**RNA viruses**

**Picornavirus**

The picornavirus family (Figure 60) comprises numerous small RNA viruses that significantly affect humans and livestock. Picornaviruses use various receptors to infect cells, and many require co-receptors or alternative receptors. This family of RNA viruses includes nine genera with diverse characteristics, causing diseases such as poliomyelitis, the common cold, hepatitis A, and foot-and-mouth disease (FMDV). The FMDV was the first identified animal virus, leading to early vaccine development. Enteroviruses and Rhinoviruses, two main groups within this family, can be found in the alimentary tract and nasopharynx, respectively.

**Classification of medically important genera:**

**Enterovirus-** Infects the intestinal tract.

**Rhinovirus-** Infects the nasopharynx.

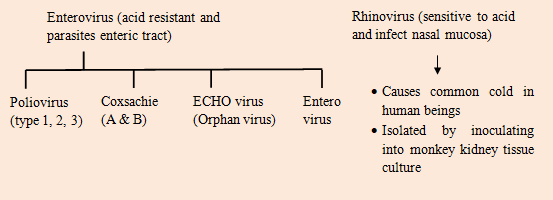
**Hepatovirus-** Causes liver infection in humans.

**Parechovirus-** Parechovirus can cause a range of illnesses, particularly in young children, including respiratory infections, gastroenteritis, and in more severe cases, encephalitis and myocarditis.

**Veterinary important genera:**

**Aphthovirus-** Causes foot-and-mouth disease in cattle.

**Cardiovirus-** Causes encephalomyocarditis in mice.

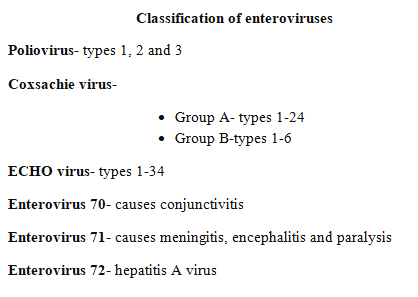
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**Figure 60: Classificaion of picornaviruses**

**Enteroviruses**

Enterovirus infect higher vertebrates and can cause a wide range of diseases, from short-term illnesses to severe conditions like central nervous system diseases, paralysis, swelling, and even death. They are notably responsible for poliomyelitis outbreaks and other serious diseases such as aseptic meningitis, enteroviral encephalitis, and enteroviral vesicular stomatitis. Enteroviruses are also a common cause of the common cold. Most enteroviruses are host-specific, infecting only one or a few related species. While there is no common group antigen for enteroviruses, some exhibit antigenic cross-reactions.

Enterovirus virions are non-enveloped, icosahedral capsids about 30 nm in diameter, composed of 60 structural subunits formed from four polypeptides, with a total molecular weight of 80 to 140 kDa, surrounding a single-stranded RNA genome. Unlike other picornaviruses such as rhinoviruses, enteroviruses remain stable across a wide pH range (3 to 10), allowing them to stay infectious through the gastrointestinal tract. Without a lipid envelope, they resist ether, chloroform, and alcohol but can be inactivated by ionizing radiation, formaldehyde, or phenol. The presence of molar MgCl2 reduces their thermolability, helping live, attenuated oral poliomyelitis (OPV) vaccines maintain potency without refrigeration.

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**Enterovirus 71 infections**

Enterovirus 71 is closely related to coxsackievirus A16. Both viruses can cause skeletal myositis in suckling mice and myelitis with paralysis in cynomolgus monkeys. Enterovirus 71 was first isolated from young children with encephalitis and aseptic meningitis in California in 1969. It has since caused infections worldwide, including large outbreaks of hand, foot, and mouth (HFM) disease, often linked with aseptic meningitis and severe central nervous system (CNS) complications in young children.

**Epidemic paralysis-** the virus is unique among non-polio enteroviruses for causing epidemic paralysis. This has included localized outbreaks involving small numbers of patients over several years and regional epidemics affecting hundreds to thousands of people in a single season.

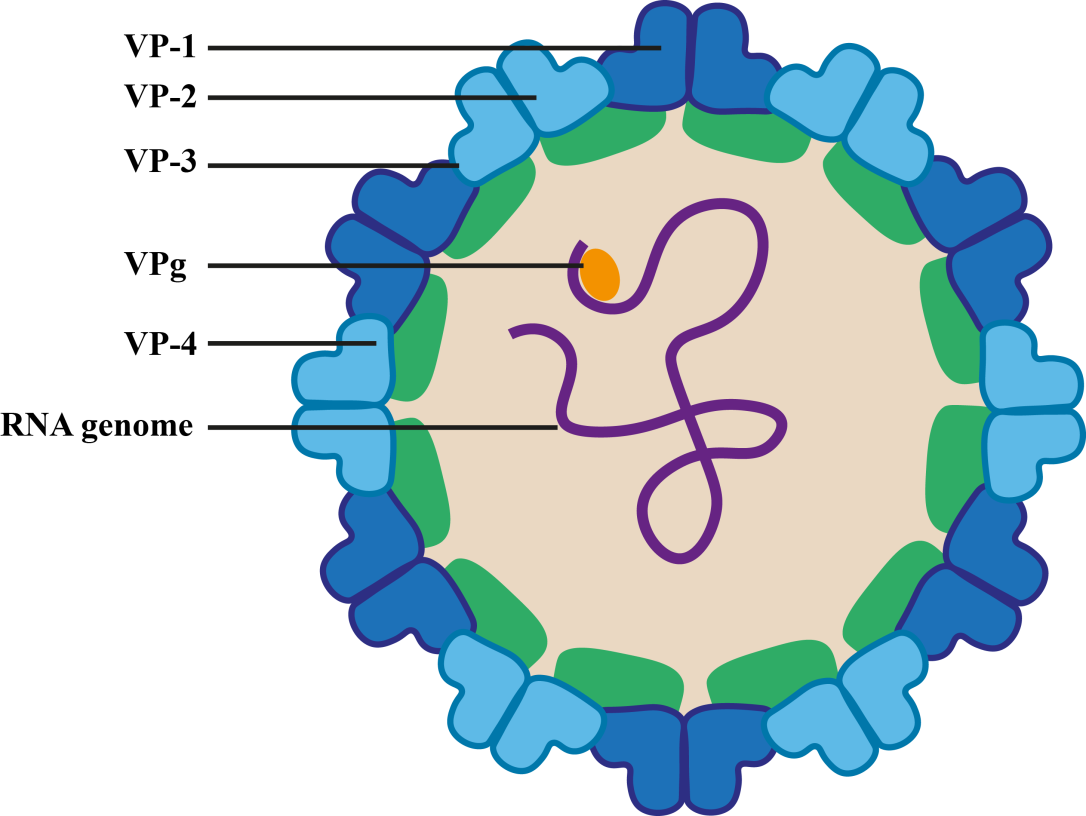
**Severe manifestations-** Infants and young children have developed brainstem encephalitis with high mortality due to rapid cardiovascular collapse and pulmonary edema. Other manifestations include generalized maculopapular rash, interstitial pneumonia, and myocarditis.

The virus can be isolated from vesicle fluid, feces, oropharyngeal secretions, urine, and cerebrospinal fluid (CSF), with the highest isolation rates from vesicle swabs and the lowest from CSF. Primary isolation is most successful in African green monkey kidney cell culture, though the cytopathic effect can take 5 to 8 days to develop. Treatment is symptomatic and supportive. Recent large-scale epidemics have been particularly notable in the Far East.

**Polioviruses**

Paralytic disease in children has been recognized since ancient times. In 1949, Enders, Weller, and Robbins discovered that polioviruses could grow in cultures of non-neural cells from human embryos, producing cytopathic effects. This groundbreaking discovery earned them the Nobel Prize. olioviruses cause poliomyelitis, a systemic viral infection that primarily affects the central nervous system (CNS) and leads to paralysis. The disease name, derived from "polios" (gray) and "myelos" (marrow or spinal cord), describes the pathological lesions in the gray matter, especially in the anterior horns of the spinal cord.

**Structure:** Poliovirus (Figure 61) is a positive-strand RNA virus in the human enterovirus C species of the Picornaviridae family. The virion is a spherical particle, about 27 nm in diameter, with 60 subunits arranged in icosahedral symmetry, each made up of four proteins (VP1-VP4). VP1, which faces outward, holds the major antigenic site for neutralizing antibodies. Viral protein genome-linked (VPG) is a small protein covalently linked to the 5' end of the viral RNA genome of poliovirus. This protein plays essential roles in the replication and translation of the viral RNA inside host cells. The genome is a single strand of positive-sense RNA, approximately 7.4 kb long, which can be directly translated by host ribosomes into a polyprotein that is then cleaved into 11 different proteins. The RNA genome itself is infectious. There are three serotypes (1, 2, and 3), identified in the 1950s, which are distinguished by their reactions with specific sera.

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**Figure 61: Structure of poliovirus**

**Antigenic types:** There are three antigenic types of poliovirus: 1, 2, and 3. The prototype strains for these types are Brunhilde and Mahoney (type 1), Lansing and MEF1 (type 2), and Leon and Saukett (type 3). Type 1 is the most common and responsible for most epidemics, type 2 typically causes endemic infections, and type 3 has also caused epidemics. Immunity to poliovirus is type-specific.

In the 1950s, it was found that poliovirus cultures fractionated using a five-step sucrose gradient (A to E) displayed two peaks of antigenic activity: one at step C and another at step D. The C antigen reacted more strongly with acute-phase human sera, while the D antigen reacted with convalescent sera. The C antigen corresponds to empty capsids, while the D antigen corresponds to full infectious virus particles.

The D antigen (native or N antigen, dense) is associated with the whole virion and is type-specific. It is protective and its measurement is used to determine the potency of inactivated polio vaccines (IPV). The C antigen (heated or H antigen, coreless or capsid) is associated with the empty, non-infectious virus. Anti-C antibodies do not neutralize infectivity and are not protective. The D antigen can be converted to the C antigen by heating the virus to 56°C.

**Pathogenesis:** Poliovirus infection involves two viremic phases: the first following gut infection and the second about a week later, spreading to other sites, such as the throat and tonsils. The presence of humoral antibodies greatly inhibits or prevents this viremic spread. Studies in the 1950s showed that passive antibodies could protect against poliomyelitis, explaining polio epidemics as improved hygiene delayed exposure until after maternal antibodies waned. In high-circulation areas like Northern India, many cases occurred despite high maternal antibody levels, indicating maternal protection can be overwhelmed by high exposure.

Poliovirus is primarily spread through the fecal-oral route, although respiratory transmission is possible. The virus initially colonizes and multiplies in the nasopharynx, throat, and feces. It then moves to Peyer's patches and lymphatic tissues of the alimentary canal. The virus spreads to regional lymph nodes and enters the bloodstream (primary viremia). In asymptomatic cases, the virus is contained at this stage, prompting type-specific antibody formation. However, in a small number of cases, the virus replicates further, causing a major viremia and the symptoms of abortive poliomyelitis.

The virus replicates further in the reticuloendothelial system, and reenters the bloodstream (secondary viremia) to reach the spinal cord and brain. The virus can also travel along peripheral nerve axons to the CNS. In the CNS, poliovirus selectively multiplies in and destroys neurons, starting with the degeneration of Nissl bodies followed by nuclear changes. Necrotic cells are lysed or phagocytosed by leukocytes or macrophages. Lesions are most prominent in the anterior horns of the spinal cord, causing flaccid paralysis, but can also affect the posterior horns and intermediate columns. Pathological changes often exceed the extent of paralysis. In some cases, encephalitis occurs, affecting the brainstem and extending to the cerebral cortex's motor areas.

**Epidemiology:** Localized paralytic polio epidemics began appearing in the United States around 1900, with a significant outbreak in Brooklyn, New York, in 1916 resulting in thousands of deaths. The summer season often brought about polio epidemics, leading to widespread panic and quarantine measures. Poliomyelitis is exclusively human and transmitted through the fecal-oral route. Patients or symptomless carriers shed the virus in feces, with temporary carriage lasting up to several months. The virus can persist in the environment, including sewage, for extended periods. Infection is often asymptomatic, with the ratio of subclinical to clinical infections estimated to be high. Certain factors, such as pregnancy and tonsillectomy, may increase the risk of paralysis.

**Clinical presentation:** In cases progressing to paralytic poliomyelitis, flaccid paralysis initially focal, spreads over 3-4 days (Table 26). Paralysis distribution classifies cases as spinal, bulbar, or bulbospinal. Mortality ranges from 5-10%, primarily due to respiratory failure. Muscle recovery occurs over 4-8 weeks, typically complete after six months, though some residual paralysis may remain. The incubation period for poliomyelitis is estimated to be 9-12 days (range 5-35 days) from contact to prodromal symptoms, and 11-17 days (range 8-36 days) until the onset of paralysis.

**Postpoliomyelitis syndrome-** Some patients who have partially or fully recovered from paralytic poliomyelitis experience a delayed onset of muscle weakness, pain, atrophy, and fatigue many years after the initial illness. Typically, the muscles affected are the same as those impacted during the original episode, though weakness can also appear in previously unaffected muscles. The progression of these new symptoms is usually gradual, and severe disability is rare.

**Table 26: Clinical symptoms of poliovirus**

|  |  |
| --- | --- |
| Disease conditions | Symptoms |
| Inapparent infection | Poliovirus infections range from asymptomatic to severe paralysis and death. Approximately 95% of infections are asymptomatic and detectable only through virus isolation or antibody titers.. Clinical illness occurs in just 5-10% of cases. The incubation period averages 10 days, ranging from 4 days to 4 weeks. |
| Abortive poliomyelitis (Minor Illness) | The earliest symptoms, appearing during primary viremia, include fever, headache, sore throat, and malaise, lasting 1-5 days. It occurs in 4-8% of cases. |
| Paralytic poliomyelitis (major illness)- | Paralysis occurs in about 0.1% of infections. It often follows a biphasic course, with minor illness symptoms similar to abortive poliomyelitis, followed by major illness (3-4 days later) characterized by a return of fever (biphasic fever), headache, stiff neck, and meningitis symptoms, indicating CNS involvement. Paralysis is flaccid and asymmetrical, commonly affecting the proximal muscles more than distal ones, with legs more involved than arms. The paralysis progresses over 2-3 days, stopping when the patient becomes afebrile. Bladder paralysis is common in adults but rare in children. Sensory loss is rare and suggests other diagnoses like Guillain-Barré syndrome. |
| Bulbar paralytic poliomyelitis | This form affects muscles innervated by cranial nerves, leading to symptoms like dysphagia and nasal speech. It can involve the ninth and tenth cranial nerves, causing pharyngeal paralysis and respiratory complications. |
| Polioencephalitis | Primarily affecting infants, polioencephalitis causes confusion and disturbances of consciousness, sometimes leading to spastic paralysis due to upper motor neuron involvement. |
| Non-paralytic poliomyelitis | Nonparalytic poliomyelitis includes signs of meningeal irritation (aseptic meningitis) and is more severe than abortive poliomyelitis. |
| Complications | The most critical complication is respiratory failure due to paralysis of respiratory muscles or medullary centers. Myocarditis, gastrointestinal issues, lymphatic hyperplasia and paralysis-related complications can also occur. |
| Risk factors | Factors influencing infection and paralysis risk include gender (boys before puberty, women in adulthood), pregnancy, and strenuous exercise during major illness. Intramuscular injections or injuries before infection can localize the disease to a specific limb. Tonsillectomy increases the risk of bulbar poliomyelitis due to potential viral spread from damaged nerve endings. |

**Laboratory diagnosis:** Specimens ofthroat swabs, feces (rectal swabs), blood, and cerebrospinal fluid (CSF) are used.

**1. Viral isolation-** The preferred method for specific diagnosis of poliovirus is virus isolation in tissue culture, which grows well in primate tissue cultures. Primary monkey kidney and continuous human/simian tissue cultures are commonly used for virus isolation and vaccine production. Infected cells show characteristic cytopathic effects within 2-3 days, including rounding, becoming refractile and pyknotic, and forming eosinophilic intranuclear inclusion bodies. Virus identification is confirmed by neutralization tests with specific antisera. However, virus isolation alone does not confirm poliomyelitis; it must be interpreted alongside clinical and serological evidence.

* **Blood:** Virus can be isolated during primary viremia (3-5 days post-infection) but is rarely practical.
* **Throat:** Virus can be isolated in the early stages of the disease.
* **Feces:** Virus isolation is effective in over 80% of patients in the first week, 50% up to the third week, and 25% up to the sixth week. Multiple samples increase accuracy, especially early in illness. Prolonged excretion can occur in immunodeficient individuals, but there are no carrier stages.
* **CSF:** Unlike other enteroviruses, poliovirus is rarely isolated from CSF but can be obtained from spinal cord and brain postmortem.

**2. Serodiagnosis-** A four-fold rise in antibody titer detected in paired sera via neutralization or complement fixation (CF) tests.

* **Neutralization test-** Neutralizing antibodies appear early and persist for life
* **CF test-** CF test antibodies to the C antigen appear first and fade quickly. The test is useful for exposure identification**.** Anti-D antibodies appear weeks later but last for about five years. but not type-specific diagnosis.

**3. Molecular diagnosis-** The primary diagnostic method for poliovirus is polymerase chain reaction (PCR). To maximize isolation, two stool samples should be collected 24 hours apart within the first two weeks. The virus appears in the oropharynx early in infection and is rarely found in CSF. During the initial viremia phase (3-5 days post-infection), the virus can be isolated from blood, but this is not diagnostically significant.

* **Reverse transcriptase PCR-** PCR-based tests enhance viral RNA detection in CSF.
* **Sequencing-** Differentiates wild virus, oral polio vaccine (OPV) virus strains, and vaccine-derived polioviruses (VDPVs) within a population. Genetic sequencing is crucial for determining the virus's origin and transmission mode during outbreaks, aiding in containment efforts.

**Prognosis:** Muscular paralysis in poliomyelitis typically progresses for 1-3 days, occasionally extending up to a week. Permanent weakness occurs in approximately two-thirds of paralytic cases, with complete recovery being less likely in severe cases requiring mechanical ventilation. Prognosis can be assessed after one month, with minimal additional recovery expected beyond nine months. Recovery from pharyngeal paralysis is usually evident by 10 days and eventually complete. Bulbar poliomyelitis rarely results in permanent sequelae in survivors.

**Management:** Specific antiviral drugs for poliomyelitis are unavailable, so management focuses on supportive care and symptom relief. During the acute phase of paralytic poliomyelitis, hospitalization is necessary. Bed rest may prevent worsening paralysis, and hot moist packs can alleviate muscle pain and spasms. Physical therapy should begin once paralysis progression stops. Bladder paralysis may necessitate catheterization.

