Category III

■ Single or multiple transdermal bites, scratches or contamination of mucous membrane with saliva (i.e., licks)
■ Use immunoglobulin plus vaccine
■ Use vaccine alone

Rabies-Related Viruses

The genus *Lyssavirus* consists of more than 80 viruses including the rabies virus, which is the prototypical human Lyssavirus pathogen. Other viruses included in this group rarely cause human disease.

Duvenhage Virus. The virus is classified as Lyssavirus serotype 4. The virus has been reported in bats from Southern Africa and many European countries. The first human case was reported in 1971 from South Africa, who died of clinical rabies after being bitten by a bat.

Mokola Virus. The virus is classified as Lyssavirus serotype 3. The virus has been isolated from many domestic and wild animals (shrew, cat, and dog) in Africa. It was first reported from shrews in Africa in the year 1968. Later, the virus has been isolated from animals in many European countries. The first report of human infection was from two children with CNS infection.

Lagos Bat Virus. The virus is classified as Lyssavirus serotype 2. The viruses have been reported from bats and cats in Nigeria and Central Africa. The virus was initially isolated in 1956 from the pooled brains of frugivorous bats from Lagos island, Nigeria. The virus causes a rabies-like illness following intracerebral inoculation in infected monkeys.

Other Rabies-Related Viruses, such as Rochambeau virus and Australian bat Lyssavirus have been reported to cause occasional rabies-like fatal disease in humans. Obodhiang and Kotonkan viruses have only been reported from mosquitoes in Sudan and Ibadan. No human infection by these viruses has been documented.

Human immunodeficiency virus (HIV)

Human immunodeficiency virus (HIV) is a retrovirus that causes acquired immunodeficiency syndrome (AIDS). AIDS is one of the most devastating epidemics ever recorded in the world. AIDS was first recognized in Los Angeles in

1981, when five cases of *Pneumocystis carinii* (now called *Pneumocystis jirovecii*) pneumonia in homosexual men and drug addicts were reported. The causative agent of AIDS was first reported by Luc Montagnier and colleagues from the Pasteur Institute, Paris, in 1983. They isolated a retrovirus from a West Asian patient with persistent generalized lymphadenopathy and named it *Lymphadenopathy-associated virus* (LAV). In 1984, Robert Gallo and colleagues from the National Institute of Health, USA, reported isolation of a retrovirus from patient with AIDS and called it *human T cell lymphotropic virus-III* (HTLV-III). The International Committee on Virus Nomenclature in 1986 gave the name *human immunodeficiency virus*, or HIV, for the same virus. HIV-1 is first isolated virus from the cases of AIDS, and HIV-2 has been isolated from some case of AIDS from West Africa.

HIV Virus Classification. HIV is a *Lentivirus*, a sub family of Lentiviridae in the family retrovirus. This family includes the viruses known for (*i*) poor host immune responses, (*ii*) latency, (*iii*) persistent viremia, and (*iv*) infection of the central nervous system. HIV, like other retroviruses, are enveloped RNA viruses, characteristically possessing an RNA-dependent DNA polymerase called reverse transcriptase.

Properties of the Virus

Morphology. HIV is a spherical, enveloped virus, which measures up to 120 nm in diameter (Fig. 68-1). It has a unique three-layered structure: (*i*) the innermost genome layer, (*ii*) middle coneshaped nucleocapsid, and (*iii*) an outer membrane of glycoprotein surrounded by lipoprotein envelope.

Viral genome: HIV genome is most complex of human retroviruses. The genome is diploid and consists of two identical copies of single-stranded positive-sense RNA genome. The HIV genome is most complex of human retroviruses. It contains three major genes *gag*, *pol*, and *env*, characteristic of all retroviruses. All these genes encode for the structural proteins. The *gag* gene encodes for internal capsid and matrix “core” proteins (p15, p18 and p24). Of these three proteins, p24 is the major antigen, which is demonstrated in serum of HIV patients during the early stage of infection and persists till the appearance of serum antibodies. Detection of p24 antigen in serum, therefore, is of diagnostic value. The *pol* gene encodes for several proteins, including the enzymes reverse transcriptase, integrase, and protease. The enzyme reverse transcriptase synthesizes DNA by using the genome RNA as a template. The enzyme integrase integrates the viral DNA into the cellular DNA, and the enzyme protease cleaves various viral precursor proteins. The *pol* gene expresses precursor protein p160, which is cleaved into three proteins: p31, p51, and p64. The *env* gene codes gp160, a precursor glycoprotein that is split to form two envelope glycoproteins, gp120 and gp41, which form the surface spikes and transmembrane tissue proteins, respectively. Apart from these genes, it also consists of six other regulatory genes (*tat*, *rev*, *nef*, *vif* and *vpr*) including an additional gene *vpu* in HIV-1 and *vpx* in HIV-2. Regulatory genes encode several proteins, which are essential for

transcription and invasion of virion into host cells. *tat* gene is the most important one, which encodes a protein called Tat protein that facilitates viral gene transcription. Tat protein along with other HIV-encoded regulatory protein called Nef suppresses the synthesis of class I MHC (major histocompatibility complex) proteins, thereby reducing the ability of cytotoxic T cells to kill the HIV-infected cells. The *rev* gene encodes another regulatory protein, which controls transport of mRNA from the nucleus into the cytoplasm.

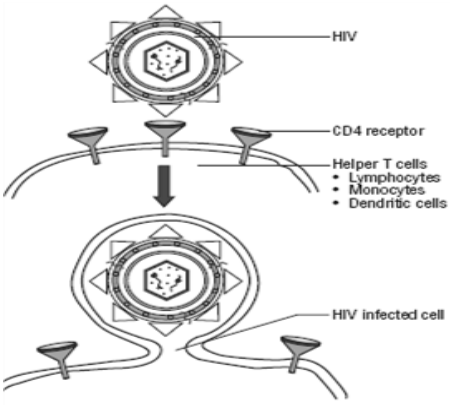
Nucleocapsid: The viral genome is surrounded by a nucleocapsid consisting of proteins. Three enzymes: (*i*) reverse transcriptase, (*ii*) integrase, and (*iii*) protease are located in the nucleocapsid.

1. *Reverse transcriptase* performs two important functions. First, it transcribes the RNA genome into the proviral genome. Second, it also has a ribonuclease H activity, which degrades the RNA when it is in the form of an RNA–DNA hybrid molecule. Degradation of the viral RNA genome is an essential step in the synthesis of double-stranded proviral DNA.

2. *Integrase* is other important enzyme, which facilitates integration of proviral DNA into the host cell DNA.

3. *Protease* is another enzyme, which splits precursor polyproteins into functional viral polypeptides.

Envelope: The virus is surrounded by a lipoprotein envelope. The lipid component is derived from the host cell membrane and glycoproteins, which are virus coded. The major virus coded envelope glycoproteins are the projecting spikes on the surface and the anchoring transmembrane pedicles. The projecting spikes combine with the CD4 receptors on susceptible host cells, and transmembrane pedicles cause cell fusion.



Invasion by HIV.

HIV genes and their products

Gene	Gene product	Description
gag	p24	Nucleocapsid core protein
	p15	Nucleocapsid core protein
	p55	Precursor of core protein
	p18	Polyprotein from *gag* gene
env	gp120	Outer envelope glycoprotein
	gp41	Transmembrane envelope glycoprotein
	gp160	Precursor of envelope glycoproteins
pol	p31	Reverse transcriptase
	p51	Reverse transcriptase
	p64	Reverse transcriptase

Viral replication. HIV replication is similar to that of other retroviruses. The virus binds to the CD4 proteins on the cell surface with the help of its gp120 envelope protein. This protein also interacts with chemokine receptors on the cell surface. Then gp41 of the virus mediates fusion of the viral envelope with the cell membrane followed by entry of the virus into the cell. CXCR4 and CCR5 are the

chemokine receptors, which are very much essential for entry of HIV into CD4_ cells. The T-cell-tropic strains of HIV bind to CXCR4 and the macrophage-tropic strains bind to CCR5. Once inside the cell, after uncoating, the virion RNA-dependent DNA polymerase transcribes the genome RNA into double-stranded DNA, which subsequently integrates with the host cell DNA. Integration is mediated by the viral enzyme integrase. The host cell RNA polymerase transcribes the viral mRNA from the proviral DNA. The viral mRNA encodes several proteins, which are cleaved by many enzymes. For example, the Gag protein is cleaved by the virus-encoded protease to form the main core protein (p24), the matrix protein (p17), and several smaller proteins. The Pol protein is also cleaved by protease to produce reverse transcriptase, integrase, and protease. The Env protein is cleaved by a cellular protease. The immature virions containing the precursor polyproteins are assembled in the cytoplasm, and cleavage by the viral protease occurs as the immature virion buds from the cell membrane, resulting in the production of mature infectious HIV.

Antigenic and genomic properties. HIV shows two distinct antigens: (*a*) group-specific antigen and (*b*) type-specific envelope glycoproteins.

Group-specific antigen: The protein p24 is the group-specific antigen present in the core of the virus. p24 is stable and does not show variations. Serum antibodies against p24 antigen are not protective and do not neutralize infectivity of HIV. But p24 is an important serological marker for diagnosis of HIV.

Type-specific envelope glycoproteins: gp120 and gp41 are the type-specific envelope glycoproteins, which are present on the surface of the virus. gp120, which protrudes from the surface, is the most important that combines with CD4 receptors as well as the chemokine receptors on surface of CD4 cells. It shows many antigenic variants due to mutations in the gene that encodes the antigen. V3 loop is the most immunogenic region of the gp120 and also shows significant antigenic variations. Antibodies against gp120 neutralize the infectivity, but the rapid emergence of gp120 variants does not confer protection against new strains; hence it is difficult to produce an effective vaccine. gp41, which is embedded in the envelope, mediates the fusion of the viral envelope with the cell membrane at the time of infection. HIV shows two distinct antigenic types: HIV-1 and HIV-2. The envelope antigens of both the types are different. Their core polypeptide shows some degree of cross-reactivity. HIV-2 is more closely related to simian immunodeficiency virus than to HIV-1. HIV-1 represents the original isolates from Americas, Europe, and other Western countries, whereas HIV-2 isolates are originated from Western Africa. HIV-2 is more closely related to simian immunodeficiency virus than to HIV-1. HIV-1 and HIV-2 strains based on sequence analysis of either *gag* or *env* genes have been classified into three groups: M (major/main), N (non-M, non-O/new), and O (other). The M group is the most prevalent group, which causes majority of HIV-1 infections. Group M consists of nine subtypes: A–D, F–H, J, and K; all of which have originated from Central

Africa and are prevalent all over the world. Groups N and O include only some HIV-1 isolates from Central Africa, which do not belong to the group M.