**Immunoprophylaxis:**

**1. Passive immunization-** The administration of human gammaglobulin for passive immunization offers limited value in preventing poliomyelitis.

**2. Active immunization-** For nearly 50 years, both IPV and live-attenuated OPV (Table 27) have effectively controlled paralytic poliomyelitis.

**a. Salk's killed vaccine-** The inactivated poliovirus vaccine (IPV) comprises formalin-inactivated poliovirus serotypes 1-3, cultured in monkey kidney cells. Early challenges with incomplete virus inactivation were addressed through modifications, enhancing safety. IPV is administered intramuscularly in a series of three doses followed by a booster. Advances in vaccine formulation and potency have significantly increased seroconversion rates, with neutralizing antibodies present in 99% of recipients after two doses and 100% after three doses. Although children vaccinated with IPV may exhibit prolonged viral shedding compared to those vaccinated with oral poliovirus vaccine (OPV), IPV still contributes to partial herd immunity.

**b. Sabin's live attenuated vaccine-** Albert Sabin developed an oral polio vaccine (OPV) utilizing attenuated poliovirus strains to induce active immunity. Attenuation criteria ensure both safety and efficacy. Administered orally, OPV provides local intestinal immunity. Molecular epidemiological methods have addressed the challenge of differentiating between wild and attenuated strains. Since its introduction in 1962, OPV has been widely adopted for mass vaccination campaigns due to its high immunogenicity, cost-effectiveness, and ease of administration. However, OPV carries a risk of vaccine-associated paralytic poliomyelitis (VAPP), prompting developed countries to prefer the inactivated poliovirus vaccine (IPV). OPV's ability to induce local intestinal immunity contributes to community-wide protection and herd immunity. The immunity duration conferred by OPV is similar to that of natural infection, potentially outlasting that provided by killed vaccines.

**c. Monovalent vaccine-** Monovalent OPV vaccines had been replaced by trivalent vaccines that were reintroduced in 2005 to enhance immunogenicity and target specific poliovirus types during outbreaks. These vaccines are particularly effective in resource-limited settings and have been instrumental in global polio eradication efforts.

**Table 27: Difference between IPV and OPV**

|  |  |  |
| --- | --- | --- |
| Vaccine difference | IPV | OPV |
| Formulation | Inactivated (killed) poliovirus strains (types 1, 2, and 3). | Live, attenuated virus strains (types 1, 2, and 3). |
| Valency | Only trivalent, targeting all three serotypes. | Available as trivalent (tOPV), monovalent (mOPV1, mOPV3), or bivalent (bOPVs) |
| Pathogenesis | Generates humoral immunity, reducing virus replication, but does not provide gut immunity. | Induces a local immune response in the intestines, providing mucosal immunity. |
| Administration | Given intramuscularly (upper arm or anterolateral thigh), alone or combined with other vaccines. | Administered orally as drops. |
| Dosage | Two fractional dose at 6 and 14 weeks of age | OPV 0- at birth immediately.  OPV 1, 2 & 3 - 6, 10 & 14 weeks (OPV cam be given till 5 years). |
| Safety | Virus is inactivated, no risk of disease from vaccine | Rarely, the vaccine virus can mutate and cause vaccine-derived poliovirus (VDPV) in immunocompromised |
| Efficacy | Highly effective in preventing paralytic polio and its transmission. | Highly effective in inducing mucosal immunity; beneficial in areas with poor sanitation. |
| Indication | Used in countries where wild poliovirus transmission has been interrupted or inactivated poliovirus outbreaks occur. | Historically used in global polio eradication efforts due to its ability to induce herd immunity. |
| Potential drawbacks | Requires trained healthcare professionals for administration, may not provide as robust mucosal immunity as OPV. | Risk of vaccine-associated paralytic polio (VAPP) in a very small number of vaccine recipients, especially in areas with low vaccination coverage and poor sanitation. |

**Global polio immunization programme:**

**A. Expanded program on immunization-** Initially, polio was seen as an epidemic mainly afflicting wealthier nations, causing it to be overlooked in developing countries where most cases, particularly type 1 poliovirus, affected children aged 6 months to 2 years. The WHO's Expanded Program on Immunization (EPI) aimed to address this, but challenges like supply disruptions and political indifference hindered progress. After the eradication of wild type 2 poliovirus, a shift from trivalent to bivalent OPV (types 1 and 3) was necessary to reduce risks associated with type 2 strains. Concerns about OPV's risks, including vaccine-derived polioviruses (cVDPVs), prompted developed countries to adopt the IPV.

**WHO eradication and containment strategy includes-**

* Detecting and interrupting poliovirus transmission.
* Strengthening routine immunization and transitioning to IPV and bOPV.
* Implementing containment plans for wild poliovirus.
* Legacy planning for the post-eradication phase.

Post-eradication strategies involve facility containment, high vaccine coverage, and hygiene measures, with phases of surveys, stock destruction, and OPV recall. Alternative IPV production methods are being explored to address containment challenges.

**B. Global polio eradication initiative-** In 1988, the World Health Assembly, the decision-making body of the World Health Organization (WHO), launched the Global Polio Eradication Initiative (GPEI) with the ambitious goal of eradicating polio worldwide by 2000. At that time, polio was endemic in 125 countries. The GPEI is a public-private partnership that includes WHO, UNICEF, the US Centers for Disease Control and Prevention (CDC), Rotary International, and the Bill and Melinda Gates Foundation. This coalition supports routine immunization programs, ensuring infants receive three doses of the OPV before their first birthday.

**GPEI key efforts-**

* National Immunization Days (NIDs): Administering two doses of OPV to all children 4-8 weeks apart, regardless of previous immunization history.
* Outbreak Response Immunization: Providing one OPV dose to children under five in the area of a detected polio case.
* Mopping-Up Immunization: Visiting homes in outbreak-prone areas to administer two OPV doses to children under five, spaced one month apart.

**Progress and challenges-** While complete eradication has not been achieved, the GPEI has dramatically reduced the prevalence of polio, eradicating two of the three wild poliovirus serotypes.

**Pulse plio programme:** Before the 1990s, India grappled with significant polio outbreaks despite being a pioneer in polio research and vaccine production. The Indian Council of Medical Research (ICMR) established polio research units in Mumbai and Vellore, revealing the country's hyperendemic status for poliovirus.In 1995, the Government of India initiated the Pulse Polio Immunization (PPI) program to vaccinate all children under 5 with oral polio vaccine (OPV) on designated national immunization days (NID). Despite initial successes, around 5-6% of children were missed, leading to house-to-house searches for vaccination.

India persisted with OPV despite its low efficacy, while inactivated polio vaccine (IPV) showed promise elsewhere but wasn't used until licensed in 2006. India's switch to IPV faced challenges due to regulatory and governmental hurdles. Despite these obstacles, India achieved a significant milestone in 2012 when the World Health Organization (WHO) declared it polio-free after sustained transmission interruption. However, global polio threats persist, with WPV circulating in other countries like Nigeria, Pakistan, Afghanistan, and Chad.

True eradication requires transitioning from OPV to IPV to eliminate both wild and vaccine-derived polioviruses. India's journey from high polio prevalence to eradication showcases substantial progress but also underscores the need for continued vigilance and vaccination efforts to prevent new outbreaks.

**Vaccine-induced polio:** In rare instances, the live poliovirus in the oral poliovirus vaccine (OPV) can mutate, regaining its ability to cause neurovirulence, or attack the central nervous system. This can result in paralysis similar to that caused by the wild poliovirus.

There are two primary ways-

**1. Vaccine-Associated Paralytic Poliomyelitis (VAPP)-** This occurs when the virus mutates spontaneously in a recently vaccinated individual. VAPP is extremely rare, with an incidence of 0.09 to 25 cases per million OPV doses. Before OPV was removed from the U.S. immunization schedule in the late 1990s, an average of eight VAPP cases were reported annually. Globally, 250 to 500 VAPP cases occur annually in countries that still use OPV.

VAPP in immunodeficient patients has distinct features, such as a longer interval (1 to 8 months, sometimes up to 7 years) between OPV administration and disease onset, chronic meningitis, progressive neurological dysfunction, and a higher risk of mortality. Most cases in this group are associated with type 2 OPV virus.

**Incidence and risk:**

* **Recent vaccinees-** Individuals who recently received OPV, typically developing paralysis 7 to 21 days post-vaccination.
* **Household contacts-** Close contacts (e.g., family members or caregivers) who develop paralysis 20 to 29 days after the vaccinated individual receives OPV.
* **First dose association-** Over 80% of VAPP cases are linked to the first OPV dose. Type 2 and 3 OPV are common cuase.
* **Immunodeficient individuals-** Approximately 25% of VAPP cases occur in immunodeficient individuals, especially those with B-cell immunodeficiencies, severe combined immunodeficiency syndrome, or common variable immunodeficiency.

**2. Vaccine-Derived Poliovirus (VDPV)-** This occurs when mutations happen over a longer period, resulting in a virus genetically similar to the vaccine strain. The weakened virus in OPV mutates and circulates in under-immunized populations, acquiring properties similar to wild polioviruses.

VDPVs are classified as (GPEI)-

* **Immune Deficiency Associated Vaccine-Derived Poliovirus (iVDPVs)-** These arise in individuals with primary immunodeficiencies.
* **Circulating Vaccine-Derived Poliovirus (cVDPVs)-** These are VDPVs that have spread within the community, causing new outbreaks.
* **Ambiguous Vaccine-Derived Poliovirus (aVDPVs)-** These are VDPVs with no clear evidence of origin from immunodeficient individuals or community transmission.

**Notable Outbreaks-**

* **Hispaniola (2000-2001)-** An outbreak of 21 paralytic cases caused by a virulent strain related to the type 1 Sabin OPV vaccine strain.
* **Other regions-** Similar outbreaks have occurred in economically deprived areas with low immunization rates, allowing the virus to mutate and circulate.

**Managing VDPV risks-** The risk of VDPVs circulating is generally low, and new effective vaccines, called novel Oral Poliovirus Vaccines (nOPV), have been developed to address these risks. Most cVDPV cases originate from the OPV against poliovirus serotype 2.

**Coxsackie virus**

Coxsackieviruses are non-enveloped, linear single-stranded RNA viruses. They are characterized by their ability to infect suckling mice but not adults and their limited growth in cell cultures. Based on the pathological changes in suckling mice, coxsackieviruses are divided into two groups: A and B. Group A coxsackieviruses are known to cause flaccid paralysis due to generalized myositis, while group B coxsackieviruses cause spastic paralysis due to neuronal tissue degeneration and focal muscle injury.

**Structure and classification:** Coxsackieviruses, belongs to the Picornaviridae family and the Enterovirus genus. They are human enteroviruses characterized by a single positive-strand RNA enclosed in a protein-based icosahedral capsid, without an envelope or enzymes. These viruses are 22–30 nm in size. They are classified into two subgroups: coxsackievirus A (CA) with 23 serotypes (1-24, excluding 23) and coxsackievirus B (CB) with six serotypes (1-6). This classification is based on the types of histopathologic lesions they cause in suckling mice.

**Epidemiology:** Coxsackieviruses are widespread globally, with each serotype predominating in specific regions. Climate plays a significant role in their spread, with activity peaking in summer and autumn in temperate regions, circulating year-round in tropical areas, and persisting at low levels with seasonal increases in Mediterranean climates. Coxsackievirus B affects both genders equally and is prevalent worldwide. Infections vary in severity with age, with neonates being more vulnerable. In low socioeconomic settings, infants often contract the virus early in life, while in more developed areas, infections may occur later. Coxsackieviruses are common human pathogens, with similar viruses found in pigs, and chimpanzees experiencing mostly subclinical infections, although CA7 can cause paralysis in monkeys.

**Pathogenesis:** Coxsackieviruses are primarily transmitted through oral contact with contaminated hands, water, or food. They can also spread via aerosols, especially during respiratory outbreaks or from individuals with mild infections. These viruses can affect various organs including muscles, heart, brain, pancreas, lungs, skin, and eyes. Certain strains like cardiotropic and pancreatropic show specific pathogenic properties. Following oral transmission, the virus multiplies in the throat, small intestine, and lymph nodes before spreading to target organs via the bloodstream or lymphatic system. Most infections are asymptomatic, with susceptibility influenced by immune status, deficiencies, genetic factors, and hormonal interactions.

**Clinical presentation:** Clinical presentations (Table 28) of Coxsackieviruses encompass a spectrum of symptoms, from mild to severe that include-

**Table 28: Clinical presentations of coxsackie viruses**

|  |  |
| --- | --- |
| Coxsackie virus | Clinical presentations |
| Group A | **Herpangina-** presents abruptly with fever, vomiting, myalgia, headache, and sore throat; the characteristic enanthem appears as punctate macules evolving into erythematous papules, which vesiculate and centrally ulcerate over 24 hours; moderately painful lesions are found on the soft palate and uvula; fever subsides in 2 to 4 days; ulcers may persist up to a week. |
| **Aseptic meningitis-** displays symptoms such as fever, headache, neck stiffness, and light sensitivity; causes inflammation of brain and spinal cord membranes; commonly affects children and young adults; symptoms resolve within a week to ten days with supportive care; coxsackievirus A7 can lead to epidemics and paralysis outbreaks. |
| **Hand, Foot and Mouth Disease (Herpetiform exanthems)-** manifests as clusters of papulovesicular lesions on the skin and oral mucosa; affects young children (<10 years old); associated with Coxsackievirus A16 and A9; symptoms include oral vesicles mainly on the cheeks and tongue, with low-grade fever. |
| **Neonatal pneumonia-** Reported in the first few days of life, always fatal, caused by echovirus types 6, 9, 11, and group A3. |
| Group B | **Epidemic myalgia (Bornholm disease/ Pleurodynia)-** involvement of skeletal muscles; severe spasmodic pain in the chest or upper abdomen; abrupt onset of localized pain, often accompanied by fever, sore throat, and headache, without cough or coryza; recovery within 4 to 6 days; children experiences milder symptoms; may appear sporadically or in outbreaks. |
| **Myopericarditis-** common in adolescents and young adults (especially males); symptoms appear 7 to 14 days after an upper respiratory infection and include dyspnea, chest pain, fever, and malaise; The chest pain can be dull and angina-like or sharp and pleuritic. |
| **Juvenile diabetes-** association with Coxsackie B4 infection; causality remains unestablished. |
| **Transplacental and neonatal infections-** Group B viruses can cause disseminated disease in neonates, involving hepatitis, meningoencephalitis, and adrenocortical involvement. Some of the common conditions are-   * **Myocarditis-** Commonly caused by group B coxsackieviruses and sometimes echovirus 11. Symptoms include heart failure, respiratory distress, tachycardia, cardiomegaly, and cyanosis. Severe cases show rapid circulatory collapse and often involve multiple organs (CNS, liver, pancreas, adrenal gland). * **Hepatitis-** Characterized by jaundice, bleeding, metabolic acidosis, and multiple organ failure. Symptoms rapidly worsen, leading to uncontrollable hemorrhage and hepatic failure. Despite intensive care, over half of affected infants die within days. Survivors may develop hepatic fibrosis and chronic liver insufficiency. |
| **Post-viral fatigue syndrome-** Associated with group B viruses, but the condition and its connection are not clearly defined. |
| Complications | Orchitis has been observed in adolescent boys infected with group B coxsackieviruses 2, 4, and 5. Additionally, splenomegaly and a heterophile-negative mononucleosis-like syndrome have been observed. |

**Lab diagnosis:** Diagnosing a Coxsackievirus infection involves a combination of clinical evaluation and laboratory tests-

**Specimen collection-** Samples can be taken from throat swabs, stool, cerebrospinal fluid (CSF), and other body fluids or tissues.

**1. Virus isolation**- Coxsackievirus isolation involves inoculating suckling mice, typically through intracerebral, subcutaneous, and intraperitoneal routes, as adult mice are not susceptible. Suckling hamsters can also be experimentally infected. Coxsackie B viruses grow well in monkey kidney tissue cultures, while only types 7 and 9 of group A Coxsackieviruses thrive in this medium. Additionally, Coxsackie A21 virus can grow in HeLa cells. Coxsackieviruses can cause a characteristic cytopathic effect in certain cell lines, aiding in their identification.

**2. Polymerase chain reaction (PCR)**-

* **RT-PCR**: Reverse transcription PCR is used to detect and amplify viral RNA from clinical specimens. This method is highly sensitive and specific, allowing for the identification of the virus even in low quantities.
* **Real-Time PCR**: This quantitative method can determine the viral load and is often used for faster and more precise diagnosis.

**3. Serology**-

* **Neutralization Tests**: These tests measure the presence of neutralizing antibodies against Coxsackieviruses in the patient's serum, indicating past or current infection.
* **Enzyme-Linked Immunosorbent Assay (ELISA)**: Used to detect specific IgM and IgG antibodies. The presence of IgM suggests a recent infection, while IgG indicates past exposure or immunity.

**4. Genotyping**- Sequencing the viral genome or specific regions can help identify the serotype of Coxsackievirus, which is useful for epidemiological studies and outbreak investigations.

**5. Histopathology and immunohistochemistry**-

* **Tissue biopsy**: In cases of severe disease such as myocarditis, a biopsy of the affected tissue may be performed. Histopathological examination can reveal characteristic changes caused by viral infection.
* **Immunohistochemistry**: This method uses antibodies to detect viral antigens in tissue sections, providing evidence of infection.

**Treatment:** No specific vaccines for Coxsackievirus prevention are currently available or planned in the near future. Preventive measures focus on general hygiene practices, especially hand washing and sanitary control of swimming pools and meals, as the virus spreads mainly through dirty hands or contaminated water and food. Currently, there are no specific antiviral agents for Coxsackievirus therapy, although γ-globulins have shown some efficacy in limited cases.

**Acute Hemorrhagic Conjunctivitis (AHC)**

AHC is a highly contagious eye infection marked by pain, eyelid swelling, and subconjunctival hemorrhage, which typically resolves on its own within a week. Predominantly it is caused by enterovirus 70 and coxsackievirus A24.

**Epidemiology:** First emerged in 1969, spreading rapidly from Ghana and Indonesia to many parts of the world. Enterovirus 70 was responsible for widespread epidemics across Africa, Europe, and Asia. Coxsackievirus A24 was identified in large outbreaks in Singapore and later spread across Southeast Asia and the Indian subcontinent. Most AHC outbreaks occur in crowded coastal tropical regions during the hot, rainy season. In economically developed regions, AHC outbreaks are less common and mostly limited to certain seasons.

**Patterns of transmission:** AHC spreads primarily through direct contact from fingers or contaminated objects to the eye. Rapid person-to-person transmission is common, especially in crowded, unsanitary conditions, and often in poor communities. Reuse of water and sharing towels further contribute to the spread. Nosocomial transmission, particularly in ophthalmology clinics, has also been noted.

**Clinical presentation:** Symptoms of AHC appear suddenly and peak within 24 hours, including burning, foreign body sensation, pain, photophobia, swelling, and watery discharge, typically affecting both eyes. Fever, malaise, and headache occur in 20% of cases. Subconjunctival hemorrhage is a key sign, present in 70-90% of enterovirus 70 cases, but less common with coxsackievirus A24. Recovery usually begins within a few days and completes in about 10 days, although some discoloration may persist.

**Complications:** Severe AHC cases can lead to keratitis lasting several weeks, though permanent scarring is rare. Secondary bacterial infections can complicate conjunctivitis. Notably, over 200 cases of acute motor paralysis, resembling poliomyelitis, have been reported in connection with AHC caused by enterovirus 70, particularly in regions like India and Thailand.

**Laboratory diagnosis:** Diagnosis involves recovering viruses from conjunctival swabs or scrapings within the first three days of illness. Isolation rates are high for coxsackievirus A24 and slightly lower for enterovirus 70. Rising antibody titers in patients can confirm infection.

**Treatment and prevention:** Treatment is primarily symptomatic, as antimicrobial agents are not effective. Preventive measures include thorough hand washing, using separate towels, and sterilizing ophthalmologic instruments to prevent contagion.

**Echovirus**

Echoviruses, initially recognized through tissue cultures, were named "enteric cytopathogenic human orphan viruses" because they couldn't be linked to any specific disease at first. These viruses, part of the Human enterovirus B (HEV-B) species in the Enterovirus genus of the Picornaviridae family, were first isolated from stool samples and shown to cause cytopathic effects in cell cultures.

**Structure:** Echoviruses are small (27-30 nm), non-enveloped viruses with a single-stranded RNA genome of about 7,400 nucleotides. Initially, 28 distinct echovirus serotypes were identified, but some were reclassified (e.g., serotype 10 as a reovirus, and serotypes 22 and 23 as Parechoviridae). Modern classification based on molecular criteria has identified over 100 distinct enterovirus types, with echoviruses forming a genetically coherent group. Echoviruses share the basic properties of the enterovirus genus, including polioviruses. They have a protein capsid (30 nm) made of 60 copies of four structural proteins (VP1-4) enclosing a single-stranded RNA genome of about 7400 nucleotides.VP1, VP2, VP3 form the outer shell of the capsid and are involved in receptor binding and immune recognition, while VP4, located on the inner surface, is crucial for stabilizing the structure and RNA packaging.

**Resistance:** Echoviruses, resistant to organic solvents and low pH, were distinguished by their ability to grow only in cell cultures and not in laboratory animals, leading to their initial classification as "orphan" viruses.

**Pathogenesis:** Echoviruses thrive in human and simian kidney cultures, where they produce cytopathic effects, but they naturally infect only humans. They are not pathogenic to laboratory animals, although some strains can cause paresis in monkeys and newborn mice. Upon entering the body, echoviruses typically target the epithelial cells of the gastrointestinal tract and can also infect the upper respiratory tract. The virus replicates in the oropharynx and intestinal mucosa, usually causing asymptomatic or mild, nonspecific symptoms. After this initial replication, echoviruses can enter the bloodstream, leading to viremia, which allows the virus to spread to various target organs.

**Epidemiology:** Echoviruses primarily inhabit the alimentary tract and spread via the fecal-oral route, with epidemics often occurring in summer. Transmission occurs through respiratory and gastrointestinal routes, with initial replication in epithelial tissue, potentially followed by viremia and secondary organ infection. Nosocomial outbreaks have been noted in neonatal units. There is no animal reservoir. Echoviruses are frequently isolated from children under 5 due to immunological reasons and higher transmission risk, often in central nervous system infections. Adults can also be infected, but they generally have milder symptoms or are asymptomatic due to previous exposure and immunity. Infections are more common in males and those of lower socioeconomic status.

**Clinical presentation:** Echovirus disease patterns are highly variable, with most infections being subclinical, similar to other human enteroviruses. The same serotype may result in different clinical outcomes in patients during the same epidemic.

* **Aseptic meningitis-** Commonly caused by echoviruses, including EV6, EV9, EV11, and EV30. Symptoms include fever, headache, nausea, neck stiffness, respiratory illness, rash, or myalgia. Rare cases of paralysis reported.
* **Neonatal infections-** Severe generalized infections resembling bacterial sepsis. Can include meningitis, meningoencephalitis, myocarditis, and hepatitis.Transmissions may occur in transplacental or soon after delivery, also from other infected infants.
* **Immunocompromised patients-** Serious chronic infections such as meningoencephalitis may occur. Sometimes may be fatal. The disease is observed in individuals with B-cell deficiencies, treated with gammaglobulin.
* **Respiratory and skin infections-** Symptoms include mild respiratory infections (common cold, bronchitis, herpangina) and maculopapular rashes. Roseola-like skin manifestations such as the 'Boston exanthem' caused by echovirus 16.Isolated cases of conjunctivitis and uveitis are seen in specific outbreaks.
* **Gastrointestinal infections-** Causative agents of acute viral gastroenteritis, despite replication in the gastrointestinal tract.
* **Cardiac involvement-** Associated with myocarditis, particularly in neonates. Suspected role in dilated cardiomyopathy.

**Protective immunity:** Mainly antibody-mediated, largely serotype-specific, potentially resulting in life-long immunity.Cell-mediated immunity is less understood but may involve cross-reactive epitopes.

**Laboratory diagnosis:** Various samples, including feces, throat swabs, or CSF, are commonly utilized for echovirus detection. Traditional methods involve inoculating these samples into monkey kidney tissue cultures to observe virus growth through cytopathic changes. However, the abundance of echovirus serotypes complicates identification via neutralization tests, although hemagglutination and serum pools can streamline this process. Additionally, echoviruses may persist in stool for an extended period after infection. RT-PCR is effective for detecting CNS infections, while sequencing the VP1 gene offers detailed information about virus types. Acute infection can be indicated by the presence of enterovirus-specific IgM antibodies or a rise in antibody titers between acute and convalescent phase samples.

**Prevention:** Preventing Echovirus infections relies on practicing good hygiene and public health measures. No specific vaccine is currently available.Surveillance systems help monitor Echovirus prevalence for timely interventions. Immunoprophylaxis, such as intravenous immunoglobulin (IVIG), may be considered for high-risk populations but is not routinely recommended. Vaccine development for Echovirus and other enteroviruses is challenging due to the large number of serotypes and the need for serotype-specific neutralizing antibodies. Chemotherapeutic agents like pleconaril have shown promise but have limited use due to side effects.

**Top of Form**

**Bottom of Form**

**Rhinovirus**

Rhinoviruses are leading cause of upper respiratory tract infections worldwide, resulting in significant economic burdens due to medical visits and absenteeism.

**Structure:** Rhinoviruses belong to the Picornaviridae family and Enterovirus genus. The capsid (30 nm) exhibits icosahedral symmetry, with 12 vertices, each containing five capsid proteins (VP1-VP4). The 7200 bp genome contains positive-sense, single-stranded RNA.. VP1, VP2, and VP3, contribute to the virus' antigenic diversity, while VP4 anchors the RNA core.

**Resistance:** They are inactivated below pH 6 but remain relatively stable at 20-37°C, capable of surviving on surfaces for days.

**Pathogenesis:** Rhinoviruses primarily infect humans. Experimental infections are observed in chimpanzees. The virus attaches to Intercellular Adhesion Molecule 1 (ICAM-1) receptors on nasal ciliated epithelial cells, causing damage and secondary bacterial infections. Local inflammation and cytokines likely contribute to common cold symptoms, with interferon production and specific antibody response aiding recovery. Rhinoviruses preferentially infect the upper respiratory tract, but recent studies suggest they can replicate in the lower respiratory tract as well. Symptomatic infections are associated with cellular inflammatory responses, including increases in white blood cell count and inflammatory cytokines.

Various inflammatory mediators, including interleukins and kinins, are present in increased concentrations during rhinovirus infections, correlating with symptom severity. Neurogenic mechanisms also play a role in illness expression, with the parasympathetic nervous system controlling nasal fluid production. Rhinovirus infections have been implicated in wheezing illnesses and asthma exacerbations, particularly in individuals with allergic airway inflammation.

**Epidemiology:** The common cold is a globally prevalent infectious disease transmitted through droplets, hand-to-hand contact and self-inoculation of the conjunctiva or nasal mucosa. The incubation period ranges from 2-7 days, and the duration of virus shedding is likely short. Contrary to popular belief, cold weather does not directly cause the common cold. Rhinoviruses cause respiratory illnesses year-round worldwide. Viruses show a peak in infections in early fall and a smaller peak in spring.

**Immune response:** Human rhinovirus infection involves both direct effects on respiratory epithelial cells and responses from the innate and adaptive immune systems. The type I interferon response produces IFN-β, IFN-γ, and proinflammatory cytokines like IL-6, IL-8 and IL-8 that play a significant role in determining the clinical outcomes of infection by attracting and activating neutrophils. Humoral immune responses, particularly the production of serotype-specific IgG and IgA antibodies, are crucial for preventing HRV infection. These antibodies appear 1-2 weeks post-infection and can remain elevated for over a year, providing protection against reinfection with the same serotype. T cells play a role in antiviral immunity by recognizing viral antigens and triggering immune responses.

**Clinical presentation:** Rhinovirus primarily causes upper respiratory illnesses known as the common cold.

**1. Upper respiratory infections**

* **Common cold-** The virus causes 50% to 66% of common colds. Symptoms appear within two days and last 7-14 days, including runny nose, nasal congestion, sore throat, cough, headache, and malaise. Rhinovirus can also affect the eustachian tube and middle ear, with abnormalities seen in about 75% of patients (viral otitis media).
* **Rhinosinusitis-** Sinus abnormalities are often seen in rhinovirus infections. The viral RNA has been detected in 50% of patients with acute sinusitis and in sinus epithelium of those with acute sinusitis. Nose blowing may spread the virus to the sinuses.

**Complications:** Complications of rhinovirus infections include-

* **Acute bacterial otitis media**- Complicates about 2% of colds in adults and up to 30% in children, often due to eustachian tube dysfunction.
* **Exacerbations of chronic bronchitis**- Rhinovirus is linked to 40% of these episodes, which can lead to hospitalizations in patients with chronic obstructive pulmonary disease (COPD).
* **Asthma exacerbations**- Rhinovirus is the main cause of asthma attacks in older children and adults, particularly in the fall when school starts.
* **Acute bacterial sinusitis**- Occurs in 0.5% to 8% of cases, potentially due to nasal secretions being propelled into the sinuses.

**2. Lower respiratory infections-**

* **Croup-** The virus occasionally causes croup, typically seen in fall. It is rare but more common in children and young adults.
* **Bronchiolitis-** Rhinovirus is the second most common cause of bronchiolitis in hospitalized children, after respiratory syncytial virus (RSV). Rhinovirus-associated bronchiolitis is linked to recurrent wheezing and asthma development. Very-low-birth-weight infants have higher rhinovirus-associated bronchiolitis rates.
* **Community-Acquired Pneumonia (CAP)-** Rhinovirus is a common cause of viral CAP in children, with rates from 18% to 26%. Coinfections with bacteria are frequent. The virus accounts for about 5% of viral CAP cases in adults but can cause significant morbidity in elderly residents of long-term care facilities

**3. Asymptomatic infections-** Asymptomatic rhinovirus infections are common in children (<4 years old). These cases might represent prolonged virus shedding after symptoms resolve, mild unrecognized symptoms, or the incubation period before symptoms appear.

### Laboratory diagnosis: For upper respiratory infections, nasopharyngeal swabs or aspirates yield the best results. For lower respiratory infections, samples like tracheal aspirates or BAL fluid are used.

**1. Virus culture**- Rhinovirus can be cultured in human cell lines such as MRC5 and WI-38. Growth indicated by cytopathic effects can take up to two weeks. Rapid methods using multichamber slides and immunofluorescence exist but are not widely used.

**2. Serology**- Due to numerous serotypes, serological tests are not practical for diagnosis.

**3. Antigen detection**- Not used routinely due to lack of a common rhinovirus antigen.

**4. Molecular methods**- RT-PCR and real-time RT-PCR are more sensitive and faster. These methods often target the highly conserved 5′UTR region but can cross-react with enteroviruses (EVs). Newer methods like NASBA show promising sensitivity and specificity.

* **Genotyping**- Viruses are categorized into groups A, B, and C based on genetic analysis. Sequencing the VP1 or VP4 gene helps distinguish HRV strains.
* **Whole-Genome Sequencing (WGS)**- Offers comprehensive genetic characterization, helping to track virus evolution, recombination, and virulence factors.

**Treatment:** Commercially available treatments for preventing or treating rhinovirus infections have not proven effective. These infections, caused by various viruses including rhinoviruses, respiratory syncytial virus, coronavirus, and others, are typically self-limited and benign. Therefore, treatments must be quick, inexpensive, and safe. Vaccination is challenging due to the diverse viral causes. Antiviral chemotherapy, such as leukocyte interferon and pleconaril, have shown promise but faced supply and safety issues. Rupintrivir targeted rhinovirus replication but had modest effects in clinical trials. Handwashing with iodine and ethanol-based sanitizers reduce virus on the skin but are not highly effective in natural settings. Acid-labile rhinoviruses may be targeted with virucidal tissues and hand treatments with organic acids, though results are mixed. Pain relievers, decongestants, and antihistamines can alleviate symptoms. Preventive measures include covering mouth and nose when coughing or sneezing and frequent handwashing with soap and water.

**Parechovirus**

Human Parechoviruses are reported worldwide. With the advent of viral RNA sequencing, it was found that the previously designated echovirus serotypes 22 and 23 were sufficiently distinct from other enteroviruses and were reclassified into a new picornavirus genus as parechovirus serotypes 1 and 2, respectively. Parechoviruses differ from enteroviruses in capsid protein organization and use a unique cell membrane receptor. The 5′ nontranslated region of the parechovirus genome is distinct enough to allow for the development of specific parechovirus PCRs. Currently, 14 parechovirus serotypes have been proposed.

Parechovirus is transmitted through fecal-oral and respiratory routes, with high viral titers in stool facilitating fecal-oral spread. The diseases attributed to parechoviruses are similar to those caused by echoviruses, including respiratory tract infections, exanthems, viral meningitis, encephalitis, myocarditis, and severe neonatal infections. In neonatal encephalitis, parechoviruses have been linked to white matter injury. Infants may also pfresent fever (39°C) and irritability. Additional symptoms included rhinorrhea, cough, and poor feeding. The virus have been frequently isolated from the stools of neonates and infants with undifferentiated febrile illnesses. While parechoviruses have been associated with mild gastroenteritis, their overall significance in gastrointestinal disease remains unclear. There has also been a reported case of fatal pneumonia caused by human parechovirus 1 in an elderly woman.

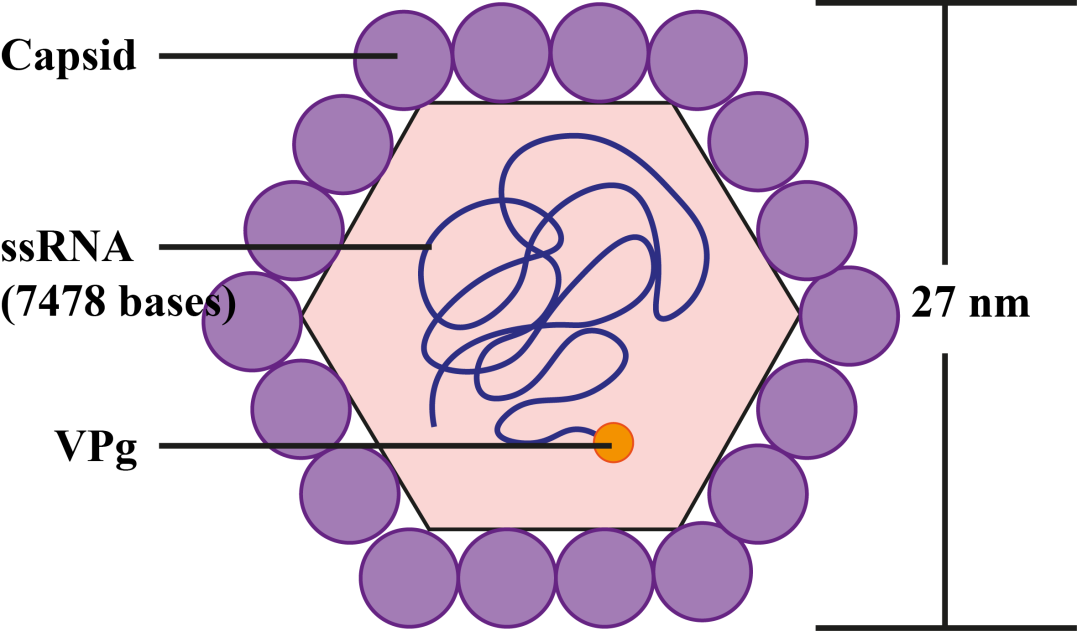
Early prevalence studies indicate that parechovirus infections are not common, but larger studies using advanced identification techniques are needed for more definitive epidemiological data. In the United States, most parechovirus infections occur during summer and fall, although the seasonality is less pronounced compared to enteroviruses

**Hepatovirus**

**Hepatitis A (HAV)**

The hepatitis A is the most important virus of the Hepatovirus genus in the Picornaviridae family. In 1973, Feinstone and colleagues identified the virus in stool samples, confirming it as the causative agent.

**Structure:** The HAV virion (Figure 62) is non-enveloped ( approximately 27-32 nm) with icosahedral symmetry. The genome is single-stranded, positive-sense RNA of 7.5 kb with one open reading frame encoding a polyprotein. This polyprotein is processed into structural proteins (VP4, VP2, VP3, VP1) and non-structural proteins (2B, 2C, 3A, 3B, 3C, 3D). HAV is classified into five genotypes, with genotypes I, II, and III (subdivided into A and B) infecting humans. A third subgenotype, IC, is proposed but not yet recognized.