Other properties. HIV is a thermolabile virus. It is readily inactivated at 60°C in 10 minutes and at 100°C in seconds. The virus in dried blood, at room temperature (20–25°C), may survive for up to 7 days. The virus has been isolated from various tissues even up to 16 days at autopsy of the patient infected with HIV. HIV is inactivated by treatment with 50% ethanol, 35% isopropanol, 0.5% lysol, 0.5% formaldehyde, 0.3% hydrogen peroxide, and 10% bleaching powder in 10 minutes. Bleaching powder is an effective disinfectant for use as surface decontaminant at a concentration of 0.5%, with free chlorine 5 g/L (5000 ppm). A 2% solution of glutaraldehyde is effective for disinfection of medical instruments. In liquid plasma or in lyophilized blood products, HIV can be inactivated by heating at 56°C for 30 minutes. It is also inactivated at a very low pH (1) and high pH (13).

Virus Isolation

Cell culture. HIV can be cultured by cocultivation of lymphocytes with potentially infected and uninfected mononuclear cells to promote viral replication. Primary isolates of HIV grow very slowly on cell lines compared with laboratory-adopted strains. Virus growth is detected by testing the culture supernatant fluid to demonstrate p24 antigen or viral reverse transcriptase activity after incubation of the culture for an average period of 7–14 days or even larger (28 days).

Pathogenesis and Immunity

HIV is primarily a sexually transmitted pathogen transmitted by high-risk behaviors, such as unprotected intercourse, male homosexual intercourse, and also by intravenous (IV) drug abuse. The tropism of the HIV for CD4-expressing T-cells and macrophages is the principal determinant of the pathogenicity of HIV. HIV shows tropism for all the cells expressing CD4 antigens on their cell surfaces. The CD4 antigens act as receptors for HIV. The virus infects helper T cells and kills them, resulting in HIV-induced immunosuppression, leading to full-blown AIDS – a key feature of the pathogenesis of HIV infection. This makes the patient most susceptible to opportunistic infections and certain cancerous conditions, such as Kaposi’s sarcoma and lymphoma. However, the virus does not directly cause any tumor, because HIV genes are not found in these tumor cells.

Pathogenesis of HIV infection. In the genital tract, infection with HIV begins in Langerhans cells, the dendritic cells that line the mucosa. This is followed by infection of the local CD4+ helper T cells in the genital tract and by the appearance of the virus in the blood 4–11 days after infection. The CD4 receptors are present on CD4 T lymphocytes and also on the cells of the macrophage lineage, such as monocytes, macrophages, and alveolar macrophages of lungs, dendritic cells of the skin, and microglial cells of the brain. gp120 protein is the principal determinant of pathogenicity of HIV. The Vy region of the gp120 determines cellular tropism of the virus. After HIV enters the host, gp120 binds selectively to the CD4 cell surface receptors and CCR5 or CXCR4 chemokine

receptors expressed on macrophage lineage cells. After gp120 binds to the receptor, the associated gp41 protein initiates the cell membrane fusion. After fusion with the host cell membrane, the virus loses its envelope and reverse transcription of RNA to DNA occurs. The reverse transcriptase ribonuclease mediates transcription of the RNA into double-stranded DNA provirus. The provirus is integrated into the genome of the infected cell causing latent infection. The long and variable incubation period of HIV is because of this latency. During the period of latency, there is a high level of viral replication. It is estimated that 10 billion HIV particles are produced and destroyed each day. The gp120 present on the surface of the infected cell leads to fusion of the cells with the formation of multinucleated syncytia. The lysis of fused cells results in replenishment of a large number of uninfected cells from the circulation. HIV also causes accumulation of nonintegrated circular DNA copies of the genome, increased permeability of the plasma membrane, and induction of apoptosis. All these contribute to killing of the infected T cells. In addition, lysis of infected cell releases progeny virions to infect new cells. The CD4 cells are decreased in number, and the CD4:CD8 cell ratio is reversed. Viral infection also suppresses the function of the infected cell without causing structural damage. The virus replicates continuously in the lymph nodes, thereby releasing the virions and infected T cells into the blood. During the course of infection, the virus causes a drastic reduction in the number of CD4 T cells, which may occur due to HIV-induced cytolysis, cytotoxin T-cell immune cytolysis, or natural terminal differentiation of T cells. The virus also infects brain monocytes producing multinucleated giant cells and significant central nervous system manifestations. The fusion of the HIV-infected cells in the brain and other sites is the key pathological finding.

Host immunity. HIV is characterized by development of both cell-mediated and humoral immunities against HIV-related proteins.

Cell-mediated immunity: Cellular immunity is characterized by the development of cellular responses produced against HIV proteins. Suppression of cell-mediated T-cell immunity is the most profound consequence of HIV infection. CD4 helper T cell, monocytes, and macrophages are important components of CMI against HIV infection. The CD4 helper T cell plays a very important role in the outcome of immune responses. The CD4 helper cells are essential for (*a*) activation of macrophages and (*b*) induction of functions of cytotoxic T cells, natural killer cells and B cells, and a variety of soluble factors that stimulate growth and differentiation of lymphoid cells. HIV binds directly to the CD4 receptors of the T helper cells, resulting in gradual depletion of the T-cell population. The deficiency or reduction of CD4 T cells leads to depression of cellular immune response and impairment of humoral responses. The reduction of CD4 T cells is responsible for producing delayed-type hypersensitivity reaction that leads to opportunistic infections caused by many opportunistic pathogens. This causes CMI to gradually fail (*i*) to mount cytotoxic T-cell response to virally infected cells, (*ii*) to form delayed-type hypersensitivity reaction, and (*iii*) to

process new foreign substances presented to the immune system. Monocytes and macrophages also play an important role in the dissemination and pathogenesis of HIV infection. CCR5 chemokine receptors are the major HIV coreceptors present on monocytes and macrophages, which appear to be the major cell types infected with HIV in the brain. These cells, therefore, contribute immensely for development of neurological manifestations associated with HIV infection. In the lungs, infected pulmonary alveolar macrophages may also play a role in the development of interstitial pneumonitis observed in some HIV patients. The macrophage-tropic strains of HIV are seen in large number early in the infection, and these strains are responsible even for transmission of the infection. The virus may enter brain through infected monocytes and release toxins to neurons as well as chemotactic factors that lead to infiltration of brain with inflammatory cells. The macrophage-tropic HIV viruses are usually not present, but may be present rarely in neurons, oligodendrocytes, and astrocytes.

Humoral immunity: Humoral immunity is characterized by the development of neutralizing antibodies produced against p24, gp120, gp41 and various proteins in most of the individuals infected with HIV. However, the level of neutralizing activities is low. In adults, antibody to gp120 develops several months after the initial viremia. The development of neutralizing antibodies is associated with slow progression of disease in adults, children, and infants. The HIV escapes from the immune system of the host in many ways.

HIV escapes from the immune system through its ability:

- to integrate viral DNA with the host cell DNA, resulting in persistent infection,
- to undergo high rate of mutation of the *env* gene,
- to produce Tat and Nef proteins that downregulate class I MHC proteins required for cytotoxic T cells to recognize and kill HIV-infected cells, and
- to infect and kill CD4+ helper T cells. Continuous virus reproduction in macrophages and CD4+ T cells also maintains the virus in an immune privileged state.

Clinical Syndromes

The course of untreated HIV infection is usually 10 years or longer. The disease progresses through the stages of (*a*) primary infection, (*b*) dissemination of virus to lymphoid organs, (*c*) clinical latency, and (*d*) a late stage of profound immunosuppression known as full-blown AIDS. HIV is associated with following clinical syndromes:

Acute HIV infection is characterized by rapid rise in plasma viremia with a concomitant drop in CD4 count after an incubation period of 3–6 weeks. The symptoms of HIV are nonspecific and include low-grade fever, fatigue, malaise, rash, headache, and lymphadenopathy; spontaneous resolution may occur within weeks. HIV antibodies are usually absent in the serum at the onset of illness, but begin to appear after 3–4 weeks of the infection. This condition is referred to as

seroconversion illness. Serum antibodies are not demonstrated, but p24 antigen can be demonstrated during the beginning of the infection.

Asymptomatic HIV infection. This period is followed by an asymptomatic or clinically latent stage during which the patient continues to remain asymptomatic for several months to years. This stage is characterized by a low level of viral replication and a gradual fall in CD4 count. The serum is positive for HIV antibodies in these patients. Another characteristic of the stage of latency is persistent generalized lymphadenopathy, which may last for several years or a period of asymptomatic infection. During this stage, virus continues to replicate in the lymph node. The persistent generalized lymphadenopathy denotes the presence of enlarged lymph nodes in two or more noncontiguous extrainguinal sites that persist for at least 3 months in the absence of any current illness or medication that may cause lymphadenopathy. This is a benign condition but may progress to AIDS-related complex (ARC) or AIDS.

AIDS-related complex is characterized by lymphadenopathy and fever. This has an insidious onset and may be associated with malaise and weight loss. Diarrhea, night sweats, fatigue, and opportunistic infections are the presenting symptoms. The patients with ARC may progress to AIDS in a few months.

AIDS is the end-stage disease of the HIV infection. It denotes the irreversible breakdown of immune system of the host, making the infected host highly susceptible to a wide range of progressive opportunistic infections or unusual malignancies, such as Kaposi's sarcoma. AIDS is characterized by deterioration of immune response as evidenced by CD4 T cell decrease response. The onset of clinical manifestations correlates with:

- a reduction in number of CD4 T cells to less than 450/L,
- increased level of virus in the blood, and
- presence of p24 antigen in the blood.