**Figure 62: Structure of hepatitis A**

**Resistance:** HAV is highly resistant to harsh conditions, such as high temperatures, acidity, and freezing, diethyl ether due to its cohesive capsid structure.

**Epidemiology:**.HAV primarily spreads through the fecal-oral route, either directly via contact with infected individuals or indirectly through contaminated food and water. Most outbreaks in developed countries are from person-to-person transmission. Food-borne sporadic cases are observed in developing countries, often linked to seafood (eg. scallops) and imported frozen foods like blackberries, strawberries, pomegranate seeds, vegetables. Rarely, HAV can also spread through blood transfusions and organ transplants.

Globally, there are approximately 100 million HAV infections and 1.5 million symptomatic cases annually, causing 15,000 to 30,000 deaths. HAV ranks highest in incidence among the major acute viral hepatitis types. The burden of HAV varies significantly, with low- and middle-income countries, particularly highest in Africa and South Asia. The risk groups include drug users, men who had sex with men (MSM), poor hygiene and homeless individuals.

**Pathogenesis:** The virus enters the liver through the portal vein, replicates, and is excreted via bile ducts. The enterohepatic cycle continues until the body produces antibodies. Immune mechanisms, particularly CD8+ T cells and natural killer cells, are involved in hepatocyte damage. Liver damage is believed to be mediated by T cells rather than antibodies.

**Clinical presentation:** Hepatitis A virus infection typically follows a course starting with a 2-4 week incubation period. The three phases of infection are -

* **Nonspecific prodromal phase-** characterized by flu-like symptoms (low grade fever, malaise, fatigue) and intestinal disorders (nausea, vomiting, abdominal pain, diarrhea, anorexia) lasting a few days.
* **The icteric phase-** marked by jaundice and elevated serum aminotransferase levels due to hepatic cytolysis. The first specific sign prompting medical attention is dark urine, followed by pale stools and yellowing of the skin, sclera, and mucous membranes due to bilirubinuria. Itching, indicating cholestasis. Physical examination may reveal an enlarged, tender liver, and in some cases, a palpable spleen. Serum aminotransferase levels often exceed 1000 U/dL, with total bilirubin typically ≤10 mg/dL and alkaline phosphatase below 400 U/L.
* **Recovery phase-** After the jaundice resolves, patients may experience a recovery phase. During this phase, symptoms such as fatigue and malaise may persist but gradually improve.

**Other conditions-**

**a. Fulminant hepatitis**- Rare, occurs in less than 1% of cases.

**b. Relapsing hepatitis-** occurs in about 3-20% of patients, typically manifests 3 to 12 weeks after the initial episode, with milder symptoms than the initial infection. HAV does not cause chronic infections, unlike other hepatitis viruses.

**c. Extra-hepatic manifestations-** Rare and include neurological symptoms (e.g., Guillain-Barre syndrome), rash, pancreatitis, arthritis, myocarditis, acute kidney injury, and hematological disorders (e.g., hemolysis and cryoglobulinemia).

Higher mortality rates are seen in older patients, males and individuals with underlying liver pathology or chronic viral hepatitis. Acute liver failure, although rare, is associated with increased mortality, particularly in patients with creatinine levels >2 mg/dL, total bilirubin >9.6 mg/dL, and albumin <2.5 g/L.

**Laboratory diagnosis:** Hepatitis A shares clinical features with other viral hepatitis forms, making diagnosis challenging, though suspected during outbreaks.

* **Blood tests-** Liver function tests, especially ALT and AST levels, indicate liver damage but lack specificity..
* **Antibody detection-** Detection of specific antibodies against HAV in serum confirms the diagnosis, with IgM anti-HAV indicating acute infection. IgM levels peak during infection and persist for up to 4 months from symptom onset. Total antibody testing is used to assess immunity post-exposure or vaccination. IgG antibodies, persisting long-term, indicate past infection.
* **Biopsy-** Liver biopsy or imaging is unnecessary for diagnosis, but if performed, may show distinct inflammation patterns compared to other viral hepatitis types.
* **Nucleic acid tests-** Stool tests for HAV RNA are rarely used for diagnosis but can detect RNA in patients and contaminated sources, aiding outbreak tracking.
* **Histopathology-** Hepatitis A exhibits pronounced portal inflammation. Detection of HAV particles in infected cells aids diagnosis.

**Prevention:** There's no specific treatment for HAV, so management focuses on symptomatic relief, while improving sanitation to curb community transmission.

**1. Passive immunization-** Immunoglobulins were once used for prevention, but the effective vaccine has largely replaced them, except for infants under 12 months.

**2. Vaccines-** Post-exposure prophylaxis with the hepatitis A vaccine is approved for immunocompetent individuals aged 12 months to 40 years.

* **Inactivated vaccines:** These vaccines contain killed hepatitis A viruses, which cannot cause infection but stimulate an immune response. The inactivated hepatitis A vaccines available in the United States are Havrix and VAQTA. They are typically administered in two doses, with the second dose given 6 to 18 months after the first.
* **Live attenuated vaccines:** These vaccines contain weakened forms of the hepatitis A virus. While less commonly used, they have been studied as potential options for hepatitis A prevention. Examples include vaccines based on strains like CR326 and HM175.

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**Astroviridae**

Astroviruses are enteric viruses that were first identified in the feces of children with diarrhea, recognizable by their star-like shape under electron microscopy, although only 10% of the particles show this morphology, complicating early diagnosis. Astroviruses are found in about 3-8% of children with diarrhea, and some individuals can carry and shed the virus asymptomatically, spreading it even after symptoms subside. These viruses belong to the Astroviridae family, which comprises two genera: Mamastrovirus, infecting mammals including humans, and Avastrovirus, infecting birds. Astroviruses, alongside Caliciviridae, are now acknowledged as significant causes of gastroenteritis in both children and adults, causing viral gastroenteritis.

**Structure:** Astroviruses (Figure 63) are nonenveloped viruses, 28-30 nm in diameter, infecting a wide range of animal species, including humans. They exhibit icosahedral symmetry and, under electron microscopy, show a characteristic star-like morphology. Human astroviruses have a positive-sense, single-stranded RNA genome with a 3′ polyadenylated tail, containing three open reading frames (ORFs) that encode the protease, polymerase, and capsid proteins. There are eight identified serotypes (HAstV-1 to HAstV-8). These viruses can be propagated in cell cultures, including primary HEK cells and various monkey cell lines.

Capsid protein VP34

VP27

VP25

**Figure 63: Structure of astrocviridae**

**Resistance:** Astroviruses are highly stable in the environment, resisting inactivation by alcohols, bleach, detergents, heat, and UV treatment. They can survive up to 90 days in marine and tap water, with greater longevity in colder temperatures.

**Pathogenesis:** The pathogenesis of astrovirus-induced illness is not well understood. In animals, astrovirus infections are linked to shortening of small intestinal villi and mild inflammatory infiltrates in the lamina propria. These infections may also decrease intestinal disaccharidase activity, leading to osmotic diarrhea, similar to rotavirus. Although stool filtrates containing astroviruses can infect volunteers after oral administration, astroviruses induce illness infrequently compared to noroviruses, suggesting they may be less pathogenic in adults.

**Epidemiology:** Astroviruses are primarily transmitted through the fecal-oral route, with contaminated food and water linked to outbreaks.The virus can be isolated from water treatment facilities and can accumulate in filter-feeding shellfish like oysters and mussels.

**A. Humans**- Astroviruses are globally distributed and detected in 3-6% of children under 2 years old with infectious gastroenteritis, with rates reaching up to 20% in some developing countries. They are often the second most common viral pathogen in young children after rotavirus. Outbreaks have also been reported among healthy adults and elderly individuals. Astrovirus infections can be asymptomatic, found in up to 2% of individuals without symptoms, and may be underreported due to their typically mild nature. Immunodeficient individuals (HIV hematopoietic cell transplantation, hematologic malignancies) are at higher risk. Astrovirus infections occur year-round, peaking in autumn and early winter, and are common in high-density environments like childcare centers and schools. They often co-infect with other enteric pathogens, notably noroviruses and rotaviruses.

**B. Animals**- Astroviruses have been identified in various mammalian and avian species. They have been found in animals such as rabbits, mice, calves, sheep, piglets, dogs, deer, kittens, mink, turkeys, ducks, chickens, and guinea fowl. Astrovirus infections in animals can predispose them to further infections by other viruses.

### Clinical presentation:

### a. Mammalian astroviruses- Astrovirus infection in mammals primarily causes gastroenteritis, with symptoms including diarrhea, vomiting, nausea, anxiety, headache, malaise, abdominal discomfort, and fever. Symptoms typically begin 2-3 days post-infection, coinciding with viral shedding in feces, which can persist even after other symptoms resolve (up to 35 days). There is also an association with intussusception, though causation is unclear.

**b. Avian astroviruses-** In avian species, astroviruses cause a wider range of diseases, including gastroenteritis in turkeys and chickens, nephritis in chickens, and severe hepatitis in ducklings.

**Laboratory diagnosis:** Unlike other recently identified viral causes of gastroenteritis, astroviruses are often excreted in high quantities in stool samples and can be easily identified using electron microscopy, even without immune aggregation. Detection in cell culture has been achieved through immune electron microscopy (IEM) or immunofluorescence, while an enzyme immunoassay (EIA) targeting the astrovirus group antigen has been extensively utilized in epidemiological research. EIA demonstrates comparable sensitivity (91%) and specificity (98%) to IEM. However, reverse transcription-polymerase chain reaction (RT-PCR) techniques have emerged as the most commonly employed methods for astrovirus detection, offering significantly higher sensitivity compared to EIA or IEM.

**Treatment and prevention:** Astrovirus infections in humans are generally mild and self-limiting, typically requiring only fluid rehydration therapy, often manageable at home, making hospital admissions rare. No vaccines are available for humans, and immunoglobulin treatments for immunocompromised individuals have shown mixed results. There is also no treatment for astrovirus-infected animals. Preventing transmission through diligent handwashing and food hygiene is crucial. Astroviruses are stable and resistant to inactivation, making them difficult to eliminate once introduced. Infection induces astrovirus-specific Th1-type CD4 cells in the gut mucosa, contributing to the immune defense.

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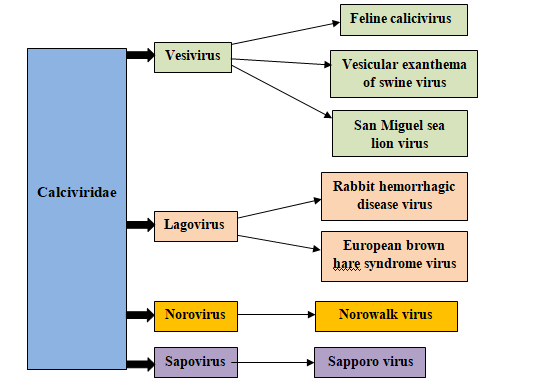
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**Calciviridae**

Noroviruses (Figure 64) are a major cause of epidemic acute gastroenteritis. The Norwalk virus, discovered in 1972 during a gastroenteritis outbreak in Norwalk, Ohio, became the prototype of this group, characterized by its small round structured morphology, inability to grow in vitro, and association with acute gastroenteritis epidemics.

**Classification:**



**Figure 64: Classification of calciviridae**

**Structure:** Caliciviruses, named for their chalice-like appearance under an electron microscope, infect a variety of animal species including marine mammals, swine, cats, rabbits, and humans. The calicivirus genome is a single-stranded, positive-sense RNA ranging from 7.3 to 8.5 kb, with a VPg protein linked to the 5' end and a polyadenylated 3' tail. The genome features a large open reading frame (ORF) encoding a polyprotein, flanked by short untranslated regions (UTRs).

**Replication:** Once inside the host cell, the viral RNA is released into the cytoplasm. The viral RNA is directly translated by host ribosomes to produce viral proteins, including RNA-dependent RNA polymerase, which replicates the viral genome. New viral particles are assembled in the cytoplasm.

**Pathogenesis:** Infection of intestinal epithelial cells leads to cell damage and apoptosis. This damage disrupts the absorptive and secretory functions of the intestine. The virus-induced damage and immune response lead to increased fluid secretion into the intestinal lumen, resulting in diarrhea. Due to the lack of an animal model, pathogenesis insights come from human studies. Acute infection with Norwalk and Hawaii results in reversible jejunal lesions, with blunted villi, intact mucosa, and leukocyte infiltration, appearing within 24 hours, peaking during illness, and resolving within two weeks, though some changes can persist up to six weeks. Diarrhea is linked to temporary malabsorption of d-xylose and fat, and decreased brush-border enzyme activity, which normalize within two weeks. These viruses do not produce enterotoxins.

**Immune Response:** Infection with the Norwalk virus triggers the production of virus-specific serum immunoglobulins IgG, IgA, and IgM, even in previously exposed individuals. IgA and IgM responses are short-lived, while IgG levels remain elevated for months. Antigen-specific cellular responses to the capsid, predominantly Th1 type, have been observed in peripheral blood. Protective immunity to Norwalk virus is poorly understood, but most individuals show resistance to reinfection for 4 to 6 months, with increased resistance after multiple exposures. Lack of HBO blood-group antigen secretion is linked to resistance to Norwalk strain infection, suggesting these antigens may act as cellular receptors for noroviruses. Cytokines and inflammatory mediators produced in response to infection contribute to the symptoms of gastroenteritis.

**Clinical presentation:**

**a. Norovirus-** The Norwalk viruses measure 26-34 nm in diameter. The viruses are heat and acid-stable, as well as ether-resistant. The prototype Norwalk virus features three ORFs encoding essential proteins for viral replication and structure. Human disease-related norovirus strains are mostly found in genogroups GI and GII. Noroviruses, primarily causes gastroenteritis in humans. Symptoms can start gradually or abruptly, with abdominal cramps or nausea often being the initial complaints. Vomiting and diarrhea are common, though they can occur independently. Other symptoms include myalgias, malaise, occasional headaches, and low-grade fever (101° to 102°F) in about half of the cases. Diarrhea typically involves four to eight non-bloody stools within 24 hours. The illness usually lasts 48 to 72 hours and resolves without long-term effects.

**b. Sapovirus-** Sapoviruses are caliciviruses that infect humans of all ages, causing acute gastroenteritis in sporadic cases and outbreaks. The virus also infect various animals such as pigs, mink, dogs, sea lions, and bats. It has 15 identified genogroups, with four (GGI, GGII, GGIV, GGV) infecting humans. The virus cannot yet be cultured from human samples, although porcine sapovirus can be grown in specific cell lines. A potential antiviral nucleoside has shown effectiveness in blocking viral replication in cell culture. Sapovirus infections are mostly seen in children under 5 years old and tend to be milder than norovirus infections.

**c. Lagovirus-** Lagovirus includes Rabbit hemorrhagic disease virus (RHDV) and European brown hare syndrome virus (EBHSV), proposed to be one species with two genogroups. Lagoviruses often recombine and use host cell histo-blood group antigens for attachment. RHDV primarily infects the liver, spleen, and blood cells, causing severe disease in rabbits but not in other species. Effective vaccines against RHDV exist, and the virus has been used for rabbit population control in Australia.

**d. Vesivirus-** Feline calicivirus is the primary vesivirus, causing respiratory and sometimes systemic diseases in cats. The virus binds to α2,6-linked sialic acid and fJAM-A receptors, leading to entry and replication in various cell types. Vesiviruses also infect dogs and sea mammals, with potential cross-species transmission.

Nebovirus, a new genus identified from the Newbury agent-1 virus in cattle, interacts with a wide range of histo-blood group antigens and shows recombination within its genome. Nebovirus infections are common in cattle globally, often co-occurring with bovine norovirus.

**Laboratory diagnosis:** A clinical diagnosis of norovirus-related illness relies on epidemiological information and the absence of other pathogens. However, signs and symptoms alone are not specific enough. Routine lab tests often do not confirm norovirus infection, but the absence of fecal leukocytes can help rule out other pathogens. Laboratory confirmation is necessary, and various methods, including RT-PCR and immunoassays, are used to detect norovirus directly in stool samples. Serological methods are also employed due to limited virus excretion in stools during outbreaks, with serum antibody titers rising within 10 to 14 days after illness onset.

**Treatment and prevention:** Treatment for norovirus infection relies on supportive care, including oral rehydration with isotonic fluids and, in severe cases, intravenous therapy. Symptomatic relief for associated symptoms is achieved with analgesics and antiemetics, while antiperistaltic agents are used to control diarrhea. Developing a norovirus vaccine is challenging due to various factors, including the inability to induce long-term immunity and the lack of suitable animal models and in vitro propagation systems. Preventing norovirus outbreaks requires proper hygiene practices, such as restricting activities of symptomatic food handlers and using disinfectants like chlorine bleach for effective decontamination.

**Reference:**

Desselberger, U., 2019. Caliciviridae Other Than Noroviruses. Viruses 11, 286. https://doi.org/10.3390/v11030286

**Reoviridae**

The Reoviridae family infects a wide range of hosts, including microorganisms, insects, plants, and vertebrates. It comprises two subfamilies and numerous genera, with notable members (table 29). The name "Reoviridae" originates from the early isolates, which were respiratory and enteric in origin but not linked to any known disease, hence termed "respiratory, enteric orphanviruses."

**Table 29: Classification of reoviruses**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genus | Rotavirus | Orbivirus | Orthoreovirus | Coltivirus |
| Subfamily | Sedoreovirinae | Sedoreovirinae | Spinareovirinae | Spinareovirinae |
| RNA segments | 11 | 10 | 10 | 12 |
| Capsid structure | 3 layers | 3 layers | 2 layers | 2 layers |
| Hosts | Vertebrates | Vertebrates, insects | Vertebrates | Vertebrates, insects |

**Structure:** Electron microscopy investigations elucidate the intricate capsid architecture of reoviruses, characterized by multi-layered structures. Virions (Figure 65) have icosahedral symmetry and contain two or three distinct capsid layers. The innermost layer encloses dsRNA segments and RNA synthesis enzymes. Certain reoviruses, for eg., Rotavirus genus, exhibit an additional protein layer adorned with glycosylated spike proteins on their surface, resulting in a distinctive wheel-like morphology. Reovirus genomes typically encompass 9 to 12 segments of linear, double-stranded RNA (dsRNA), each predominantly encoding a singular protein. Notably, genomes of rotavirus and orbivirus encompass 11 segments encoding 12 proteins. These segments exhibit size variability, collectively comprising approximately 18,500 to 19,200 base pairs in coding capacity. Each genomic segment harbors brief untranslated regions at both termini.

**Replication:** The replication process of reoviruses is generally preserved across different genera.

**1. Viral attachment-** Reovirus attachment (Table 30) is facilitated by capsid proteins that interact with various cell surface receptors, including protein and carbohydrate receptors (Table ). Rotavirus VP4, cleaved by extracellular proteases, interacts with histo blood group antigens (HBGAs). Membrane-interacting proteins aid in penetration across cellular membranes, followed by disassembly of outer capsid layers.

**Table 30: Reoviruses and their receptors**

|  |  |
| --- | --- |
| Viruses | Receptors |
| Rotavirus | **VP4** |
| Orthoreovirus | **σ1** |
| Orbivirus | **VP2** |

**2. Amplification-** Penetration and uncoating trigger the transcriptional activities of the core, leading to mRNA synthesis within the core and subsequent release through pores.. Translation of mRNAs occurs in the cytosol or on the rough endoplasmic reticulum (ER), where certain proteins undergo glycosylation. Cores assemble in viral organelles, known as viroplasm, capable of transcription and mRNA generation.

**3. Replication-** The mechanism underlying the selection and packaging of reovirus genome segments occurs within the viroplasm, with mRNA secondary structures potentially guiding selective packaging. Each mRNA serves as a template for synthesizing complementary negative strands, forming double-stranded genome segments.

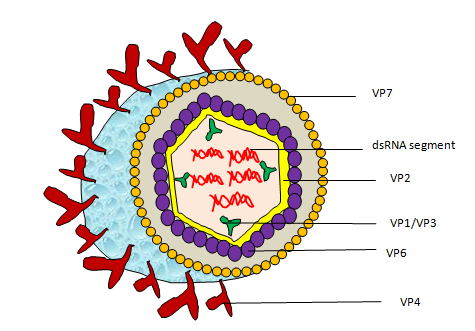
**4. Assembly and release-** Rotavirus assembly takes place in the viroplasm, where double-layered particles (DLPs) form through interactions of inner core proteins. These DLPs bud into the endoplasmic reticulum (ER) lumen, transiently acquiring an envelope before shedding it. This process is facilitated by the ER-localized nonstructural protein 4 (NSP4). The incorporation of VP4 and VP7 into the outer capsid shell requires high calcium ion concentrations. Virions are eventually released following host cell lysis.

**5. Syncytia formation-** Orthoreoviruses can induce the formation of syncytia, or fused cells, mediated by fusion-associated small transmembrane proteins that are not included in virions. This cell fusion process may enhance virion release by promoting cell death.

**Discussion of different Reoviruses:**

**1. Rotavirus-** Rotaviruses cause gastrointestinal infections in humans and other mammals. While many cases are mild, some can result in severe diarrhea and vomiting, particularly in infants and young children. There are seven rotavirus species, labeled A to G. Groups A, B, and C infect humans and animals, with Group A causing endemic gastroenteritis in children and Groups B and C causing gastroenteritis epidemics. Group B is associated with severe adult diarrhea in China.Rotaviruses frequently reassort their genomes, leading to a variety of strains, and can exchange genes between human and animal viruses.

Rotavirus infection is nearly universal, with most children acquiring antibodies by age 2 to 3. Severe gastroenteritis from rotavirus primarily affects infants and children between 6 months and 2 years, as maternal immunity wanes and the gastrointestinal tract matures. Mortality is higher in poorer countries due to malnutrition, limited treatment access, larger viral doses, and co-infections. However, global mortality has decreased by 30% since 1985 due to improved treatment access, nutrition, and fewer comorbidities.

****

**Figure 65: Structure of rotavirus**

**a) Pathogenesis-** Rotaviruses replicate in the small intestine, infecting nondividing enterocytes and causing mild diarrhea due to viral enterotoxin, nutrient malabsorption, and secretion mediated by the enteric nervous system (ENS).. Severe diarrhea is often linked to the viral protein NSP4, which disrupts calcium levels in cells, leading to chloride, sodium, and water release, resulting in secretory diarrhea. Additionally, damage to the intestinal mucosa leads to the loss of absorptive cells and decreased disaccharidase activity, contributing to malabsorption.

**b)** **Clinical preentation-** These include vomiting, diarrhea, abdominal pain, and fever. While most natural infections in adults are asymptomatic, severe cases can occur. Rotavirus gastroenteritis typically begins with vomiting and fever before progressing to profuse diarrhea. Laboratory findings include isotonic dehydration and metabolic acidosis, while viral shedding in stool can persist for several days. Dehydration and electrolyte abnormalities are the primary causes of death from rotavirus gastroenteritis, with seizures and aspiration also contributing. Rotavirus can also cause extraintestinal manifestations, particularly in immunocompromised children, including chronic diarrhea and extraintestinal infections..

**c) Immune response-** The immune response to rotavirus involves innate, cellular, and humoral mechanisms, with neutralizing antibodies (especially IgA in the gut) playing a crucial role in protection against severe disease. Natural rotavirus infection provides partial protection against subsequent episodes of gastroenteritis.

**d) Epidemiology-** In temperate climates, the disease peaks in winter months and drier periods in tropical regions. Rotavirus spreads primarily via the fecal-oral route, though respiratory transmission is possible. Transmission can also occur through contaminated water and possibly airborne routes. High-risk settings include daycare centers and nursing homes, and the virus is a significant cause of nosocomial infections and traveler's diarrhea. The virus is highly contagious and stable in the environment.

**e) Laboratory diagnosis-** Various techniques, such as antigenic assays, RT-PCR, electron microscopy, immune electron microscopy, polyacrylamide gel electrophoresis (PAGE), and viral culture, can detect rotavirus. Commercial antigenic assays like ELISA and latex agglutination are commonly used for practical diagnosis. Multiplexed RT-PCR is valuable for epidemiologic studies, while electron microscopy and PAGE can identify unusual strains. However, cell culture, although capable of detecting rotavirus, is less sensitive, especially for human strains.

**f) Treatment:** The Centers for Disease Control and Prevention (CDC) provides guidelines for treating rotavirus gastroenteritis, emphasizing:

* **Rehydration and electrolyte balance**- Primary therapies focus on rehydration and restoring electrolyte balance.

1. **Oral rehydration solutions (ORSs)**: Preferred over intravenous (IV) rehydration for mild to moderate dehydration, even with moderate vomiting, due to their effectiveness in damaged intestines.
2. **Low osmolarity ORS**: Recommended by the World Health Organization (WHO) for reduced vomiting and stool output.
3. **Intravenous (IV) hydration**: Recommended for severe dehydration or when oral hydration is not feasible.

* **Breastfeeding and diet**- Breastfeeding should continue during rehydration, and a diet should be resumed as soon as tolerated, avoiding foods high in simple sugars.
* **Zinc supplementation**- Recommended by the WHO during and after illness for reducing diarrhea prevalence in developing countries (not used by CDC).
* **Racecadotril**- Shows promise as an adjunct to ORS in reducing stool output and duration of diarrhea but requires further evaluation.
* **Oral immunoglobulins**- May be beneficial for treating chronic rotavirus diarrhea or providing prophylaxis in high-risk settings.
* **Other agents**- Antimotility agents like loperamide are not recommended, while bismuth subsalicylate and probiotics may provide modest benefits in reducing diarrhea duration.

**g) Preventive measures:** Preventing rotavirus infection through immunization is crucial, particularly in areas where access to treatment is limited. Key points include:

* **Vaccine history**- The first human rotavirus vaccine, RotaShield, was withdrawn in 1999 due to an association with intestinal intussusception.
* **Subsequent vaccines**- Rotarix and Rotateq have proven safe and effective in clinical trials, with no observed association with intussusception.
* **Vaccine recommendations**- Both vaccines have been recommended for routine immunization by the Advisory Committee on Immunization Practices (ACIP), with Rotarix administered at 2 and 4 months of age and Rotateq at 2, 4, and 6 months of age.

**2.** **Orbivirus-** Orbiviruses are transmitted by insects like ticks, mosquitoes, sand flies, and biting midges. They infect a wide range of animals and birds, causing cytopathic infections in animal cells but noncytopathic ones in insects. In humans, four serogroups of orbiviruses have been linked to diseases such as neurologic infections (encephalitis and meningitis) and acute febrile illnesses, with clinical presentations resembling those of rickettsial illnesses. The genus includes 22 species, with notable pathogens like Bluetongue virus, Epizootic hemorrhagic disease virus, and African horse sickness virus.

**a) Bluetongue virus-** Bluetongue virus, transmitted by Culicoides biting midges, originated in Africa but has since spread globally. It affects sheep, goats, and cattle, with sheep being the most severely impacted. Infections can be asymptomatic or cause severe symptoms such as edema, fever, depression, and cyanosis of the tongue. The virus expanded from Africa and Cyprus to Europe and other regions after 1950. While livestock vaccines are available, the presence of multiple serotypes complicates prevention efforts.

**b) Epizootic hemorrhagic disease virus-** Thevirus is transmitted by Culicoides midges. It primarily affects wild ruminants, particularly white-tailed deer in North America. The disease has a sudden onset, causing high fever, salivation, rapid pulse, and hemorrhage, and can be fatal within 36 hours.

**c) African horse sickness virus-** Thevirus affects horses, donkeys, mules, and zebras (the natural reservoir). It causes severe disease in horses and mules but is often asymptomatic in zebras. The virus is transmitted by Culicoides midges and is endemic to Africa.

**3. Orthoreoviruses**: Human infection typically causes mild symptoms like upper respiratory illness and gastroenteritis, though severe cases with neurological (paediatric meningitis), respiratory (severe acute respiratory syndrome), and gastrointestinal manifestations (diarrhea in children) have been reported. Transmission occurs through fecal-oral and airborne routes. Moreover, reoviruses have shown promise as oncolytic agents, inducing apoptosis in various cancer cells and demonstrating efficacy in preclinical studies and human clinical trials.

**4. Coltiviruses**: Include Colorado tick fever virus, which infects various animals and humans, causing flu-like symptoms and potentially severe complications. It is transmitted by ticks, particularly active at high altitudes in spring and summer.

**a) Colorado Tick Fever Virus (CTFV)-** CTFV is the second most common arboviral infection in the United States, with about 400 cases reported annually. It is transmitted primarily by ticks, especially Dermacentor andersoni, though other vectors include mosquitoes, rodents, and humans. Most prevalent in regions like the Rocky Mountains and parts of southwestern Canada, affected states include Colorado, Montana, Utah, and Wyoming. Transmission peaks between April and July during tick activity. Symptoms usually last about a week and include fever, chills, headache, retroorbital pain, myalgia, weakness, and lethargy. Neurological complications are more common in children, with rare complications like myocarditis and pericarditis. Hospitalization is necessary in up to 20% of cases, with deaths mostly reported in children. Lab findings often show leukopenia, thrombocytopenia, and relative lymphocytosis, with prolonged viremia aiding diagnosis. Various methods, including immunofluorescence assay and ELISA, are used to detect CTFV antibodies. Treatment is supportive, as there's no specific antiviral therapy, and aspirin is avoided due to thrombocytopenia risk. Tick prevention is crucial, as CTFV infection may confer long-lasting immunity.

**b) Salmon river virus-** It is considered a serotype of CTFV. The virus was isolated from a viremic patient with a CTFV-like illness and acquired near Idaho’s Salmon River.Many mammals carry the virus naturally, but only humans show clinical symptoms.

**c) Eyach virus-** The virus isantigenically related but distinct from CTFV and was initially found in Ixodes sp. ticks in France and Germany. It has been linked to febrile illnesses and neurological conditions such as encephalitis and polyradiculoneuritis, especially in patients from the former Czech Republic. Isolating and propagating the virus is challenging, often necessitating the use of intracranial injection in suckling mice. Diagnosis typically relies on ELISA targeting recombinant VP6 protein and PCR, offering high specificity for EYAV without any cross-reactivity with CTFV.

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**Reference:**

Payne, S., 2017. Family Reoviridae, in: Viruses. Elsevier, pp. 219–226.

https://doi.org/10.1016/B978-0-12-803109-4.00026-X

**Togaviridae**

The term "toga" in "Togaviridae" originates from the Latin word meaning "cloak" or "covering," referring to the virus's characteristic envelope that encloses the virion. Togaviridae (Figure 66) consists of two genera: Alphavirus and Rubivirus. Rubella virus, the only species in Rubivirus, causes widespread fever and rash0. Alphaviruses are found worldwide and infect hundreds of thousands each year, causing a range of illnesses from mild fevers to severe encephalitis and hemorrhagic fever.

Togaviridae

Rubiola virus

Alphavirus

Rubella virus (German measles)

Old World virus

New World virus

Chikungunya

(Africa & Asia)

Eastern equine encephalitis (US)

O'nyong-nyong

(Africa)

Western equine encephalitis (US)

Mayaro

(South America)

Venezuelan equine encephalitis (US)

Ross river (Australia & Oceania)

Sindbis

(Africa, Scandinavia & Asia)

Barmah forest virus

(Australia)

**Figure 66: Classification of Togaviridae**

**Alphavirus**

Alphaviruses are transmitted by arthropod vectors, primarily mosquitoes, and often have non-human hosts crucial to their life cycles. The viruses are identified by their antigenic and nucleotide sequences as well as their geographic distribution. The primary clinical manifestations of ‘Old World’ alphaviruses are fever, rash, and joint pain. The ‘New World’ alphaviruses primarily impact the central nervous system, leading to encephalitic symptoms.

**Structure:** Alphaviruses (Figure 67), part of the Togaviridae family, encompass at least 30 viruses classified into seven serocomplexes. These lipid-enveloped viruses measure 50-60 nm in diameter and contain an 11-12 kilobase positive-strand RNA genome. This RNA is encased in a nucleocapsid with glycoproteins E1 and E2 projecting from the surrounding lipid membrane, facilitating virus entry into host cells via receptor-mediated endocytosis.

Capsid

Viral RNA (+)

Membrane

E1/E2 envelope glycoprotein

**Figure 67: Structure of Chikungunya**

**Pathogenesis:** The alphavirus life cycle involves two main stages: the production of nonstructural proteins from the RNA genome to initiate replication and evade host defenses, followed by the synthesis of structural proteins from a subgenomic RNA, which are then assembled into new virions at the plasma membrane. Alphaviruses are categorized into antigenic complexes and subgroups based on serological tests and genetic sequencing, which correspond to their geographic distributions and disease profiles, such as encephalitis or fever-rash-arthralgia syndromes.

Initial replication likely occurs in subcutaneous tissue and local muscles. The systemic effects of alphavirus infections are not well understood, but joint disease caused by viruses like Ross River virus (RRV) is well-documented. RRV induces a monocytic inflammatory response in human synovial fluid, with T-cell-derived interferon-gamma detected in joint fluid. Infections in synovial monocytes/macrophages cause symptoms such as swelling, effusion, and pain. Host factors, such as HLA-DR7 positivity, influence disease outcomes by potentially reducing cytotoxic T-cell responses to the virus.

**Epidemiology:** The epidemiology is discussed below (Table 31).

**Table 31: Geographical distributions of alphaviruses**

|  |  |
| --- | --- |
| Alphaviruses | Geographical distributions |
| Old World viruses (causing Fever, Rash, and Polyarthritis) | **Chikungunya virus-**   * Global epidemics with Aedes mosquito transmission. * Epidemics in Africa, India, and other regions. * Adaptation to Aedes albopictus mosquitoes seen. * Human reservoir contributes to epidemics. |
| **O'nyong-nyong virus-**   * Outbreaks in Uganda and the Ivory Coast. * Resurgence in southern Uganda and the Ivory Coast. * Related to CHIK virus, transmitted by Anopheles mosquitoes. |
| **Sindbis virus-**   * South African outbreaks linked to rainfall and flooding. * Spread by Culex mosquitoes, shares hosts with West Nile virus. * Northern European cases known as Pogosta disease. |
| **Ross river virus-**   * Common in Australia and Pacific Islands. * Causes epidemic polyarthritis. * Transmitted by mosquitoes, with long-lasting joint symptoms. |
| **Mayaro virus-**   * Epidemics in the Caribbean and South America. * Transmitted by Haemagogus mosquitoes. * Occurs in marmosets and other primates. |
| **Other alphaviruses-**   * Bebaru, Cabassou, and Semliki Forest viruses are either non-pathogenic or cause rare fever-arthropathy diseases. |
| New World viruses  (causing encephalitis) | **Eastern Equine Encephalitis (EEEV) Virus-**   * Limited to eastern and Gulf coasts of the United States, occasionally seen in Canada and South America. * High case-fatality rates in humans and horses. * Transmitted by Culiseta melanura mosquitoes with birds as reservoirs. * Winter theoretically interrupts epidemics, but virus persists and migrates along the east coast. * Aerosol transmission poses a risk mainly in laboratory settings. |
| **Western Equine Encephalitis (WEEV) Virus-**   * Primarily affects the Americas, transmitted by Culex tarsalis mosquitoes. * Risk factors include rural residence and outdoor occupations. * Significant decrease in cases after 1996, unlike EEE. * Cyclic outbreaks, particularly severe in Massachusetts. |
| **Venezuelan Equine Encephalitis (VEEV) Virus-**   * Causes equine and human diseases in South and Central America. * Multiple mosquito species serve as vectors. * Periodic outbreaks documented in Venezuela and other countries. * Disease severity in humans linked to equine incidence. * Cannot spread directly from person to person. |

**Chikungunya virus (CHKV)**

CHIKV disease was first identified in 1952 during an arthritis outbreak in Tanzania.The name "chikungunya" is derived from a Swahili word meaning "that which contorts," referring to the severe joint pain and stiffness associated with the illness. The virus was isolated from human serum and Aedes and Culex mosquitoes.

**Epidemiology:** CHIKV is widespread across sub-Saharan Africa, Saudi Arabia, the Indian subcontinent, and Southeast Asia. Two major genetic lineages exist: one restricted to West Africa, and the other subdivided into Asian and East African sublineages. The Asian sublineage remains genetically conserved.

Epidemics occur when the virus reemerges in areas of prior activity or moves into new areas. Chikungunya originated in Kenya and spread to Reunion Island, where it affected 265,000 of the 770,000 inhabitants and caused 237 deaths. The virus then spread to India, Mauritius, Seychelles, Mayotte, Madagascar, Italy, and Malaysia. After being inactive since 1973, CHIKV re-emerged in 2006, leading to a significant outbreak in Andhra Pradesh and Tamil Nadu, affecting nearly a million people. In Asia, significant outbreaks occurred in the 1960s, with reemergence in Indonesia (1982), Malaysia (1988-89), Thailand (1995), and Indonesia (2001-03).