When CD4 count falls less than 200/L, the patient develops full-blown AIDS. This stage is characterized by development of HIV wasting syndrome with weight loss and diarrhea for 1 month. This is also associated with many opportunistic infections, such as tuberculosis, *Pneumocystis carinii* pneumonia, toxoplasmosis, cryptococcal meningitis, and other diseases. Patients with AIDS show clinical manifestations in different ways. They can manifest as lymphadenopathy with fever, opportunistic infections, malignancies, and neurological manifestations of HIV, such as dementia.

Opportunistic infections: The opportunistic infections are the severe infections induced by the agents that rarely cause serious disease in immunocompetent individuals. These opportunistic infections are the predominant causes of morbidity and mortality among the patients with late-stage HIV infection and full-blown AIDS. These are usually associated with HIVinfected patients when their CD4 cell count falls to less than 200 cells/L. The most common opportunistic infections caused by various pathogens, such as protozoa, bacteria, viruses, and fungi. In patients with AIDS, coinfection with DNA virus often leads

to enhanced expression of HIV in cells *in vitro*. Coinfection with herpes virus and cytomegalovirus (CMV) has been shown to contribute to enhance expression of HIV in cells. The CMV has shown to produce a protein that acts as chemokine receptor and facilitates HIV to infect the cells.

Malignancies: The patients with AIDS show a marked susceptibility to the development of malignancies. Human herpes virus-8–associated Kaposi's sarcoma is the most noted malignancy associated with AIDS. Kaposi's sarcoma is much more common in untreated AIDS patients than in general population. It is a vascular tumor suggested to be of endothelial origin, which is found in the skin, mucous membrane, lymph node, and visceral organs. Other AIDS-associated malignant conditions include non-Hodgkin's lymphoma, Hodgkin's lymphoma, cervical cancer, and anogenital cancer. Burkitt's lymphoma has been shown to be even much more common in AIDS patients than in general population.

Neurological diseases: AIDS patients are associated with several distinct neurological syndromes. These include AIDS dementia complex, subacute encephalitis, vacuolar myelopathy, aseptic meningitis, and peripheral neuropathy. AIDS dementia complex is the most common neurological manifestation of HIV and occurs due to HIV infection of the microglial cells and neurons of the brain. This condition is characterized by poor memory, inability to concentrate, apathy, automotor retardation, and behavioral changes. Toxoplasma encephalitis, cryptococcal meningitis, and John–Cunningham (JC) virus-induced progressive multifocal leukoencephalopathy are some of the common infectious diseases of the brain associated with patients of AIDS.

Pediatric AIDS is an important condition acquired from infected mothers. AIDS in pediatric populations usually occurs:

by vertical transmission of HIV from infected mother or

by perinatal transmission of HIV through breast-feeding.

Children develop clinical manifestations by 2 years of age and subsequently die of AIDS in the following 2 years. The condition is more severe in neonates because the immune system is very poor during the time of birth. Clinical manifestations of AIDS in children include pneumonia, severe oral candidiasis, interstitial pneumonitis, encephalopathy, wasting, generalized lymphadenopathy, hepatosplenomegaly, diarrhea, growth retardation, and bacterial sepsis. The progression of vertically acquired HIV infection in children appears to have a trimodal distribution. Approximately, 15% of children have rapidly progressive disease and the remainder have a chronic progressive course or an infection pattern typical of that seen in adults.

Children with perinatally acquired HIV infection, if remain untreated, show a very bad prognosis. The progression of the infection is very fast in the first year of life and is believed to be associated with higher viral load of HIV-1 in the blood. The replication of the virus in children shows a different pattern than that seen in adults. Viral RNA load is usually low at birth, but the level of virus then increases rapidly within the first 2 months of life, followed by slow decline at the age of 2

years. Progression of infection from acute HIV infection to AIDS occurs usually at a median 11 years after the infection. Earlier, many of the patients used to die within 1–2 years following diagnosis of AIDS, but now with the introduction of highly active antiretroviral therapy (HAART) and better management of opportunistic infections, the death rates from AIDS have begun to decline significantly. In India, mean duration of survival of a patient after diagnosis of HIV is 7 years 8 months.

Epidemiology

HIV infection is epidemic throughout the world.

Geographical distribution. HIV-1 is the most common cause of HIV infection in the Americas, Europe, Africa, and Asia. HIV-1 subtypes show differences in their geographical distribution. Subtype A predominates in West Africa; subtype B in the United States, Europe, and Australia; subtype C in India, China, and Southern Africa. HIV-2 is the most common cause of HIV epidemics in West Africa. This virus is also found in European countries. Since the first recognition of AIDS in the United States in 1981, the AIDS has become a worldwide disease, affecting a large number of population. The current estimate of worldwide disease prevalence is more than 38 million HIV infections. As per estimation of the World Health Organization (WHO), 5 million new HIV infections are occurring each year; 90% of which are occurring in developing countries, generally in sub-Saharan Africa and Southeast Asia. The HIV seroprevalence rate among pregnant women is highest in sub-Saharan Africa, which ranges from 35% to 45%. The seroprevalence rate in pregnant women in Asia is 2%, and the vertical transmission of HIV from infected mothers to infants is 24% without breast-feeding. It has been estimated that Indian mothers infected with HIV can have a transmission rate as high as 48% with breast-feeding. More than 4.4 million children are infected worldwide, and the death of 3.2 million children has been reported due to HIV infection.

India: The first incidence of HIV infection was documented among sex workers in Chennai, Madurai, and Vellore of Tamil Nadu in 1986–1987. The first case of AIDS was detected in the year 1986 from Mumbai. Since then, HIV infection has been documented from each part of India. The National AIDS Control Organisation (NACO), India has estimated that by the year 2008, nearly 2.4 million people were living with HIV, next only to South Africa. The national HIV prevalence is varied from 0.4% to 1.3%. Distribution of HIV epidemic in India is varied and is based on the prevalence of HIV infection in the high- and low-risk groups. Different states in India have been classified as high-, medium-, and low-prevalence areas. Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra, and Goa are classified as high-prevalence states with HIV prevalence over 1% even in lowrisk population. Both HIV-1 and HIV-2 have been shown to occur in India. Various studies have shown a high prevalence of subtype C in India. Nearly 78.4% strains in north India and 95% of strains in Kolkata and south India have been shown to be HIV 1C subtype.

Reservoir, source, and transmission of infection. HIV is primarily a human infection. Humans infected with HIV and AIDS are the reservoir of infection. The high titer of HIV is found in the blood, semen, and vaginal secretions of the infected people; hence these are important sources of infection. The virus is also present in the breast milk of an infected mother.

Transmission of HIV infection. HIV infection occurs either by the transfer of HIV-infected cells or free HIV not associated with cells. HIV transmission occurs in following ways:

Sexual transmission: HIV is transmitted primarily through sexual contact and constitutes more than 70% of the HIV transmission. Sexual transmission is more common in heterosexual women and men than in homosexual men worldwide. Varied sexual behaviors, such as (*a*) more number of sexual partners, (*b*) sex with commercial sex workers, homosexuals, and (*c*) receptive anal sex have been reported to be increasingly associated with HIV infection. The presence of other sexually transmitted disease, such as gonorrhea, syphilis, or herpes simplex virus type 2 infection increases the risk of sexual HIV transmission by more than 100 times. This is due to preexisting inflammation and ulcers caused by these diseases, which facilitate the transfer of HIV through mucosa. The risk to HIV significantly increases with an increase in the number of sexual contact with multiple partners.

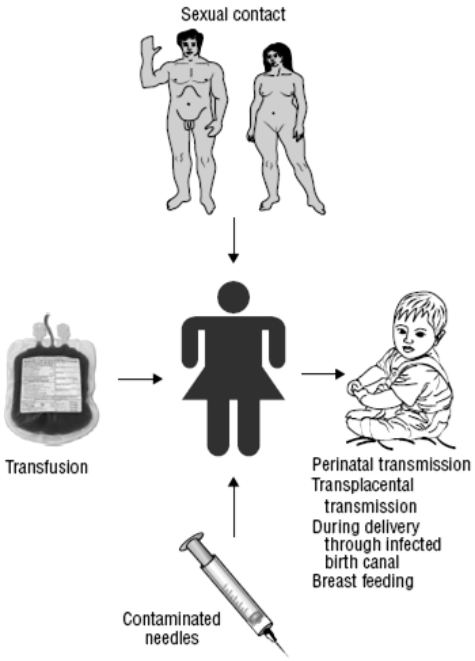
Transmission by blood transfusion: HIV is also transmitted by transfusion of infectious blood or blood products, such as serum, plasma, and cells from HIV-positive individuals. It can also be transferred by the organs donated from the HIV-positive individuals. HIV among health workers has also been documented following a needle stick injury with contaminated blood. However, the reported cases are relatively few, and the estimated risk of transmission is as low as 0.3%.

Parenteral transmission: Parenteral transmission occurs largely among IV drug users. Injection users of illicit drugs are commonly infected through the use of contaminated needles. IV drug users constitute a substantial proportion of new cases of HIV in north-eastern states of India, Manipur. The prevalence of HIV among injecting drug users in Manipur is increasing rapidly: from 2–3% in 1989 to more than 50% in 1991 and nearly 75% in early 2000. This increase is due to transmission of HIV through sharing of needles when injecting equipment is shared.

Mother-to-child transmission: Mother-to-infant transmission can occur by vertical transmission or by perinatal transmission. Vertical HIV infection occurs in the following ways:

1. The fetus *in utero* can be infected by vertical transmission of virus through the placenta or through the amniotic membrane if the membranes are infected or inflamed. Vertical transmission is most common during delivery of the baby of the infected mother. The risk of vertical transmission is greatly increased with an increase in duration of contact with the maternal blood and cervical vaginal secretions.