**Transmission:** In Africa, CHIKV is maintained in rural areas through a cycle involving Aedes mosquitoes, nonhuman primates and possibly rodents. Nonhuman primates serve as a reservoir, though humans can also provide a reservoir for mosquito infection. In Africa, nonhuman primates serve as a reservoir, though humans can also provide a reservoir for mosquito infection. Epidemic activity is associated with the rainy season and an increase in *Aedes aegypti* and *Aedes albopictus* mosquitoes. Other potential vectors include *Mansonia* and *Culex* species, as shown in laboratory studies.

**Clinical presentation:** CHIKV causes severe acute illness, characterized by high fever, severe joint pain, lymphadenopathy, conjunctivitis, common maculopapular rash. and a greater incidence of severe disease compared to other alphaviruses. The fever is typically biphasic with a remission period after 1-6 days. Most patients recover within days to weeks, but about 10% experience chronic joint pain lasting months to years. Hemorrhagic manifestations are rare but can lead to misdiagnosis as dengue.Fatalities are rare, typically affecting the very young, the elderly, or those with severe hemorrhagic disease or co-morbidities.During the Indian Ocean outbreak, cases of CHIKV encephalitis and deaths were reported, primarily in elderly individuals or those with co-morbid conditions.

**Laboratory diagnosis:** Detection involves using ELISA to identify IgM or IgG in serum samples, or reverse transcriptase PCR to detect viral RNA.

**Treatment:** No specific antiviral treatment or vaccine is currently available for chikungunya.

**O'nyong-nyong virus (ONNV)**

O'nyong-nyong virus (ONNV) is closely related to Chikungunya virus (CHIKV) both virologically and clinically. It first appeared in Uganda in 1959, deriving its name from a tribal word meaning "painful joints." The virus was isolated from human serum and mosquitoes, leading to an epidemic affecting over 2 million people in Uganda, Kenya, Tanzania, Zaire, Malawi, Mozambique, Senegal, and Zambia. The spread was attributed to the movement of infected humans.

**Epidemiology:** ONNV has also been detected in Cameroon and the Central African Republic. A variant known as Igbo-Ora was identified in West Africa, including Nigeria, the Central African Republic, and Côte d’Ivoire. After disappearing for 35 years, ONNV re-emerged in Uganda in 1996/1997, with subsequent cases in Kenya. Recent infections from the major strain (not Igbo-Ora) have been reported in Côte d’Ivoire and Chad.

The initial ONNV epidemic spread across multiple African countries by the mid-to-late 1960s. The virus reappeared in 1996 in southern Uganda and was genetically similar to the 1959 strain. In the fall of 2003, a third outbreak occurred in Côte d'Ivoire, initially mistaken for measles. Both ONNV and CHIKV are closely related to another African virus, Igbo-Aura.

**Transmission:** Transmission of ONNV occurs via Anopheles funestus and Anopheles gambiae mosquitoes. It is presumed that a nonhuman mammalian host maintains the virus between epidemics, although this host has not been identified. Risk factors for ONNV include living in rural areas where Anopheles mosquitoes are prevalent. ONNV is primarily localized to Uganda and other parts of Africa.

**Clinical presentation:** The clinical illness caused by ONNV is similar to that of CHIKV, with milder fever and prominent cervical lymphadenopathy. Joint pain lasting several months has been reported, though its frequency is not well documented.

**Laboratory diagnosis:** The laboratory diagnosis of ONNV involves a combination of serological tests, molecular techniques, and virus isolation. The presence of IgM indicates a recent infection, while IgG signifies past exposure or infection.

**Treatment and prevention:** Treatment is primarily supportive, focusing on symptom relief and prevention of complications. Preventive measures, including vector control and community education, are essential in managing and reducing the incidence of ONNV infections.

**Sindbis virus**

Sindbis Virus (SINV), initially identified in Egypt, has since been found in various regions worldwide, including Europe, Africa, Asia, and Australia. It was first isolated from mosquitoes in Egypt but later linked to human disease, notably in Uganda and South Africa.

**Epidemiology:** SINV primarily causes disease in humans in specific regions such as Sweden, Norway, Finland, Russia, and South Africa. SINV exhibits two major lineages: the Paleoarctic/Ethiopian and the Oriental/Australian. These lineages likely spread through migratory birds, with local adaptation and evolution contributing to genetic heterogeneity within each lineage. Unique lineages have also been identified in specific regions, such as southwest Australia.

Epidemics of SINV, including variants like Ockelbo and Pogosta diseases, typically occur during summer and autumn in northern Europe, coinciding with mosquito breeding seasons. Factors like increased rainfall and flooding in usually arid regions have been associated with higher infection rates in areas like South Africa. SINV shares hosts with other flaviviruses like West Nile Virus, particularly in regions like South Africa, the Nile Valley of Egypt, and Israel. Individuals with antibodies to SINV often also have antibodies to West Nile Virus, indicating some degree of cross-reactivity or co-infection.

**Transmission:** Birds serve as reservoirs for SINV, with various species identified as hosts in different regions. Mosquitoes, particularly Aedes, Culiseta, and Culex, act as vectors, transmitting the virus from birds to humans. In some areas, the virus has been isolated from multiple mosquito species.

**Clinical presentation:**Although it infects many vertebrates, clinical disease is predominantly observed in humans. The symptoms include joint pains, rash, fever, headache, and muscle pain, with recovery typically occurring within a few weeks.

**Pogosta disease-** Pogosta Disease, a variant of SINV infection, is characterized by arthritis, rash, fatigue, and mild fever. It primarily affects adults who work or vacation in forested areas of northern Europe, with outbreaks occurring in cycles, typically every 7 years.

**Laboratory diagnosis:** SINV can be isolated from patient samples using cell culture techniques. Serological tests like ELISA detect antibodies, while PCR assays detect viral RNA directly.

**Treatment and prevention:** Treatment for SINV infection focuses on supportive care since there are no specific antiviral medications available. Prevention includes using insect repellents, wearing protective clothing, and controlling mosquito populations through insecticide use and environmental management.

**Ross River Virus**

Ross River Virus (RRV) was named after an area near Townsville in northeastern Australia, where it was first isolated from mosquitoes in 1966. It has since been found in various regions including Papua New Guinea, Irian Jaya, and the Solomon Islands. An epidemic in 1979–80 involving over 50,000 cases spread to various Pacific Islands, likely originating from a viremic air traveler from Australia.

**Epidemiology:** RRV disease occurs every year in Australia, with most cases reported between 2000 and 8000 annually. In tropical northern regions, activity peaks during the wet season from December to May, while in temperate regions, infections occur predominantly in late spring, summer, or autumn. Major outbreaks occur every 2–4 years, with occasional outbreaks in arid inland regions following heavy rainfall and flooding.

Three genotypes of RRV have been identified. Genotype 1 was present in Queensland until the mid-1970s, while Genotype 2 has become the major circulating type throughout Australia. Genotype 3, previously dominant in southwest Australia, has been largely replaced by Genotype 2. Pacific islands isolates belong to Genotype 2.

**Transmission:** RRV is primarily maintained in a cycle between mosquitoes and vertebrate hosts, particularly macropod marsupials like kangaroos and wallabies. Mosquito species like Ae. vigilax and Ae. camptorhynchus are important vectors in coastal areas, while freshwater breeding species like Cx. annulirostris also contribute to transmission.

**Clinical presentation:** RRV disease typically presents with symptoms such as joint pains, muscle pains, and fatigue, with tenosynovitis and fasciitis being common. Most cases occur in individuals aged 20 to 60 years, with clinical illness appearing to be less common in children. Joint symptoms can persist for months or even years after the initial infection, making RRV a significant cause of epidemic polyarthritis and myalgia in humid northern tropical areas of Australia.

**Laboratory diagnosis:** Serological tests and molecular detection are used to diagnose the viral infection.

**Treatment:** Treatment for RRV primarily focuses on managing symptoms since no specific antiviral therapies are available. Supportive care is crucial, including rest, hydration, and pain relief through analgesics like acetaminophen or nonsteroidal anti-inflammatory drugs (NSAIDs).

**Mayaro Virus**

Mayaro Virus (MAYV) was first isolated from a forest worker in Trinidad in 1954 and is now known to be widely distributed in Central America, northern South America, and the Amazon Basin. It has caused documented epidemics in Brazil and Bolivia.

**Epidemiology:** Occasional outbreaks and sporadic cases occur in endemic areas, usually following human contact with forest environments. The illness caused by MAYV is very similar to chikungunya virus (CHIKV), and can include symptoms like febrile illness with rash and occasional arthropathy, and in some cases, hemorrhagic disease. There are two genotypes of MAYV, one of which is widespread, while the other is found only in Brazil.

**Transmission:** MAYV is primarily transmitted by forest-dwelling Haemagogus mosquitoes, with potential vectors including Mansonia venezuelensis and Ae. aegypti. The virus is likely maintained in a sylvatic cycle between mosquitoes and wild vertebrates, including marmosets and other nonhuman primates.

**Clinical presentation:** Mayaro fever, presents with symptoms similar to chikungunya virus (CHIKV). Patients experience sudden high fever with chills, followed by a maculopapular rash. Severe joint pain and swelling, which can persist for weeks to months and sometimes lead to chronic arthritis, are common. The incubation period is 1 to 12 days, with the acute phase lasting one to two weeks, though joint pain and fatigue may last much longer.

**Laboratory diagnosis:** Diagnosis is confirmed through serology and molecular tests. Treatment focuses on symptom relief, as no specific antiviral therapy exists.

**Treatment:** Supportive care is essential. Analgesics such as acetaminophen or nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to alleviate fever, joint pain, and muscle pain. Preventative measures, such as avoiding mosquito bites, are crucial in reducing the risk of infection.

**Bebaru virus**

Bebaru virus, an alphavirus, was first isolated from mosquitoes in Southeast Asia. Although it can cause fever and joint pain in humans, reports of illness are rare, and it is not commonly associated with significant outbreaks or severe disease. The virus primarily circulates between mosquitoes and vertebrate hosts in its natural environment. Compared to other alphaviruses, such as chikungunya or Ross River virus, Bebaru virus has a much lower profile in terms of its impact on human health

**Cabassou virus**

Cabassou virus is an alphavirus found in South America. It was isolated from mosquitoes, suggesting it is part of a sylvatic transmission cycle involving mosquitoes and possibly wild vertebrate hosts. However, there is limited information available on Cabassou virus, and it is not commonly associated with human disease. Unlike more prominent alphaviruses such as chikungunya or Ross River virus, Cabassou virus appears to cause milder or less frequent illnesses in humans, if at all. Consequently, its impact on public health is considered minimal.

**Semliki forest viruses**

Semliki Forest Virus (SFV), first isolated in 1942 from mosquitoes in Uganda, is found throughout sub-Saharan Africa. Serological surveys indicate that human infection is common in endemic regions. However, SFV has been linked to only one reported human outbreak, which occurred in the Central African Republic in 1987, causing symptoms such as fever, headache, and joint pains.

**Barmah forest virus**

Barmah Forest Virus was first isolated from mosquitoes in southeastern Australia in 1974 and later found in other parts of eastern and northern Australia. Human infections have been reported throughout mainland Australia, with seasonal and regional variations in incidence. BFV has been found in many of the same mosquito species that carry RRV, and it is believed that marsupial hosts play a key role in its maintenance and amplification. The virus shows genetic homogeneity across Australia, indicating a widely spread single strain.

BFV disease affects individuals aged 5 to 73, most commonly those between 20 and 60. The clinical presentation is similar to RRV disease but with less common and severe joint involvement. Rashes, which are more likely to be vesicular or urticarial, are more common in BFV. Chronic illness can follow BFV arthritis, although it is less frequent compared to RRV, occurring in about 10% of cases.

**Encephalitis causing alphavirus**

Encephalitis-causing alphaviruses are geographically limited by their arthropod vectors.

**Eastern Equine Encephalitis Virus (EEEV)**

Eastern Equine Encephalitis Virus (EEEV) was first identified in 1933 from infected horses in Virginia and New Jersey.

**Epidemiology:** EEEV is commonly found in freshwater hardwood swamps in the Atlantic and Gulf Coast states, as well as the Great Lakes region. The highest number of cases has been reported in Florida, Georgia, Massachusetts, and New Jersey. EEEV can persist in cold climates, likely through overwintering, and may migrate along the East Coast of the United States. The virus is also highly infectious via the aerosol route, posing a significant risk in laboratory settings.

**Transmission:** EEEV can cause severe disease in horses, some bird species, and dogs, though horses do not amplify the virus during epidemics. However, horses often serve as early indicators of an outbreak. Transmission to humans and horses is primarily through *Aedes*, *Coquillettidia*, and *Culex* species.

**Clinical presentation:** From 1964 to 2004, there were 220 confirmed human cases of EEE in the U.S., with a high case-fatality rate estimated at 50-70%. Symptoms typically appear 4-10 days after infection and include sudden fever, muscle pain, and headache. Severe cases may progress to encephalitis, causing vomiting, seizures, coma, and potentially death. Imaging studies of EEEV-infected individuals often show brain changes indicative of edema, ischemia, and hypoperfusion.

**Laboratory diagnosis:** Serum, CSF and tissue samples (brain tissue, if available) are commonly collected for diagnosis.Early collection of samples post-onset of symptoms is crucial for accurate diagnosis.

1. **Serological tests-**
   1. **IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (MAC-ELISA)-** Detects IgM antibodies against EEEV in serum or CSF. Presence of IgM suggests recent infection.
   2. **Paired Serology-** Detection of a four-fold rise in IgG antibody titers between acute and convalescent serum samples.
2. **Nucleic acid amplification tests-** RT-PCR detects EEEV RNA in serum, CSF, or tissue samples. RT-PCR is highly sensitive and specific, allowing for early detection of viral RNA.
3. **Virus isolation-** EEEV can be isolated from serum or CSF by culturing in mosquito or mammalian cell lines. Virus isolation is less commonly used due to its lower sensitivity and longer turnaround time compared to molecular methods.
4. **Immunohistochemistry-** Used to detect EEEV antigens in tissue samples, particularly brain tissue, during autopsy.
5. **Pathological examination-** Pathological examinations reveal significant damage to the brain and visceral organs, characterized by edema, vascular congestion, hemorrhage, vasculitis, and encephalitis.
6. **Animal studies-**. In both mice and hamsters, EEEV rapidly invades the brain, primarily affecting the basal ganglia and brain stem.

**Treatment:** Currently, there is no specific antiviral treatment for EEEV. Management focuses on supportive care to alleviate symptoms and prevent complications. Severe cases often require hospitalization, especially for patients presenting with encephalitis. Mechanical ventilation may be necessary for patients with respiratory failure. Anticonvulsants are administered to control seizures. Use of corticosteroids, though controversial, may be considered in some cases to reduce inflammation.

**Prevention:** No licensed human vaccine for EEEV is available. However, Horses are vaccinated with a formalin-inactivated virus and available as a combined vaccine with WEEV. Individuals are advised to use insect repellent, wear long-sleeved clothing, and avoid outdoor activities during peak mosquito activity (dawn and dusk).

**Western Equine Encephalitis Virus (WEEV)**

Western Equine Encephalitis (WEEV) virus is prevalent in the Americas, particularly in North America during the summer months, with a notable presence west of the Mississippi River.

**Transmission:** The WEE virus is transmitted mainly by the mosquito *Culex tarsalis*, which thrives in rural areas, especially those involved in agriculture and irrigation. People living in or frequently visiting these areas are at higher risk of infection. Infants and older adults are particularly vulnerable to severe forms of the disease, such as encephalitis. Risk factors for WEE infection include rural residence and outdoor work, which increase exposure to mosquito bites.

**Epidemiology:** Significant outbreaks of WEEV occurred in the mid-20th century. However, control efforts have dramatically reduced the incidence of WEEV since 1996. Between 1964 and 2005, there were 639 confirmed human cases of WEEV in the U.S., with a notable decline in cases after 1988. This reduction is attributed to improved irrigation practices and effective mosquito control programs. The overall case-fatality rate is higher in young children, whereas older adults may also suffer from significant morbidity.

**Clinical presentation:** WEEV infections are often asymptomatic or cause mild illness. Common symptoms include fever, headache, nausea, vomiting, anorexia, and malaise. In severe cases, symptoms can escalate to altered mental status, weakness, meningeal irritation, encephalitis, or encephalomyelitis, which can lead to seizures, coma, and death. The case-fatality rate for WEE is estimated to be between 3-7%.

**Laboratory diagnosis:** Common specimens include serum, CSF, and tissue samples from affected areas such as the brain in severe cases.

1. **Serological tests-**

* **MAC ELISA-** used to detect WEEV-specific IgM and IgG antibodies in serum or CSF. IgM antibodies indicate recent infection, while IgG antibodies indicate past exposure or vaccination.
* **Hemagglutination inhibition**- Measures the ability of antibodies to prevent the agglutination of red blood cells by the virus, helping to confirm infection.
* **Complement fixation**- Detects the presence of antibodies that bind to and "fix" complement proteins, which can indicate WEEV infection.
* **Neutralization assay**- Determines the presence of neutralizing antibodies by measuring their ability to inhibit viral infection of cultured cells.

1. **Molecular assay-**. RT-PCR is highly sensitive and can identify the virus even in early stages of infection.Real-Time RT-PCR quantifies viral RNA in real-time, providing both diagnostic and prognostic information. It is useful for monitoring viral load and disease progression.
2. **Virus isolation-**

* **Cell culture**: Clinical specimens are inoculated into susceptible cell lines, such as Vero or BHK cells. Cytopathic effects observed in these cultures indicate viral presence.
* **Animal inoculation**: In some cases, the virus can be isolated by inoculating specimens into newborn mice, which are highly susceptible to WEEV. This method is rarely used due to ethical considerations and the availability of more rapid techniques.

1. **Immunohistochemistry-** Involves staining tissue sections with antibodies specific to WEEV antigens. This method can detect viral proteins in tissue samples, providing evidence of infection in severe cases, particularly encephalitis.
2. **Pathological examination-** Encephalitis caused by WEEV is characterized by vasculitis and focal hemorrhages in the basal ganglia and thalamus. Small hemorrhages in the white and gray matter of the brain can occur, which may be mistaken for resolved infarcts, especially in elderly patients.
3. **Clinical indicators-** Elevated protein levels and leucocytosis in CSF may suggest infection.

**Treatment:** Treatment for Western Equine Encephalitis Virus (WEEV) focuses on supportive care, as no specific antiviral medications are available. Antibiotics may be used to prevent or treat secondary bacterial infections if suspected or confirmed. Horses are typically vaccinated with a formalin-inactivated WEEV vaccine, often combined with Eastern Equine Encephalitis Virus (EEEV) vaccine. Due to the poor immunogenicity of the inactivated vaccine, horses may need biannual vaccinations to maintain protection. Monitoring the development of neutralizing antibodies helps assess vaccination effectiveness and provides insight into the horse's immune response to the viru

**Prevention:** Mosquito control programs, including the elimination of breeding sites and insecticide spraying, can help reduce mosquito populations and the risk of transmission. Vaccination against WEEV is available for horses but not for humans.

**Venezuelan Equine Encephalitis (VEE)**

Venezuelan Equine Encephalitis Virus (VEEV) was first isolated in 1938 from the brain of an infected animal in Venezuela.

**Epidemiology:** Melanoconion mosquitoes are widely distributed across Central and South America, including regions where VEEV is endemic. They are commonly found in forested areas, swamps, and other environments with abundant vegetation and standing water, which provide ideal breeding conditions. Their preference for these habitats aligns with the presence of rodents and other small mammals that act as reservoirs for VEEV.

**Transmission:** It is a zoonotic pathogen transmitted between vector mosquitoes and vertebrate hosts. The enzootic cycle involves rodents and humans, while the epidemic or epizootic cycles involve horses and humans.

1. **Enzootic cycle-** In the enzootic cycle of VEEV, Melanoconion mosquitoes, particularly species within the *Culex* (Melanoconion) subgenus, serve as vectors transmitting the virus between rodents, the primary vertebrate hosts, and other mosquitoes. These mosquitoes thrive in forested and swampy areas, where they encounter and feed on a variety of small mammals that harbor the virus. This cycle allows the virus to persist in nature, often without causing large-scale outbreaks.
2. **Epizootic and epidemic cycles-** Other mosquito species, such as *Ochlerotatus taeniorhynchus*, are responsible for epizootic or epidemic outbreaks. These outbreaks typically occur when environmental conditions favour a shift in the virus's transmission dynamics, allowing it to spill over from enzootic hosts to equines and humans. Horses, in particular, develop high viremia levels and become key amplifiers of the virus, further facilitating transmission to humans.

**Clinical presentation**: Infection varies depending on the severity of the disease and the host's immune response. In mild cases, VEEV infection typically manifests as flu-like symptoms, including fever, headache, myalgia, and malaise. These symptoms usually appear after an incubation period of 2 to 5 days.In children and immunocompromised individuals, the disease can progress to encephalitis. Symptoms of encephalitis include high fever, severe headache, vomiting, confusion, disorientation, ataxia, seizures, and, in severe cases, coma. Neurological symptoms are more common and severe in children, who may also experience convulsions and long-term neurological sequelae.

**Laboratory diagnosis:** Diagnosis of VEEV is primarily based on clinical presentation, epidemiological history, and laboratory testing. Laboratory confirmation includes serological assays to detect specific IgM and IgG antibodies (MAC ELISA), virus isolation, and nucleic acid amplification tests such as RT-PCR to detect viral RNA.

The plaque reduction neutralization test (PRNT) is another useful method to distinguish VEEV infections from other alphaviruses, though it cannot identify the serotype. Recently, a VEEV-specific blocking ELISA has been developed to detect serotype-specific antibodies in the sera of humans, equids, or rodents.

**Treatment and prevention:** There is no effective antiviral treatment for VEEV. Supportive care focuses on alleviating symptoms and preventing complications. Controlling mosquitoes in their natural habitats is crucial for preventing the disease from transitioning from enzootic to epidemic cycles.

**Rubella virus (German Measles)**

Rubella virus infection, known as rubella or German measles, can lead to severe birth defects if contracted during pregnancy. The association between gestational rubella and congenital cataracts was first reported by Norman Gregg in 1941, establishing rubella virus as a significant teratogen. The virus was first isolated in 1962 and belongs to the Togaviridae family, specifically in the genus Rubivirus. It has two clades and up to 13 genotypes.

**Structure:** Rubella virions are spherical particles, 60–70 nm in diameter, with an electron-dense core separated from the lipid envelope by an electron-lucent zone, exhibiting significant pleomorphism. The icosahedral capsid, composed of the C protein (34 kDa), forms disulfide-linked homodimers. The virus contains structural polypeptides E1, E2, and C, along with nonstructural proteins related to replication and transcription-

* **E1-** It has a molecular weight of 59 kDa. The glycoproteins primarily form heterodimers, which are easily disrupted by standard preparation techniques. E1 is more exposed on the virion surface, contains the viral hemagglutinin and receptor site, and is immunodominant in the humoral response. It also possesses neutralization activity.
* **E2-** Ranges from 44 to 50 kDa, less than E1 due to differential glycosylation. The glycoproteins primarily form heterodimers, which are easily disrupted by standard preparation techniques.
* **C-** It is considered as capsid protein that surrounds viral RNA.

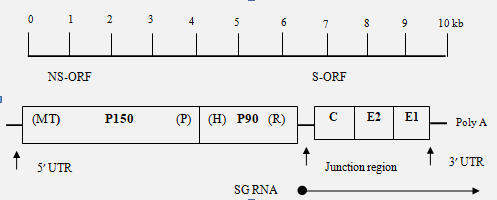
**Genome organization:** The RUBV genomic RNA is 9762 nucleotides long, featuring a 5' terminal cap and a 3' terminal poly (A) tract. Notably, it has the highest guanine (30%) and cytosine (39%) content among RNA viruses. The genome comprises two long, non-overlapping open reading frames (ORFs) and untranslated regions (UTRs) at the 5' and 3' ends, and between the ORFs.

**Resistance:** The virions are stable at physiological pH and can be frozen below -20°C for years without losing infectivity. The live, attenuated vaccine virus is stored in lyophilized form. Rubella virions are susceptible to common inactivating agents like formaldehyde, UV light, and lipid solvents.

### Virus replication:

**1. Virus entry-** The specific receptor for RUBV is unknown. The virus enters cells via receptor-mediated endocytosis. In the acidic environment of the endocytic vesicle, the viral envelope fuses with the vesicular membrane, releasing the capsid and genomic RNA into the cytoplasm.

**2. RNA translation and replication-** The genomic RNA (Figure 68) is translated into a non-structural protein precursor, which is cleaved into P150 and P90 proteins.These proteins facilitate the synthesis of a genome-length, minus-sense RNA, used as a template for producing genomic and subgenomic RNAs.RNA synthesis is asymmetric, producing more plus-sense RNA than minus-sense RNA.The uncleaved non-structural protein precursor is active in minus-strand RNA synthesis, while the cleaved P150/P90 complex is active in plus-strand RNA synthesis.



**Figure 68: Coding strategy of Rubella virus**

**3. Structural Protein translation-** Structural proteins E2 and E1 are directed into the endoplasmic reticulum (ER) by signal sequences, where the C–E2 cleavage is mediated by signal endopeptidase. In the ER lumen, E2 and E1 heterodimerize, undergo complex folding, and acquire high-mannose glycans. Following this, E1 and E2 migrate to the Golgi for further glycosylation. Late in the infection process, E1 and E2 move to the cell surface, where viral budding occurs.

**4. Capsid morphogenesis and budding-** Rubella virus capsid morphogenesis is associated with cell membranes, likely mediated by the E2 signal sequence. The capsid protein associates with the E2–E1 heterodimer and migrates from the ER to the Golgi.The capsids become visible only during budding, associated with deformed membranes.Phosphorylation of the C protein regulates genome encapsidation.

**5. Replication and effects on host cells-** Virus replicates (Figure 69) in the cytoplasm without involving the nucleus.Infection does not grossly inhibit cell macromolecular synthesis but may induce specific gene perturbations.Infected cells appear similar to uninfected cells microscopically but show cytoskeletal and organelle rearrangements.Rubella inhibits growth in primary human cell cultures by affecting mitosis.In cell lines exhibiting CPE, such as Vero and RK-13 cells, cell death occurs through apoptosis.

### Pathogenesis: The only natural host is humans, and there is no reliable animal model for studying its pathogenesis.

### a. Uncomplicated rubella pathogenesis- The pathogenesis of uncomplicated rubella remains poorly understood due to its typically mild nature. The incubation period ranges from 12 to 23 days, with primary and secondary viremia. The virus has been detected in leukocytes a week before symptoms appear, and the rash manifests as immunity develops and the virus is cleared from the blood, suggesting an immunological basis.

**b. Postinfectious encephalitis and arthritis-** Complications such as postinfectious encephalitis are thought to be autoimmune since the virus is not found in or brain tissue at autopsy, despite the absence of extensive inflammation and demyelination. In rubella arthritis, the virus has been detected in synovial fluid and cells, suggesting that viral persistence may play a role.

**c. Fetal infection and congenital rubella syndrome-** Following fetal infection, the virus can be found in nearly every organ of aborted fetuses or infants who die shortly after birth, despite infecting only a small fraction of cells. This low infection rate can still cause significant birth defects associated with congenital rubella syndrome. Early infection during organogenesis inhibits cell division, leading to developmental delays and abnormalities. Virus shedding usually stops by six months of age

**Epidemiology:** Historically, Rubella virus was endemic worldwide, affecting middle childhood in temperate regions and children under five in tropical areas, with seasonal peaks in spring and epidemics every 5-9 years. Known as "third disease" after measles and scarlet fever, it was distinguished in the late 19th century. The last major U.S. epidemic in 1964 infected about 12.5 million people. Large-scale epidemics ceased in countries with widespread vaccination, though limited outbreaks persisted in specific settings. In 2005, rubella was declared no longer endemic in the U.S. due to a 95% vaccination rate among school-aged children.

Penetration and unencapsidation

Genome RNA (+)

S-ORF

NS-ORF

(+) strand synthesis

(-) strand synthesis

Translation and processing

P150

P90

Genome complement (-)

SG-RNA synthesis

SG-RNA (+)

S-ORF

Translation and processing

Encapsidation

Budding

C

E1

E2

Modified cell membrane

**Figure 69: Replication cycle of rubella virus**

**Transmission:** Rubella virus spreads through droplets from respiratory secretions of infected individuals. Patients are most contagious during the rash's eruption, but they can shed the virus from their throat from 10 days before to 15 days after the rash appears. Infants with congenital rubella excrete large amounts of the virus from body secretions for many months, potentially infecting their caregivers. This occurs despite the presence of high levels of neutralizing antibodies, suggesting possible immune tolerance due to fetal infection

**Clinical presentation:** Age plays a pivotal role in the severity of rubella infections. While postnatal rubella is typically mild, especially in children, fetal exposure during early pregnancy can lead to severe complications due to maternal rubella.

**a. Postnatal rubella-** Most postnatal rubella cases are asymptomatic. However, symptomatic patients, particularly adults, may experience malaise, fever, and anorexia before developing symptoms like adenopathy and rash. These symptoms are not exclusive to rubella and may resemble other viral infections.

**Complications-** Complications of postnatal rubella are rare.. Arthritis or arthralgia may occur, especially in women, with symptoms lasting up to a month. Encephalitis, though extremely rare, carries a higher mortality rate in adults.

**b. Congenital rubella syndrome-** Congenital rubella syndrome results from fetal damage caused by maternal rubella, leading to fetal death, premature delivery, or congenital defects. The severity depending on the stage of pregnancy-

* In very early pregnancy, infection often leads to abortion.
* In the first trimester, there is a high risk of congenital malformations, reported to be up to 90%.
* In later stages of pregnancy, damage may be more subtle, manifesting as communication defects or developmental retardation, which may not become apparent until the child is older.

**Manifestations and persistence**: The most common malformations associated with congenital rubella syndrome are deafness, cataracts, congenital heart disease, and mental retardation. Other features include hepatosplenomegaly, thrombocytopenic purpura, myocarditis, and bone lesions, collectively known as the "expanded rubella syndrome." Infants with congenital rubella shed the virus in all bodily secretions, with some continuing to shed for up to six months or more. The virus may persist in tissues such as cataractous lenses for several years, making infected babies a significant source of infection in nursery staff.

**Immune response:** After acute rubella infection, IgM antibodies become detectable around the onset of rash and persist for 1-2 months, aiding in the diagnosis of acute infection. Subsequently, IgG1 antibodies persist indefinitely, primarily targeting the E1 glycoprotein. Rubella-specific cellular immune responses develop within 1-2 weeks of illness onset, with CD4+ epitopes mapped to all three virus structural proteins. In fetal infection, detectable IgM antibodies appear at 18-20 weeks of gestation, and maternal IgG crosses the placenta, exhibiting virus-neutralizing activity. Post-birth, the presence of IgM or a lack of decline in IgG titer confirms fetal infection.

### Laboratory diagnosis: Acute rubella symptoms are easily mistaken for other common illnesses, necessitating specific diagnostic methods.

**1. Rubella diagnosis in pregnancy-** Laboratory confirmation is crucial when rubella is suspected in pregnant women to assess potential risks to the fetus.

* **Serology (ELISA):** IgM indicates current acute infection, while IgG alone denotes past infection or vaccination, indicating immunity.
* **Paired serum testing:** Paired serum samples tested 10 days apart can demonstrate a significant rise or fall in antibody levels.
* **TORCH panel screening:** Pregnant women are screened for TORCH [Toxoplasmosis, (Others-syphilis, varicella-zoster, parvovirus B19), Rubella, Cytomegalovirus, Herpes simplex virus] panel infections, including rubella, to rule out congenital infections.
* **Virus isolation:** Rubella virus isolation from blood or throat swabs is less common due to technical challenges and delays.

**2. Congenital rubella diagnosis-**

* **Serology:** IgM antibodies in newborns indicate intrauterine infection, as IgM does not cross the placenta. Paired serum samples from mother and child can demonstrate IgG antibodies, with persistent IgG in the newborn indicating congenital infection.
* **Virus isolation:** The virus may be isolated from various sources such as urine, throat swabs, leucocytes, bone marrow, or cerebrospinal fluid in cases of congenital rubella.

**Treatment:** Postnatal rubella is typically mild, requiring no specific treatment. Symptom management is recommended for fever, arthritis, or arthralgia. Immune globulin was previously suggested for exposed pregnant women, but it only suppresses symptoms without preventing viremia.

**Rubella vaccination:** The live-attenuated vaccine was licensed in the United States in 1969. The vaccine aimed to prevent congenital rubella by controlling postnatal rubella. Live attenuated vaccine, RA 27/3 strain, has been developed through serial passage in tissue culture and are administered subcutaneously.

* **Dosage-** This vaccine can be given alone or combined with measles and mumps components as the MMR vaccine. The first dose is typically administered to children between 12-15 months of age, with a second dose given at 4-6 years of age.
* **Complications-** Generally well tolerated, it may cause minor reactions such as lymphadenopathy, rash, and arthralgia.
* **Contradiction-** The vaccine is contraindicated for immunodeficient individuals and pregnant women, who should also avoid pregnancy for three months post-vaccination. However, the vaccine is not teratogenic, so accidental vaccination during pregnancy is unlikely to cause congenital defects.
* **Effectiveness**- The MMR vaccine provides lifelong immunity in most individuals. It has significantly reduced the incidence of rubella and congenital rubella syndrome in countries with high vaccination coverage.
* **MR vaccine-** India switched to MR (Measles-Rubella) vaccine in its immunization campaign. This switch allows for a more focused approach to combating these diseases and ensures comprehensive coverage among children aged 9 months to 15 years. The first dose is usually given around 9 months of age, while the second dose follows around 15 months of age.

**Vaccination camp in India:** The MR (Measles-Rubella) vaccine campaign in India targets the elimination of measles and control of rubella. It aims to vaccinate children aged 9 months to 15 years, with the goal of achieving measles elimination by 2023 and reducing rubella cases significantly. The campaign is conducted in phases across various states, utilizing school-based immunization and community outreach to ensure comprehensive coverage. Existing healthcare infrastructure is leveraged for vaccine delivery, alongside public awareness campaigns to educate parents and caregivers. Collaboration with government departments, NGOs, and international organizations is crucial. Challenges include vaccine hesitancy, cold chain maintenance, and reaching remote areas. Continuous follow-up and booster campaigns are essential to maintain high immunity levels. In 2024, efforts are ongoing, focusing on reaching children.

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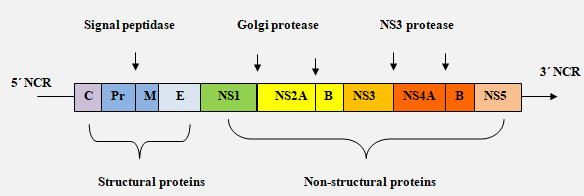
**Flaviviridae**

The term "Flavivirus" originates from the Latin "flavus," meaning yellow, attributed to the yellow fever virus. Flavivirus, a globally distributed genus comprising over 70 types of small envelope viruses, includes well-known pathogens such as dengue, Zika, West Nile, yellow fever, Japanese encephalitis, and Tick-borne encephalitis viruses. Flavivirus infections are crucial in diagnosing various illnesses, especially in travellers, with careful evaluation of epidemiological and clinical data aiding diagnosis.

**Structure:** The flavivirus virion is a small spherical particle with a diameter of about 50 nm, carrying an 11 kb genome. This genome (Figure 70) begins with a type I cap structure at the 5′-terminal portion and typically lacks a poly-A tail at the 3′-end, except for certain strains like tick-borne Encephalitis virus. The genome contains a single large open reading frame that codes for a polyprotein, later cleaved into ten proteins (Table 32), including three structural (capsid, pre-membrane, and envelope) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).