2. Perinatal infection in HIV occurs during the birth process or through breast-feeding. Transmission of HIV during breastfeeding usually occurs by 6 months. Several studies have shown that nearly one-third to half of perinatal HIV infection in Africa is caused by breast-feeding. Untreated women infect 13% and 40% children in Europe and Africa, respectively. Mother-to-infant HIV transmission rate varies between 36% and 40% in India. Relatively, the rate of postnatal transmission in Africa and other developing countries is higher because of the practice of breast-feeding. Sexually active person, both heterosexual and homosexual, IV drug abusers and their sexual partners, and the newborns of HIV-positive mothers are at highest risk for HIV infection.



4. Transmission of HIV infection.

Laboratory Diagnosis

Laboratory diagnosis of HIV infection is useful to:

1. Confirm the diagnosis of AIDS
2. Detect the individuals with HIV infection
3. Identify carriers who may transmit infections to others
4. Perform seroepidemiological studies in the community

Specimens. These include serum and plasma for HIV serology and lymphocytes for isolation of HIV.

Isolation of the virus. Virus isolation is not a routinely used method for diagnosis of HIV infection because it is time-consuming and laborious. It is used mostly for research purpose. The virus can be isolated mostly from lymphocytes in the peripheral blood and occasionally from bone marrow, plasma, and other body fluids. In patients with AIDS, high titer of virus is found in the plasma and in lymphocytes than in the peripheral blood. Viruses are found mostly within CD4 cells.

Cocultivation: It is the most sensitive method for isolation of virus. It is performed by cocultivating potentially infected and uninfected mononuclear cells to facilitate the replication of HIV. The viral growth in culture fluid is demonstrated by the presence of HIV p24 antigen and HIV reverse transcriptase. The test becomes positive after 7–14 days of culture or even may require a longer period of 28 days. The virus titer in asymptomatic infection is low, hence may not be positive for virus by culture. Viral culture is useful for detection of high virus titer, which is found early in infection before the presence of HIV antibodies.

Serodiagnosis includes demonstration of antibodies and viral antigens.

Demonstration of antibodies. Detection of specific antibodies to HIV in serum is the most commonly used method for serodiagnosis of patients with HIV and AIDS. Detectable level of antibodies is demonstrated in most individuals within 6–12 weeks after infection and in all the individuals within 6 months of infection.

The diagnosis of HIV infection is established by demonstration of specific antibodies to envelope glycoproteins gp41, gp120, and gp160 and to viral core p24 antigens. The antibody response against these viral proteins is variable during the progression of HIV infection to AIDS. Antibodies to envelope glycoproteins persist in the serum, but those directed against Gag protein (p17, p24, p55) decrease. The antibody-based serological testing in HIV is of two types: (*a*) screening tests and (*b*) supplementary or comfirmatory tests.

Screening tests: Screening tests are otherwise known as ERS which is an acronym for enzyme-linked immunosorbent assay (ELISA), rapid test, and simple test. These tests are usually highly sensitive tests and are used for initial screening of the serum samples for the presence of HIV antibodies.

ELISA: ELISA is the most frequently used test for detection of both HIV-1- and HIV-2-specific antibodies in the serum. This test is highly sensitive and specific, and commercial ELISA kits are available, which detect both HIV-1 and HIV-2 antibodies in the serum. ELISA can also be used for demonstration of antibodies in the saliva. This is very useful for testing injectable drug users from whom it may be difficult to collect blood due to collapsed blood vessels. ELISA in HIV serology are of four types: first generation, second generation, third generation, and fourth generation depending on the nature of the antigen used and detecting both antibodies and antigen in the serum.

Rapid tests: Rapid tests include dot blot assay, latex agglutination, gelatin agglutination, HIV spot, and comb test, etc. These tests are simple tests, which can be performed in any laboratory without requiring any expensive instrument or skilled manpower. Moreover, test results can be read rapidly within 30 minutes of receipt of the specimen.

Simple tests: These tests are simple, do not require any expensive equipment, and can be performed within 1 or 2 hours.

Supplementary or comfirmatory tests: These tests are used as confirmatory tests for detection of HIV antibodies. These tests are designed for a

higher specificity than the screening tests, hence are used as the test of choice to verify the results of screening tests. Western blot, line immunoassay, and indirect immunofluorescence assay are the most commonly used serologic confirmatory tests.

Western blot: It is the most common confirmatory test used in HIV serology. In this test, HIV viral antigens are separated as gp160, gp120, p66, p55, p51, gp41, p31, p24, p17 and p15 depending on their electrophoretic mobility by polyacrylamide gel electrophoresis. These antigens are then blotted onto strips of nitrocellulose paper. These strips are treated with test serum. Antibodies to these HIV proteins, if present in test serum, combine with different fragments of HIV and then react with enzyme conjugated antihuman globulin. These strips are washed, followed by addition of a suitable substrate, which produces colored bands. The position of the colored band on the strip indicates the antigen with which the antibody has reacted. The demonstration of multiple bands indicates a positive test.

The test is considered positive if it shows bands against at least two of the three viral proteins, namely, p24, gp41, and gp120 or gp160.

The test is also considered positive if multiple bands are seen with multiple proteins, which are encoded by three genes (*gag*, *pol* and *env*). This represents p24 of *gag* gene core protein, p31 of *pol* gene reverse transcriptase, gp41, gp120 or gp160 of *env* gene surface antigens.

The development of bands at one site as with p24 or gp120 is considered equivocal and may happen in early infection and also may be nonspecific.

Absence of any band indicates negative test.

It was earlier practice to confirm positive result of any one screening test by Western blot, but since the test is cumbersome, expensive, and is not readily available, other strategies are available for confirmation of the cases. So the practice followed now is to perform either two different types of ELISA or an ELISA with any of the rapid tests. If a serum is positive for HIV antibodies by both these tests, the serum is then considered positive for HIV. In case of doubt, serum samples are retested after a period of 1 or 2 months.

Other confirmatory tests: Line immunoassay in principle is similar to Western blot; however, it differs from Western blot by having artificial HIV antigens used on the strips than using viral lysis protein separated by polyacrylamide gel electrophoresis. Indirect fluorescent antibody (IFA) is another test used as a confirmatory test.

Demonstration of viral antigen. The antibody sandwich ELISA using specific monoclonal antibodies to HIV p24 is used to detect the viral capsid core antigen (p24 antigen) in blood. The p24 antigen appears much earlier than HIV antibodies during acute HIV infection, hence can be detected during window period, during which antibodies are not demonstrated in the serum. The p24 antigen appears usually 16 days after infection. The antibody sandwich ELISA using specific monoclonal antibodies to HIV p24 is used to detect p24 antigen. The

p24 antigen often becomes undetectable after HIV antibodies develop in the blood. This is due to formation of immune complexes by p24 with the antibodies. However, p24 antigen may appear later in the course of infection, suggesting a very poor prognosis.

Molecular Diagnosis. The molecular methods include reverse transcriptase polymerase chain reaction (RT-PCR), nucleic acid-based amplification (NASBA), transcription-mediated amplification (TMA), and branched chain DNA (bDNA). These methods are useful for quantitative estimation of viral load in the plasma. In a positive test, a five- or threefold change in the viral load indicates reliably a significant change in children younger than 2 years or in those older than 2 years, respectively. These tests should not be used until the diagnosis of HIV has been confirmed with a nonquantitative serodiagnostic method because these tests may falsely show low viral loads in individuals who are HIV negative. HIV DNA PCR is a sensitive method for detection of HIV provirus present inside mononuclear cells by employing oligonucleotide directed at highly conserved regions of the virus genome. The advantage of this test is that it can be used to diagnose HIV within 24 hours of infection and it has a sensitivity and specificity of 95% and 97%, respectively. The RT-PCR and NASBA for plasma are sensitive methods for detection of HIV-1 subtype B viruses, whereas bDNA method is sensitive for detection of other HIV subtypes.

Monitoring status of HIV infection. The laboratory monitoring of the status of HIV infection can be carried out by analysis of (*i*) T-cell subset, (*ii*) measurement of HIV RNA, and (*iii*) measurement of B2 microglobulin and neopterin.

CD4+ T cell count: This is an important indicator for monitoring HIV infection. This count reflects the immunological competence of the patients with HIV or AIDS. A rapid decrease in the CD4 count in adults and in infants is a poor prognostic sign and requires the initiation or alteration of antiviral therapy. The absolute number of CD4 lymphocytes and the ratio of helper T inducer lymphocytes (CD4:CD8 ratio) are very low in HIV-infected people. The CD4:CD8 T-cell ratio is reversed to 0.5:1 from a normal level of 2:1. If the CD4 count is below 500/L, it indicates progression of the disease and hence requires specific therapy against HIV. When the count falls below 200/L, it indicates a very poor prognosis and shows the increased risk for serious infection of patient, particularly opportunistic infections.

Measurement of HIV RNA: HIV RNA level in serum is an important predictive marker of disease progression and are used as prognostic marker to monitor the effectiveness of anti-HIV therapies. The test is also useful for early diagnosis of HIV infection in infants born to infected mothers.

Measurement of B2 microglobulin and neopterin:

B2 microglobulin and neopterin can be demonstrated in the serum or urine of HIV-infected people. The titer is low in asymptomatic HIV infection, but is elevated with the progression of HIV disease.

Other tests. Tuberculin test or other tests for cell-mediated immunity is negative, indicating a depressed cell-mediated immunity. Both IgE and IgA serum levels are elevated in the blood. Hematological parameters are altered in patients with HIV. There is a leucopenia with lymphocyte count less than 400/_L and thrombocytopenia in patients with AIDS. Hyperimmunoglobulinemia is associated with the progression of disease. Hypoimmunoglobulinemia is observed in end-stage disease and is associated with poor prognosis. Thrombocytopenia is a common finding in patients with HIV. Anemia occurs in as many as 25% of cases at diagnosis and occurs in 80% of cases after sometime. Neutropenia is observed in 10% of early asymptomatic HIV infections and in 50% of patients with AIDS.

Strategies for HIV testing in India. The following are the three different strategies followed for HIV testing in India:

Strategy 1: In this strategy, test serum is subjected once to ERS test, and if positive the sample is considered as HIV infected and if negative the serum is considered to be free of HIV. For this purpose usually highly sensitive and very reliable diagnostic kits are used. This strategy is used for screening of blood, organ, and tissue before transfusion and transplantation.

Strategy 2: In this strategy, a serum sample is considered negative if the first ERS test report reports it so. But if positive, it is retested with a second ERS test based on different antigen preparation and different test principles. The serum specimen if also found positive with second ERS test, it is reported as positive, and otherwise it is considered as negative. This strategy is used for HIV surveillance.

Strategy 3: It is similar to strategy 2, but with an added confirmation by a third ERS test. The third test should be based on different antigen preparation or test principle. A serum showing positive result on all three ERS tests is reported positive. The test is considered equivocal if the serum is negative in the third ERS. In such cases, serum specimens are retested on collection after 3 weeks. If this specimen also shows an equivocal result, the person is considered to be negative for HIV antibodies. This strategy is used for diagnosis of HIV infection in asymptomatic persons. The tests used in second and third strategies are of higher specificity in order to rule out any false positivity.

Treatment, Prevention and Control

Antiretroviral treatment is the mainstay in HIV treatment. The goals of antiretroviral therapy are to inhibit replication of HIV and to reduce morbidity and death.

Anti-HIV drugs. The anti-HIV drugs can be broadly classified as: (*a*) nucleoside analog reverse transcriptase inhibitors (NRTIs), (*b*) nonnucleoside reverse transcriptase inhibitors (NNRTIs), or (*c*) protease inhibitors. Antiretroviral agents against HIV are summarized in Table 5.

TABLE 5 - Classes of antiretroviral agents.

Class	Abbreviation	Mechanism of action	Specific action
Nucleoside and nucleotide reverse transcriptase inhibitors	NRTIs and NtRTIs	Reverse transcriptase inhibition	Nucleic acid analogues mimic the normal building blocks of DNA, preventing transcription of viral RNA to DNA
Non-nucleoside reverse transcriptase inhibitors	NNRTIs	Reverse transcriptase inhibition	Alter the conformation of the catalytic site of reverse transcriptase and directly inhibit its action
Protease inhibitors	PIs	Protease inhibition	Inhibit the final maturation stages of HIV replication, resulting in the formation of non-infective viral particles

Nucleoside analog reverse transcriptase inhibitors (NRTIs): Azidothymidine (AZT), didanosine (DDI), and other nucleoside analogs inhibit the enzyme reverse transcriptase and alter their incorporation into DNA to cause chain termination. These agents prevent the spread of the virus to uninfected cells. AZT is recommended for treatment of asymptomatic or mildly symptomatic people with CD4 count of less than 500/L. This is also recommended for treatment of pregnant women to reduce the possibility of transmission of the virus to the fetus. The toxicity associated with high doses of AZT and the emergence of resistance to AZT is the main disadvantage of monotherapy with AZT. Zidovudine is also used effectively to reduce significant transmission of HIV from mother to infant. The treatment decreases vertical transmission at all levels of maternal viral load.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs): NNRTIs, such as nevirapine, delaviridine, and efavirenz, inhibit the enzyme by blocking the morphogenesis of the virion by inhibiting the cleavage of the Gag and Gag core polyproteins. This in turn prevents activation of the virion.

Protease inhibitors: Protease inhibitors, such as ritonavir, indinavir, saquinavir, nelfinavir, and amprenavir, prevent the maturation of viral particle during late stage of viral replication. Monotherapy with antiretroviral therapy failed to produce significant clinical benefits including survival of the patient. The failure is partly due to the development of drug-resistant variants of HIV because resistance develops rapidly during monotherapy and cross-resistance between related drugs is also being increasingly reported. Multidrug therapy reduces morbidity due to the disease and death in many patients due to AIDS is delayed.

Antiretroviral drugs against HIV

Nucleoside reverse transcriptase inhibitors (NRTIs)

- Zidovudine (AZT)
- Didanosine (ddI)

– Zalcitabine (ddc)
– Lamivudine (3TC)
– Stavudine (d4T)
– Abacavir (1592)

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)

– Nevirapine (NVP)
– Delaviridine
– Efavirenz

Protease inhibitors (PI)

– Ritonavir
– Indinavir
– Saquinavir
– Nelfinavir
– Amprenavir

Nucleotide reverse transcriptase inhibitor

– Tenofovir

Fusion transcriptase inhibitor

– Enfuvirtide

Prevention and Control. These include the following steps: (*a*) health education, (*b*) screening of blood and blood products, (*c*) infection control, and (*d*) vaccine development.

Health education plays a key and important role for the prevention of AIDS in the absence of a suitable vaccine. Health education is aimed at behavioral changes and maintenance of a lifestyle that minimizes or eliminates the risk of transmission. Health education includes the following:

– Safe sexual practice by using a condom, which prevents transmission of the virus.
– Not sharing unsterile needles or syringes.
– Information to HIV-positive women regarding the risk of vertical transmission of HIV to infants.

Screening of blood and blood products. It is essential to screen potential blood donors before they donate blood or blood products before use. The infected persons who are tested positive for HIV should refrain from donating blood, plasma, body organs, other tissues, or sperm. The blood of the donors should be screened for HIV-1 and HIV-2 antibodies by screening tests, such as ELISA. Regular screening of blood for HIV antibodies before transfusion has reduced considerably the transmission of HIV by contaminated blood.

Infection control methods include the use of universal blood and body fluids precautions. These universal precautions include wearing protective clothings, such as gloves, masks, gown, etc., and using other barriers to prevent exposure to blood products. These also include disinfection of contaminated

surface with 10% household bleach, 70% ethanol or isopropanol, 2% glutaraldehyde, 4% formaldehyde, or 6% hydrogen peroxide. Washing clothes in hot water with adequate detergents is effective to kill HIV.

Vaccine development. A safe and effective vaccine is yet to be available against HIV. An ideal HIV vaccine is that which would:

- prevent acquisition of the virus by an adult during sexual intercourse,
- prevent transmission of virus to infants of HIV-positive mothers, and
- also block the progression of the disease.

There are many scientific obstacles to the development of AIDS vaccine. This is mainly due to various problems unique to the HIV as mentioned below:

1. Antigenic diversity and hypervariability of the virus, as the antigenicity of the virus changes readily through mutation.

2. Transmission of the disease by mucosal route – initial protection should require the production of secretory antibodies to prevent sexual transmission and acquisition of the virus.

3. Transmission of the virus by infected cells.

4. Latency of the virus – the virus can be spread through syncytium and remain latent, thereby remaining protected from antibodies.

5. Integration of the virus genome into the host cell chromosome.

6. Rapid emergence of viral escape mutants in the host – the high error rate of the viral reverse transcriptase leads to continuous mutations in the HIV genome.

Vaccines

Most of the HIV vaccines have been evaluated by using gp120 or its precursor gp160 as immunogen. The gene for this protein has been cloned, expressed in different eukaryotic systems and developed as a subunit vaccine. In India, the National AIDS Research Institute (NARI), Pune is evaluating DNA – pox virus (canary pox, fowl pox) and MVA (modified vaccinia Ankara), a highly attenuated host-range-restricted strain of vaccinia virus. The vaccine has undergone phase I clinical trial with AAV (adeno-associated virus) AIDS vaccine in 2005. The second AIDS vaccine clinical evaluation is being set up at TRC (Tuberculosis Research Centre), Chennai.

Slow viruses and prions

Introduction. Slow infectious diseases are a large group of related neurodegenerative conditions, which affect both humans and animals. The diseases are caused by a heterogeneous group of agents consisting of conventional viruses and the unconventional agents that are called *prions*. The term “slow” refers to the disease process and not to the replication of the viruses that cause the slow disease. Replication rate of virus is similar to that of most other viruses.

Slow Diseases Caused by Prions

The agents causing slow diseases were earlier called as slow virus, infectious protein, infectious amyloids, or crystal protein. These agents are now

being named prions, and this term has gained wide acceptance, replacing all the previously used terms. The prions are unconventional agents that are not viruses.

Slow diseases caused by prions and conventional viruses

Humans

Prion - Kuru
Prion - Creutzfeldt–Jakob disease (CJD)
Prion - Variant CJD
Prion - Gerstmann–Straussler–Scheinker syndrome
Prion - Fatal familial insomnia and sporadic fatal insomnia
Virus - Subacute sclerosing panencephalitis
Virus - Progressive multifocal leukoencephalopathy
Virus - Acquired immunodeficiency syndrome (AIDS)

Animals

Prion - Scrapie
Prion - Bovine spongiform encephalopathy
Prion - Feline spongiform encephalopathy
Prion - Transmissible mink encephalopathy
Prion - Chronic wasting disease of deer, mule, and elk
Virus - Visna

The diseases caused by prions are a large group of related neurodegenerative conditions, which affect both humans and animals. These diseases belong to a family of diseases known as the transmissible spongiform encephalopathies (TSEs).

There are six human TSEs caused by prions: (*a*) kuru, (*b*) Creutzfeldt–Jakob disease (CJD), (*c*) variant CJD (vCJD), (*d*) Gerstmann–Straussler–Scheinker (GSS) syndrome, (*e*) fatal familial insomnia (FFI), and (*f*) sporadic fatal insomnia.

Animal TSEs caused by prions include (*a*) scrapie and visna (diseases of sheep), (*b*) bovine spongiform encephalopathy (BSE; mad cow disease), (*c*) transmissible mink encephalopathy, and (*d*) chronic wasting disease of deer, mule, and elk.

Transmissible spongiform encephalopathies in humans caused by the prions show following characteristics:

1. Long incubation period of several years.

2. Course of illness lasting for months to years.

3. A progressive debilitating neurological syndrome that is invariably fatal.

4. Associated with pathological changes typically restricted to the central nervous system (CNS).

5. Absence of specific immunological response in the host.

6. The agents are resistant to conventionally used inactivation methods.

History. Kuru was the first human disease known to be caused by a slow virus, or prion (1959). This condition was found among “Fore” people, a tribe living in the remote highlands of New Guinea. This virus was linked to ritualistic cannibalism prevalent among these tribal people. Hallow (1959) suggested that

kuru could be a possible human form of scrapie, a slow viral disease seen in sheep. Gajdusek and his colleagues (1966) first demonstrated that kuru was transferable to chimpanzee after a long period of incubation, for which they were awarded the Nobel Prize. Since then many TSEs, linked to slow viruses, are being described both in humans and animals.

Properties of the Prions

Prions are small protein-containing infectious particles with no detectable nucleic acid. They were suspected to be viruses but otherwise do not conform to the standard definition of viruses. They differ from the viruses in their many properties. The prions show following characteristics:

1. Prions like viruses are filterable.
2. They apparently lack any virion structure or genome.
3. Unlike viruses, they are unusually resistant to inactivation by heat, disinfectants, and radiation.
4. They do not elicit any specific immune response in the infected host.

Morphology. Different hypotheses have been suggested for the makeup of prions. Initially many research workers considered prions to be (*a*) nucleic acid only, (*b*) protein only, (*c*) lacking both protein and nucleic acid, and (*d*) polysaccharide. Currently, the most widely accepted hypothesis is the "*protein only hypothesis"* first suggested by Griffith (1965) and re-established subsequently by Prusiner to indicate that scrapie is related to a proteinaceous infectious particle (PrP). Prions show following features:

- The prion does not contain any nucleic acid.
- It contains an aggregate of hydrophobic glycoprotein, resistant to protease.
- The protein present in humans and other animals is called cellular prion protein. It is similar to glycoprotein in scrapie known as scrapie prion protein, which has a molecular weight ranging from 27,000 to 30,000 Da. It is similar to scrapie prion protein in its protein sequence but differs from it in being sensitive to protease (scrapie protein is protease resistant) and being present in plasma membrane (scrapie prion protein is present in cytoplasmic vesicles).

Prion replication. In experimental animal studies carried out in chimpanzees, Prusiner demonstrated conclusively that the prion, an aberrant protein, could cause disease, for which he was awarded Nobel Prize in 1997. Prusiner hypothesis suggests that prions do not contain any nucleic acid. The normal prion protein known as *prion protein cellular* or PrPC has a significant amount of helical configuration. In its alpha-helical configuration, PrPC is usually sensitive to degradation by the activity of enzyme protease. Disease occurs when the PrPC is reconfigured into the betasheet configuration known as *prion protein scrapie* or PrPSC, which is resistant to degradation by protease. These abnormal proteins are resistant not only to protease degradation but also to radiation, heat, and other agents that destroy proteins. These aggregate into filaments that disrupt

neuron functions and cause death of cells. Both the normal alphahelical form and the abnormal beta-pleated sheet form have the same amino acid sequence but differ in their configuration.

Other properties. Prions, unlike viruses, are unusually resistant to heat at 80°C, ultraviolet radiation, and disinfectants, such as formaldehyde. They are, however, inactivated by phenol, ether, sodium hydroxide, and hypochlorite.

Prion Isolation. Prions cannot be cultivated in any tissue culture or isolated in any experimental animal models.

Pathogenesis and Immunity

Prion disease is transmitted either orally or transcutaneously. But how the prions reach the CNS, their target, is yet to be understood fully. It is believed that a functional immune system is essential for replication of prions and their transport to the reticuloendothelial cells. In these cells, large amounts of prions are present in the follicular dendritic cells as well as in the sympathetic nerve endings. From these sites, the prions reach the CNS – but how they reach the CNS is still an important issue. It is suggested that the prions may reach the CNS via splanchnic nerves at the level of the thoracic spinal cord and via parasympathetic fibers connecting with the brain. The other possible route is by the blood circulation. In experimental animal studies carried out in chimpanzees, Prusiner demonstrated that scrapie protein (PrPSC) binds to the normal cellular prion protein (PrPC) of the host. PrPC is found in most tissues of the body but is expressed in higher quantities in the CNS particularly the neurons, the main site of action of prions. PrPSC is ingested by neurons and phagocytic cells but continues to remain intact without being degraded. This may contribute to vacuolation of the neuron, a key pathological change observed in encephalitis caused by prions. In addition, accumulation of prions in high concentration causes damage in the brain tissue. These include the formation of amyloidcontaining plaques and fibrils, a proliferation and hypertrophy of astrocytes, and fusion of neurons and adjacent glial cells.

Host immunity. The prions cause encephalopathy, a condition which denotes a pathologic process in which no tissue inflammation or no immune responses are induced in the host, by which it is differentiated from that of viral encephalitis. In the symptomatic patients, a protein called 14–3–3 brain protein has been detected in the cerebrospinal fluid (CSF).

Pathogenesis and transmission of prion-related slow diseases in human

Disease	Pathogenesis	Transmission
Kuru	Cannibalism	Ingesting or handling brain tissue
Creutzfeldt–Jakob disease		
Infectious	Contact with or handling prion-containing materials	Cuts in the skin Transplantation of contaminated tissue, such as cornea Use of contaminated medical devices, such as brain electrodes Possibly through ingestion of infected tissue
Hereditary	Mutations in the germ cells	Hereditary predilection
Sporadic	No relationship to any cause	Spontaneous PrP^{C} Conversion to PrP^{SC}
Variant Creutzfeldt–Jakob disease	Infection from BSE	Probably by eating meat or nervous tissue from animals with mad cow disease Blood transfusion
Gerstmann–Straussler–Scheinker syndrome	Mutations in the PrP gene	Hereditary/genetic
Fatal familial insomnia	D 178N mutation in the PrP gene, with M129 polymorphism	Hereditary/genetic
Sporadic fatal insomnia	No relationship to any cause	Spontaneous PrP^{C} Conversion to PrP^{SC}
Progressive multifocal leukoencephalopathy		Central nervous system demylination

Clinical Syndromes

Slow diseases caused by prions in humans. Prions cause following diseases in humans: (*a*) kuru, (*b*) CJD, (*c*) variant CJD, (*d*) GSS syndrome, and (*e*) fatal familial insomnia.

Kuru: Kuru is a fatal neurological disease described only among Fore tribe inhabiting the highlands of New Guinea. The disease was spread by cannibalistic funeral practice of the tribal population. This involved the ritualistic practice by the closest female relatives and children usually consuming the brain of the person following his or her death. The brain which contained most of the infectious pathogen was the source of infection. Kuru has largely disappeared today because cannibalism has been abolished now among the Fore tribal people. Kuru is a fatal disease characterized by progressive cerebellar ataxia and tremors. The condition manifests initially as difficulty in walking, followed by cerebellar tremor, hence the name *kuru*, which means “trembling in fear”. Eventually the tremor worsens, followed by progressive cerebellar ataxia and eventual death within a year of onset of symptoms. The clinical course lasts for 3 months to 2 years.

Creutzfeldt–Jakob disease: CJD is the most common prion disease responsible for nearly 85% of all human prion diseases. The condition was described by Creutzfeldt (1920) and Jakob (1921), after whom the disease is named. Creutzfeldt–Jakob disease is a subacute progressive encephalopathy characterized by a rapidly progressive dementia, associated with myoclonic jerks. Memory loss, behavioral changes, and confusion are the common clinical manifestations. The condition is also associated with ataxia, aphasia, visual loss, and hemiparesis. The condition progresses and in the terminal stage of the disease, the patient becomes mute and comatose. The condition is associated with extensive cortical spongiosis, gliosis, and neural loss. The condition is invariably fatal and death occurs in about 8 months.

Variant CJD: The vCJD is a new disease affecting mostly adults aged 45 years. The condition was first documented in Britain in 1985. It is BSE zoonoses, probably originating from a double species switch from sheep scrapie to BSE and then from BSE to human variant. The vCJD is believed to be caused by ingestion of BSE-infected beef products contaminated by neural tissues. This is because neural tissues have a much higher concentration of PrPSC compared with any other non-neural tissue.

Gerstmann–Strдussler–Scheinker syndrome: The condition was originally described in 1936 as affecting humans. The main clinical findings of the condition are slowly progressive limb and truncal ataxia, as well as dementia. Death of the patient usually occurs within 3–8 years of presentation of symptoms.

Fatal familial insomnia: Patients with this illness present with intractable insomnia, dysautonomia, dementia, and motor paralysis. Death occurs within 6 months to 3 years following presentation. The clinical presentation of the disease, however, varies widely; hence definitive diagnosis of the condition is made by genotyping.

Slow diseases caused by prions in animals

Prions cause following diseases in animals: (*a*) scrapie, (*b*) bovine spongiform encephalopathy, (*c*) feline spongiform encephalopathy, (*d*) transmissible mink encephalopathy, and (*e*) chronic wasting disease of deer, mule, and elk.

Scrapie: Scrapie is a prototype prion disease of animals. It is a slow virus disease of the sheep, known for centuries. The infection is transmitted vertically from ewe to lamb and less frequently by direct contact. Incubation period is nearly 2 years. The condition manifests as intense irritation, and to relieve that the infected animals scratch themselves, against trees and rocks, hence the name scrapie. The condition progresses to emaciation and paralysis and finally leads to death of the animal. Autopsy of the infected brain shows spongiform degeneration without inflammation in the brain tissue. The condition has been documented extensively.

Bovine spongiform encephalopathy (BSE): Also known as mad cow disease. It is a disease of cattle. The condition has an incubation period of 2–8

years. The disease is relentlessly progressive until the animal dies. The cattle feed containing prion-contaminated meat and bone meal, which was used as a protein source, was the source of infection for trans mission of BSE to cattle. The causative agent is believed to be originated from either scrapie affected sheep or cattle with unidentified prion-associated slow virus disease. The mad cow disease, documented in 1986, has been described in cattle in the European countries and in the United States. Till 2004, nearly 190,000 confirmed clinical cases of BSE in cattle have been reported worldwide; the majority of which were from the United Kingdom alone.

Transmissible mink encephalopathy: Transmissible mink enceph alopathy is a scrapie-like disease of mink. The causative agent is believed to be a strain of scrapie virus, which is transmitted to mink by feeding the animals on scrapie-infected sheep meat.

Chronic wasting disease of deer, mule, and elk: Chronic wasting disease is a prion disease of deer, mule, and elk. It is a progressive wasting disease of these animals in the United States.

Epidemiology

Prion-related diseases are rare but worldwide.

Geographical distribution. Creutzfeldt–Jakob disease is rare but is found throughout the world with an incidence of about one case per million populations. An autosomal dominant pattern of inheritance linked to mutation in the PrP gene is found in nearly 10% cases of familial CJD. Till 2003, a total of 167 definite and probable cases of vCJD have been reported worldwide. Most of the cases of vCJD have been reported in the United Kingdom. The condition is yet to be documented from Asia. Creutzfeldt–Jakob disease syndrome and fatal familial insomnia (FFI) in humans are rare but occur in different parts of the world.

Reservoir, source, and transmission of infection. The spongiform encephalopathies caused by prions may be infectious, hereditary, and sporadic. The CJD, GSS, and FFI are inheritable. The CNS tissue in CJD has the highest concentration of prion agent, hence is the most important source of infection. Unlike the prion disease, in vCJD, lymphoid tissues show highest concentration of prions. Contaminated blood is another source of infection. Families with genetic history of the disease have been identified in cases of GSS syndrome.

Laboratory Diagnosis

The diagnosis of prion diseases is always clinical. It is usually confirmed by the histopathology of the brain tissue showing characteristic histological changes. No serological tests are available, as prions are inert agents and do not elicit any immune response in infected hosts. The prions cannot be detected in the infected tissue by any method including the electron microscopy, antigen detection, and genomic methods, such as DNA probe or polymerase chain reaction (PCR). Detection of protein 14–3–3 in the CSF by Western blot is a sensitive and specific method in cases of sporadic CJD and in vCJD. A specific reduction in uric acid

level in the CSF has been shown in vCJD but not in sporadic CJD, thus facilitating in the differential diagnosis of vCJD.

Treatment, Prevention and Control

No specific treatment is available for any prion disease.

Prions are highly resistant to inactivation by disinfectants used for other viruses, such as formaldehyde, detergents, and ionizing radiations. Hence, the materials from patients with CJD or vCJD must be handled with special care. For prevention of these diseases, special disinfection protocols have been developed by the World Health Organisation (WHO). These include autoclaving at 15 lbs for 1 hour (instead of 20 minutes) or treatment with 0.1 M sodium hydroxide and 5% hypochlorite solution. Since cases of blood transfusion-associated CJD have been reported, it is a matter of great concern. However, currently no method including PCR is available for demonstration of the agent in the blood.

Slow Diseases Caused by Conventional Viruses in Humans

Subacute sclerosing panencephalitis, progressive multifocal leukoencephalopathy (PML), and acquired immunodeficiency syndrome (AIDS) are the three slow diseases caused by conventional viruses in humans.

Subacute Sclerosing Panencephalitis. The subacute sclerosing panencephalitis is a degenerating disease of the CNS caused by persistent measles infection. It is a persistent infection by a variant measles virus that fails to complete its replication. The disease is characterized by the development of behavioral and intellectual deterioration and seizures after many years (mean incubation period is 10.8 years) of infection by measles. This is a serious and late neurological sequel of measles that affects the CNS. The condition occurs in about seven in every one million patients. The condition is associated with the presence of an extremely high measles antibody titer in the blood and CSF. Inclusion bodies, which react with antibodies to measles virus, are present in the affected neurons.

Progressive Multifocal Leukoencephalopathy is a fatal demyelinating disease of the white matter of the brain. The condition is usually fatal. It occurs primarily in persons with compromised cell-mediated immunity, such as patients with AIDS and those receiving immunosuppressive drugs and anticancer therapy. JC virus or John Cunningham virus (JCV) is the causative agent of PML. The virus following the infection kills oligodendroglia and causes syncytia in astrocytes. Reactivation of the virus in immunocompromised patients causes activation of latent virus, thereby causing the disease. Changes in mental status, alteration in the vision, and weakness are the initial clinical manifestations. The condition progresses rapidly to blindness, dementia, and coma. The patient usually dies within 6 months. Laboratory diagnosis of the condition is made by PCR for viral genome in the brain tissue or CSF. Serum antibodies to JC are demonstrated in approximately three fourth of normal sera; hence serology is not useful. No specific antiviral treatment is available.

Acquired Immunodeficiency Syndrome. AIDS caused by human immunodeficiency virus has a long latent period. Hence, the disease is also considered as an example of slow disease with a progressive course and involvement of the CNS.

Slow Diseases Caused by Conventional Viruses in Animals

Visna. Visna is a disease of sheep caused by visna virus, a lentivirus. It is a single-stranded RNA virus with an RNA-dependent DNA polymerase in the virion. Integration of the DNA provirus into the host cell DNA appears to be responsible for persistence of the virus within the host. Clinically, the condition manifests after a long incubation period with a prolonged progressive course. It has an insidious onset with paresis, progressing to total paralysis and death. The viruses are present in the saliva, blood, and CSF of the infected animal. High levels of serum antibodies are present in the serum, but these are not protective.

CONTROL QUESTIONS

1. Features of viruses. Morphology of viruses. Replication of viruses. Nomenclature of viruses. Viroids. Prions.
2. Virus-host interaction. Pathogenesis of viral infections. Host responses to virus infections.
3. Cultivation of viruses. Laboratory diagnostics of viral infections. Rapid tests. Cultural method. Detection of virus and its effects in animal, hen embryo, tissue culture. Application of neutralization test for identification of virus. Serological method.
4. *Poxvirus*. Morphology and composition. Antigenic structure. Cultivation. Viruses causing human infections and their features: Variola virus, Vaccinia virus, Cowpox virus. Eradication of small pox. Vaccination against small pox.
5. Orthomyxoviridae. *Influenza virus*. Morphology and composition. Antigens. Classification. Antigenic drift and shift. Epidemiology. Pathogenesis. Clinical manifestations. Complications. Causes of death. Lab diagnostics. Prophylaxis and treatment. Vaccines.
6. Herpesviridae. Classification. Morphology and composition. *Human herpesvirus*. Herpes simplex viruses 1 and 2. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis and antiviral treatment. Vaccines.
7. Herpesviridae. Classification. Morphology and composition. *Human herpesvirus*. Varicella-zoster virus. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis and antiviral treatment. Vaccines. Planned immunization.
8. Herpesviridae. Classification. Morphology and composition. *Human*

herpesvirus. Cytomegalovirus and Epstein-Barr virus. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics.

9. Adenoviridae. *Adenovirus.* Classification. Morphology and composition. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. *Rhinovirus*. Classification. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics.
10. Picornaviridae. Classification. *Poliovirus.* Morphology and composition. Antigens. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis. Vaccines. Planned immunization.
11. Paramyxoviridae. *Human parainfluenza virus*. Morphology and composition. Classification. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics.
12. Paramyxoviridae. Morphology and composition. *Mumps virus*. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis. Vaccine. Planned immunization. *Measles virus*. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis. Vaccines. Active and passive immunization.
13. Arboviruses. Classification. General properties. Flaviviridae. Encephalitis viruses. Classification. *Japanese encephalitis virus.* Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis. Vaccine. *Russian spring summer encephalitis virus*. *Kyasanur Forest Disease virus*. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis. Vaccine.
14. Arboviruses. Classification. General properties. Flaviviridae. *Yellow fever virus*. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis. Vaccines. *Dengue virus*. Serotypes. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics.
15. Rhabdoviridae. Classification. *Rabies virus*. Morphology and composition. Antigens. Street virus and fixed virus strains. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Preexposure and postexposure prophylaxis. Active and passive immunization. Vaccination schedules.
16. Hepatitis viruses. Classification and differences. *HAV*. Morphology and composition. Serotypes. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. Prophylaxis. Vaccines. Planned immunization. *HEV*. Morphology and composition. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics.
17. Hepatitis viruses. Classification and differences. *HBV*. Morphology and composition. Antigenic structure and serotypes. Epidemiology. Pathogenesis. Clinical manifestations. Immune response. Lab

diagnostics. Prophylaxis. Vaccines. Planned immunization.

18. Hepatitis viruses. Classification and differences. *HCV*. Morphology and composition. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. *HDV*. Morphology and composition. Epidemiology. Pathogenesis. Clinical manifestations. Lab diagnostics. *HGV*. Features and lab diagnostics.
19. Retroviridae. *Human immunodeficiency virus*. Morphology and composition. Antigenic structure and serotypes. Epidemiology. Pathogenesis. Cell tropism. Clinical manifestations. Laboratory diagnostics. Lab parameters at every stage. AIDS and its manifestations. Strategies for HIV testing in India. Prevention. Antiretroviral therapy.
20. *Rubella virus*. Morphology. Epidemiology. Pathogenesis. Clinical manifestations. Laboratory diagnostics. Prophylaxis. Vaccine. Planned immunization. Slow virus infections. Basic characteristics. Prions and mechanism of their pathogenic action. Pathogenesis and features of Creutzfeldt-Jacob disease and Kuru. SSPE, role of viruses in its onset.

TESTS

MODULE VIRUSOLOGY

Variant I

1. RNA ONCOGENIC VIRUS IS:

a) Human papilloma virus
b) Epstein Barr virus
c) Human T-cell leukemia virus
d) Hepatitis B virus

2.INTRANUCLEAR AND INTRACYTOPLASMIC INCLUSION BODIES ARE SEEN IN:

a) Pox virus
b) Rabies virus
c) Measles virus
d) Herpesvirus

3. MOLLUSCUM CONTAGIOSUM IS CAUSED BY:

a) Bacteria
b) Fungus
c) Protozoa
d) *Virus*

4. ALL ARE DNA VIRUSES EXCEPT:

a) Varicella zoster virus
b) Dengue virus
c) HBV
d) Parovirus B19

***5. HIV** CONTAINS:*

a) Integrase
b) RNA directed DNA polymerase
c) Ribonuclease
d) All of the above

6. KAPOSI SARCOMA IS CAUSED BY:

a) HHV 6
b) HHV 7
c) HHV 8
d) All of the above

7. ALL ARE ASSOCIATED WITH EPSTEIN BARR VIRUS EXCEPT:

a) Burkitts lymphoma
b) Nasopharyngeal carcinoma
c) Infectious mononucleosis
d) Kaposi sarcoma

8. EBV CAUSES:

a) Infectious mononucleosis
b) Nasopharyngeal carcinoma

c) Glandular fever
d) All of the above

9. DOUBLE STRANDED RNA VIRUS:

a) Polyomavirus
b) Parvovirus
c) Rotavirus
d) Poliovirus

10. MOLLUSCUM CONTAGIOSUM IS CAUSED BY WHICH OF THE FOLLOWING:

a) Polyomavirus
b) Reovirus
c) Flavivirus
d) Poxvirus

11. MOST COMMON CAUSE OF DIARRHEA IN INFANTS AND CHILDREN IS:

a) Staphylococcus
b) Streptococcus
c) E. coli
d) Rotavirus

12. MULTINUCLEATED GIANT CELLS ARE SEEN IN SMEAR FROM THE LESIONS OF:

a) Poliovirus
b) Herpes simplex virus
c) Rabiesvirus
d) HIV

13. EBV (EPSTEIN BARR VIRUS) CAUSES ALL EXCEPT:

a) *Glandular fever*
b) Burkitt's lymphoma
c) Pancreatic carcinoma
d) Nasopharyngeal carcinoma

14. MOST SERIOUS COMPLICATION OF MEASLES IS:

a) *Croup*
b) Meningo-encephalitis
c) Otitis media
d) Pneumonia

15. CARRIERS FOR HERPES SIMPLEX VIRUS IS:

a) Man
b) Monkey
c) Both
d) None

16. ALL OF THE FOLLOWING BELONG TO ARBOVIRUSES GROUP EXCEPT:

a) Bunyavirus

b) Paramyxovirus
c) Reovirus
d) Alphavirus

17. ALL OF THE FOLLOWING IS TRUE REGARDING HIV VIRUS EXCEPT:

a) Belongs to the subgroup lentivirus
b) Double stranded DNA virus
c) Characterised by the presence of reverse transcriptase enzyme
d) Acts on CD4 cells

18. SSPE (SUBACUTE SCLEROSING PANENCEPHALITIS) IS ASSOCIATED WITH:

a) *Tetanus*
b) Meningitis
c) Cholera
d) Measles

19. DENGUE HEMORRHAGIC FEVER IS CAUSED BY:

a) Alphavirus
b) Flavivirus
c) Bunyavirus
d) Orbivirus

20. VIRUS THAT SPREADS BY BOTH HEMATOGENOUS AND NEURAL ROUTE IS:

a) Rabies virus
b) Polio virus
c) EB virus
d) Enterovirus

21. GENE THAT ENCODES THE TOXIN FOR CHOLERA IS TRANSMITTED BY:

a) Protozoa
b) Bacteria
c) Bacteriophage
d) Fungus

22. PAUL BUNNEL TEST IS DONE FOR:

a) Malta fever
b) Typhus fever
c) Enteric fever
d) Infectious mononucleosis

23. WHICH OF THE FOLLOWING IS NOT PRION ASSOCIATED DISEAS

a) Scrapie
b) Kuru
c) Creutzfeldt-jakob disease
d) SSPE

MODULE VIRUSOLOGY
Variant II

1. INTRANUCLEAR AND INTRACYTOPLASMIC INCLUSION BODIES ARE SEEN IN:

e) Pox virus
f) Rabies virus
g) Measles virus

Herpesvirus

2. RNA ONCOGENIC VIRUS IS:

e) Human papilloma virus
f) Epstein Barr virus
g) Human T-cell leukemia virus
h) Hepatitis B virus

3.MOLLUSCUM CONTAGIOSUM IS CAUSED BY:

e) Bacteria
f) Fungus
g) Protozoa
h) *Virus*

4. HIV CONTAINS:

e) Integrase
f) RNA directed DNA polymerase
g) Ribonuclease

All of the above

5. ALL ARE DNA VIRUSES EXCEPT:

e) Varicella zoster virus
f) Dengue virus
g) HBV
h) Parovirus B19

6. KAPOSI SARCOMA IS CAUSED BY:

e) HHV 6
f) HHV 7
g) HHV 8
h) All of the above

7. EBV CAUSES:

e) Infectious mononucleosis
f) Nasopharyngeal carcinoma
g) Glandular fever
h) All of the above

8. ALL ARE ASSOCIATED WITH EPSTEIN BARR VIRUS EXCEPT:

e) Burkitts lymphoma
f) Nasopharyngeal carcinoma
g) Infectious mononucleosis

h) Kaposi sarcoma

9. DOUBLE STRANDED RNA VIRUS:

e) Polyomavirus
f) Parvovirus
g) Rotavirus
h) Poliovirus

10. MULTINUCLEATED GIANT CELLS ARE SEEN IN SMEAR FROM THE LESIONS OF:

e) Poliovirus
f) Herpes simplex virus
g) Rabiesvirus
h) HIV

11. MOLLUSCUM CONTAGIOSUM IS CAUSED BY WHICH OF THE FOLLOWING:

e) Polyomavirus
f) Reovirus
g) Flavivirus
h) Poxvirus

12. EBV (EPSTEIN BARR VIRUS) CAUSES ALL EXCEPT:

e) *Glandular fever*
f) Burkitt's lymphoma
g) Pancreatic carcinoma
h) Nasopharyngeal carcinoma

13. CARRIERS FOR HERPES SIMPLEX VIRUS IS:

e) Man
f) Monkey
g) Both
h) None

14. ALL OF THE FOLLOWING BELONG TO ARBOVIRUSES GROUP EXCEPT:

e) Bunyavirus
f) Paramyxovirus
g) Reovirus
h) Alphavirus

15. ALL OF THE FOLLOWING IS TRUE REGARDING HIV VIRUS EXCEPT:

e) Belongs to the subgroup lentivirus
f) Double stranded DNA virus
g) Characterised by the presence of reverse transcriptase enzyme
h) Acts on CD4 cells

16. MOST SERIOUS COMPLICATION OF MEASLES IS:

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g) Otitis media
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g) Cholera
h) Measles

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e) Malta fever
f) Typhus fever
g) Enteric fever
h) Infectious mononucleosis

20. MOST COMMON CAUSE OF DIARRHEA IN INFANTS AND CHILDREN IS:

e) Staphylococcus
f) Streptococcus
g) E. coli
h) Rotavirus

21.GENE THAT ENCODES THE TOXIN FOR CHOLERA IS TRANSMITTED BY:

e) Protozoa
f) Bacteria
g) Bacteriophage
h) Fungus

22. WHICH OF THE FOLLOWING IS NOT PRION ASSOCIATED DISEAS

e) Scrapie
f) Kuru
g) Creutzfeldt-jakob disease
h) SSPE

23. VIRUS THAT SPREADS BY BOTH HEMATOGENOUS AND NEURAL ROUTE IS:

e) Rabies virus
f) Polio virus
g) EB virus
h) Enterovirus

Keys

1. c	**1. c**
2. c	**2. c**
3. d	**3. d**
4. b	**4. d**
5. d	**5. b**
6. c	**6. c**
7. d	**7. d**
8. d	**8. d**
9. c	**9. c**
10. d	**10. b**
11. d	**11. d**
12. b	**12. c**
13. c	**13. a**
14. b	**14. b**
15. a	**15. b**
16. b	**16. b**
17. b	**17. a,d**
18. a,d	**18. b**
19. b	**19. d**
20. b	**20. d**
21. c	**21. c**
22. d	**22. d**
23. d	**23. b**

SITUATIONAL TASKS

1.Task. A 52-year-old woman came to the regional emergency station with lacerations in her left arm and forearm. A stray dog attacked the patient on the way to work. A woman is very afraid of getting rabies.

1. Explain the epidemiological chain of rabies (reservoir in nature, transmission routes, entry gates).
2. Describe the pathogenesis of rabies. What is the relationship between the localization of the bite and the length of the incubation period?
3. What preventive measures should be taken in this patient?
4. Who is the author of the first rabies vaccine, how was it obtained, and what was the name of the first vaccine strain?

2. **Task.** Patient K., 32 years old, is in a hospital with clinical signs of Kaposi's sarcoma. When studying the immune status, a decrease in Tx CD4 to 100 cells per ml was detected, the immunoregulatory index was reduced to 0.5.

1. Make a preliminary diagnosis.
2. What laboratory methods need to be performed to confirm the diagnosis?

3.Task. A woman, 23 years old, came to the infectious diseases hospital with rashes on the inflamed skin and genital mucosa. The eruptions had the appearance of grouped vesicles with a diameter of 0.1-0.3 cm. Some of them were eroded. The disease is accompanied by fever, soreness, itching and burning in the affected areas. About a year and a half ago, she had a similar disease in a milder form, but she did not go to the doctor.

Was diagnosed with a relapse of genital herpes?

1.Describe the structure of the genome of the herpes virus.

2.What is the source and route of transmission of herpes.

3.Explain what causes relapses of herpes, where the virus persists in the inter-relapse period?

4.List the goals and methods of laboratory diagnosis of herpes. What method will allow you to quickly make the final diagnosis?

RECOMMENDED TEXTBOOKS

1. Khaitov R.M., Yarilin A.A., Pinegin B.V. Immunology. Atlas. M. 2011 - 624 pp.
2. Edited by L.V. Kovalchuk, G.A. Ignatieva, L.V. Gankovskoy Immunology.Workshop Publisher: GEOTAR-Media, 2010 - 192 pp.
3. Lectures in immunology / Лекции по иммунологии (на английском языке). А.Н Маянский. Издательство: НГМА – 2004, 256 стр.

USED TEXTBOOKS

1. R. Koiko, D. Sunshine, E. Benjamini. Immunology. - Publisher: Academy, 2008 - 368 pp.
2. Khaitov R.M. Immunology: textbook 2nd ed. 10-11g. CD // M., GEOTAR-Media. 2011 – 528 pp.
3. G.-R. Burmester, A. Petsutto, T. Ulrichs, A. Eicher. Visual immunology. Color Atlas of Immunology. Publisher: Binom. Knowledge Laboratory, 2009 - 320 pp.