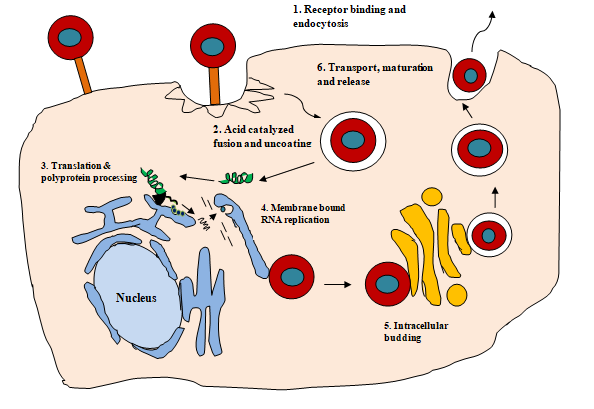
**Table 32: Classification of flavivirus proteins**

|  |  |  |  |
| --- | --- | --- | --- |
| Protein classification | Proteins | Proteases involved | Functions |
| Structural | Capsid (C) | NS2B–NS3 protease   |  | | --- | |  | | Responsible for packaging viral RNA during assembly and nucleocapsid formation. |
| Pre-membrane (PrM) | |  | | --- | | Furin | | Acts as a scaffold, preventing premature fusion of the virus during cell detachment. Its glycosylated form aids in E protein synthesis and forms heterodimers in immature virus particles. |
| Membrane (M) | Signalase | Transmembrane protein, cleaved portion of prM. |
| Envelope | Signalase | Mediates receptor-mediated endocytic fusion, cell entry, viral assembly, and budding. Comprises stem-transmembrane domain pair and three ectodomains. |
| Non-structural | NS1 | Signalase | Facilitates RNA replication and immune evasion. |
| NS2A | ER-bound protease | Facilitates replication complex formation, virus assembly, and evasion of host immune response. |
| NS2B | NS2B–NS3 protease | Serves as a cofactor for NS3 protease. |
| NS3 | NS2B–NS3 protease | Exhibits trypsin-like serine protease, helicase NTPase, and RNA triphosphatase activities. |
| NS4A | NS2B–NS3 protease | Involved in replication complex formation and RNA replication. |
| NS4B | Signalase | Participates in replication complex formation and RNA replication. |
| NS5 | NS2B–NS3 protease | Central to viral RNA replication and may act as an interferon antagonist in flavivirus pathogenesis. |

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**Figure 70: Genome structure of flavivirus**

**Replication:** Flaviviruses infect target cells through bites from an infected vector. They enter host cells via receptor-mediated endocytosis and are transported to endosomes. The acidic conditions in endosomes trigger the fusion of the viral and endosomal membranes, leading to the release of viral RNA into the host cell cytoplasm. There, the RNA uses the host's machinery to produce three structural and seven nonstructural proteins, completing viral replication and assembly. Initially, immature viral particles with a precursor M protein (prM) are synthesized and later matured by the cellular protease furin. The mature viral particles are then released into the extracellular medium through exocytosis (Figure 71).



**Figure 71: Replication of flavivirus**

**Yellow fever (YF) virus**

Yellow fever originated in Africa, the disease was first recognized during an outbreak in the New World in 1648. The virus causes yellow fever, an infectious disease also known as "Blood Vomit." From the 18th to the early 20th century, YFV caused recurrent epidemics, particularly in North America, the Caribbean, and Europe, posing a major threat to humans.

**Epidemiology:** Yellow Fever is endemic to sub-Saharan Africa and South America but has not been documented in Asia. However, it could potentially spread to *Aedes aegypti*-infested areas, including the southern United States. Epidemic (urban) YF is transmitted by Aedes aegypti mosquitoes, which become infected after feeding on viremic humans and then spread the virus. The risk of epidemic transmission increases when a person with a forest-acquired infection travels to an *Aedes aegypti*-infested area while still viremic.

**Pathogenesis:** Early stages of yellow fever (YF) infection show increased cytokines TNF-a, IL-1RA, and IL-6, seen in both vaccine studies and wild-type infections. The virus replicates in lymph nodes then spreads to organs like the liver and kidneys, causing characteristic damage. Recovery results in lifelong immunity. Prior immunity to related viruses might offer partial protection, but not through antibody enhancement. Risk factors for symptomatic illness include youth and advanced age, while hepatitis B carriage doesn't increase risk.

**Clinical presentation:** YF ranges in severity from mild flu-like symptoms to potentially fatal hemorrhagic fever in around half of cases. About half of those infected show no symptoms. Symptoms typically begin abruptly 3 to 6 days after infection, including fever, headache, muscle pain, conjunctival redness, and low white blood cell count. Some patients experience initial relief followed by a recurrence of fever. Severe cases can develop jaundice due to hepatitis and bleeding tendencies. Laboratory findings commonly include albumin in urine, elevated bilirubin levels, and increased liver enzymes. In severe stages, complications such as shock, metabolic acidosis, and neurological symptoms may arise. Fatal outcomes typically occur within 7 to 10 days in severe cases.

**Prevention:** No specific antiviral therapy exists for yellow fever. Treatment primarily involves supportive care such as oxygen, fluids, and pressors to manage hypotension and metabolic acidosis. Fresh-frozen plasma and vitamin K are used to address clotting deficiencies.

**Vaccination:** The 17D vaccine offers effective protection against yellow fever, inducing immunity in over 95% of recipients with a single subcutaneous dose lasting at least 10 years, possibly lifelong. Rare but serious adverse reactions include anaphylaxis and vaccine-associated diseases resembling wild-type infection. Despite these risks, vaccination remains crucial for travelers visiting endemic regions with revaccination every 10 years. In India, the 17D vaccine is produced at the Central Research Institute in Kasauli.

**Dengue**

The earliest English description of dengue, referred to as "break-bone fever," was by Benjamin Rush during the 1780 Philadelphia epidemic. Sporadic outbreaks were subsequently reported throughout the tropics and subtropics. Albert Sabin isolated the virus in 1944 and showed that different viral strains do not cross-protect. After World War II, a pandemic began in Southeast Asia with intensified transmission of multiple serotypes, leading to outbreaks of dengue hemorrhagic fever.

**Epidemiology:** Dengue virus, consisting of four serotypes (1-4), is primarily transmitted in tropical regions by *Aedes aegypti* mosquitoes. Other mosquitoes, like *Aedes albopictus* and *Aedes* *polynesiensis*, can also spread the virus under certain conditions. Although enzootic transmission among forest monkeys occurs in Asia and Africa, human-to-human transmission via mosquitoes maintains the virus. Mosquitoes require a 1-2 week incubation period after feeding on an infected person before they can transmit the virus. They breed near homes in water containers and bite indoors during early morning and late afternoon. Infants born to immune mothers are at risk of developing hemorrhagic fever if infected within their first year, before maternal antibodies diminish.

**Pathogenesis:** Most dengue virus infections go unnoticed, resulting in self-limiting dengue fever. However, Following a mosquito bite, the virus replicates in lymph nodes and spreads through the blood to various tissues. Patients typically experience fever and malaise due to cytokine responses, with myalgia indicating potential muscle pathology.

* **Dengue Hemorrhagic Fever** (**DHF)-** DHF can lead to vascular leakage and hemorrhagic diathesis, exacerbated by immune complex formation and cytokine release. Secondary dengue infections are associated with higher levels of certain cytokines (TNF-α, IL-2). and memory T cell activation. Secondary dengue infection (mostly in children) involves a process known as antibody-dependent enhancement (ADE), where antibodies from a previous infection facilitate increased viral uptake in cells with Fc receptors.
* **Dengue Shock Syndrome (DSS)-** DSS involves extensive immune activation, with elevated levels of cytokines such as TNF-α, IL-8, and IFN-γ, and the release of mediators like RANTES and platelet-activating factor. This results in significant endothelial cell apoptosis and dysfunction, causing severe plasma leakage. The loss of plasma volume leads to circulatory failure, manifested by hypotension and a rapid, weak pulse.

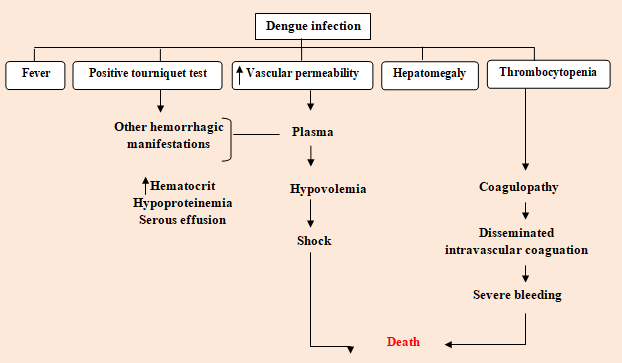
**Clinical presentation:**

**1. Classic Dengue Fever (DF)-** The infection often goes unnoticed or presents with nonspecific symptoms, particularly in children. Symptoms typically appear 4 to 7 days after infection. Initial symptoms include fever, chills, severe headache, and retro-orbital pain, which quickly progress to severe musculoskeletal pain, abdominal tenderness, nausea, and vomiting. A rash appears within 3 to 4 days, followed by petechiae as it fades. Minor mucosal bleeding is common. Vertical transmission can lead to acute neonatal dengue.

**2. Severe dengue complications-**

**a. Dengue hemorrhagic fever-** DHF begins acutely with high fever and symptoms similar to DF, but with increased drowsiness and lethargy. As the fever subsides after 2-7 days, signs of circulatory insufficiency may emerge. Increased vascular permeability and abnormal hemostasis can result in hypovolemia, hypotension, and potentially severe hypovolemic shock with internal bleeding. Petechiae on the trunk, limbs, and axillae appear by the third day. Neurological symptoms may indicate infectious encephalitis, with the virus detectable in cerebrospinal fluid and brain tissue. Thrombocytopenia can be severe, with platelet counts as low as 20,000/cubic mm, and hemoconcentration is marked by a hematocrit rise of 20% or more.

**b. Dengue shock syndrome-** DSS is DHF with signs of circulatory failure, including a narrow pulse pressure (<20 mm Hg) or frank shock (Figure 72). Patients with DSS present with signs of shock, including cold, clammy skin, rapid and weak pulse, narrowing pulse pressure, hypotension and enlarged liver. If not promptly treated, DSS can lead to multiple organ failure and death. Early detection and adequate volume replacement can lead to rapid recovery, with case fatality rates as low as 0.2%. Laboratory criteria include thrombocytopenia (platelet count below 100,000/mm³) and evidence of plasma leakage indicated by hemoconcentration (hematocrit rise of 20% or more for age, sex, and population), pleural effusion, and ascites.



**Figure 72: Clinical symptoms of dengue**

**Prevention:** There is no targeted treatment for dengue. Supportive care involves measures like using cold tepid sponges, administering paracetamol for fever (avoiding aspirin and NSAIDs such as Ibuprofen due to risks of gastritis, vomiting, acidosis, platelet dysfunction, and severe bleeding), replacing fluids and electrolytes, and considering platelet transfusion if platelet counts drop below 10,000. Dengue shock is managed with whole blood transfusion and appropriate shock management techniques.

**The Japanese encephalitis virus (JE)**

The JE virus was first isolated in Japan in 1934. Since the 1970s, the incidence of JE has risen in Southeast Asia, India, Nepal, and Sri Lanka, likely due to changes in agriculture and increased disease recognition. JE annually causes an estimated 35,000 to 50,000 cases and 10,000 to 15,000 deaths, making it the leading cause of epidemic viral encephalitis worldwide.

**Epidemiology:** JE is widespread across Asia, from Pakistan to far eastern Russia, and is both endemic and epidemic in Southeast Asia, China, and the Asian subcontinent. Major outbreaks in Northern India in 2005-2006 caused thousands of deaths. JE transmission is seasonal, with sporadic cases in temperate regions from July to September and hyper-endemic activity from March to October in subtropical Asia. The virus is primarily spread by *Culex tritaeniorhynchus* mosquitoes, which transmit it to pigs and aquatic birds, with humans and horses as incidental hosts. Rural areas with rice paddies are high-risk zones due to favourable mosquito breeding conditions.

**Outbreaks in India:** Japanese encephalitis was first identified in India in 1955 when the virus was isolated from mosquitoes of the *Culex vishnui* complex during an outbreak in Tamil Nadu's Vellore. The virus remains active in Tamil Nadu and Andhra Pradesh, predominantly affecting children, highlighting its endemic status. Most cases occur between October and November.

Since 1976, the highest incidence of human disease has been reported in several states including Andhra Pradesh, Assam, Bihar, Haryana, Goa, Karnataka, Kerala, Tamil Nadu, Pondicherry, Uttar Pradesh, and West Bengal. Sporadic cases have also been documented across the country, with exceptions in Dadra, Daman, Diu, Gujarat, Himachal Pradesh, Jammu and Kashmir, Lakshadweep, Meghalaya, Nagar Haveli, Punjab, Rajasthan, and Sikkim. Japanese encephalitis has emerged as a significant public health concern of national importance in India.

**Pathogenesis:** Once transmitted to humans the virus targets the central nervous system, causing inflammation and damage to brain tissue, leading to the characteristic symptoms of encephalitis. The immune response to the virus contributes to tissue damage, and severe cases can result in coma and death.

**Clinical presentation:** Symptomatic cases of Japanese encephalitis (JE) are rare but severe, often leading to coma and death in 25% of cases. Early symptoms include lethargy, fever, headache, and nausea, progressing to coma. Clinical features include high fever, altered consciousness, facial paralysis, and seizures. Recovery typically occurs after 1 week, with gradual neurological improvement, though long-term complications can persist. Laboratory findings include peripheral leukocytosis and abnormal brain imaging.

**Prevention:** Preventive strategies include controlling mosquitoes and relocating piggeries away from human habitats.

Vaccine options for Japanese encephalitis (JE) include:

1. A **formalin-inactivated** **mouse brain vaccine**, using the Nakayama strain, has been successfully used for immunization in Japan and on a limited scale in India. The vaccine regimen consists of two doses administered two weeks apart, followed by a booster dose 6-12 months later. However, immunity provided by this vaccine is short-lived.
2. A **live attenuated** **vaccine** developed in China from the JE strain SA 14-14-2, after passage through weanling mice. This vaccine is administered in two doses given one year apart and has shown effectiveness in preventing clinical disease.

**West Nile virus (WNV)**

West Nile virus was first isolated from a febrile woman in Uganda's West Nile region in 1937. Mosquito transmission between vertebrate hosts, especially birds, was soon confirmed. Although the annual number of cases in the U.S. has decreased since 2003, more than 1000 neurologic cases are still reported each year.

**Epidemiology:** WNV is prevalent across Africa, southern Europe, the Middle East, Asia, Australia, and the Americas. The virus spreads through an enzootic cycle involving birds and mosquitoes, with over 300 bird species and 62 mosquito species implicated. *Culex* mosquitoes are key vectors. Birds in the Corvidae family are particularly susceptible, often signaling the virus's presence with mass die-offs. The virus can also be transmitted through organ transplants, blood products, and possibly breast milk, though blood screening has reduced transfusion risks.

**Pathogenesis:** The virus enters the bloodstream, replicates in target organs, and causes viremia. It crosses the blood-brain barrier, invading the CNS and infecting neurons, leading to inflammation and brain tissue damage. The immune response involves activation of various immune cells, contributing to neuronal destruction and inflammatory lesions.

**Clinical presentation:** Most WNV infections are asymptomatic, but symptoms typically emerge within 2 to 6 days. WN fever, characterized by sudden onset flu-like symptoms such as fever, chills, malaise, headache, and body aches, without evident neurological signs. Lab tests commonly reveal peripheral leukocytosis or leukopenia, CSF pleocytosis, and occasionally hyponatremia,

**Prevention:** There is currently no specific antiviral medication available for WNV. Treatment mainly focuses on managing symptoms. There is no commercially available vaccine for humans

**Zika virus (ZIKV)**

Zika virus was first isolated in 1947 in Uganda's Zika Forest and subsequently identified in humans in 1952. Initially considered benign, its dramatic spread across the Americas in 2016 and its link to congenital abnormalities in infants prompted the WHO to declare it a global health emergency in February 2016.

**Epidemiology:** Zika virus (ZIKV) is primarily transmitted by Aedes mosquitoes and exists in two distinct lineages: African and Asian. In Africa, it circulates between mosquitoes and non-human primates, whereas humans are the main hosts in the Asian lineage. Serological diagnosis is complicated by cross-reactivity with other Flaviviruses. Significant outbreaks occurred in Yap Island, Micronesia (2007), French Polynesia (2013), and New Caledonia (2014). The virus reached Brazil in 2015, rapidly spreading throughout the Americas to various countries.

**Transmission:** ZIKV primarily spreads through the bite of infected mosquitoes, particularly *Aedes aegypti*. It can also be transmitted sexually and has been detected in various body fluids including blood, urine, saliva, and breast milk. Maternal-fetal transmission can lead to congenital infections and neurological complications in infants.

**Clinical presentation:** Approximately 20% of ZIKV infections result in symptomatic illness, characterized by fever, rash, joint pain, and conjunctivitis. Symptoms typically resolve within a week. Infants and children show similar symptoms, with joint pain often manifesting as limb pain or refusal to move limbs.

**Complications:** ZIKV infection is associated with severe complications such as congenital microcephaly in infants born to infected mothers, Guillain-Barre syndrome, and fetal losses during pregnancy.

**Prevention:** Currently, there is no specific treatment for ZIKV infection. Management focuses on supportive care, including rest, hydration, and symptom relief. Preventive measures include vector control, avoiding mosquito bites, and safe sexual practices to prevent transmission.

**St. Louis Encephalitis (SLE)**

First reported as an epidemic of unknown cause in St. Louis, Missouri in 1933. The disease remains prevalent in the U.S., particularly among the elderly in southern states, although evidence suggests that West Nile virus is displacing SLE in California and Texas.

**Epidemiology:** St. Louis Encephalitis (SLE) is prevalent across the United States, reported in almost all states, and also found in the provinces of Ontario and Manitoba in Canada, as well as Sonora State in Mexico. The virus is transmitted to birds in three distinct cycles by different mosquito species: *Culex pipiens* and *Culex quinquefasciatus* in the midwestern and eastern states, *Culex nigripalpus* in Florida, and *Culex tarsalis* in the Great Plains and farther west. Humans are incidental hosts in this enzootic cycle, similar to West Nile Virus (WN) infections.

**Pathogenesis:** Following infection, the virus enters the bloodstream and disseminates throughout the body, including the central nervous system (CNS). Once in the CNS, the virus infects neurons, leading to inflammation and damage to brain tissue. The immune response is activated, involving various immune cells that contribute to the destruction of infected neurons and the formation of inflammatory lesions.

**Clinical presentation:** SLE manifests as febrile headache, aseptic meningitis, or fatal encephalitis, more severe in older adults. Symptoms start with malaise, fever, headache, and myalgias, progressing to confusion, tremors, and ataxia. Generalized weakness and abnormal movements are common. Complications like pneumonia can arise, with higher mortality in older patients. HIV-positive individuals may be at greater risk. Lab findings include elevated white cell count and abnormal CSF composition.

**Prevention:** There is currently no commercially available vaccine. Treatment focuses on managing symptoms and providing supportive care. Drinking fluids (to avoid dehydration) and avoiding peak mosquito hours (during dawn and dusk) are helpful.

**Tick-Borne Diseases**

**A. Tick-Borne Encephalitis (TBE)**

Descriptions of a disease compatible with TBE appeared in Austria in the early 1930s, but the virus was not isolated until 1948. Similar cases in Russia in 1932 led to descriptions of Russian spring-summer encephalitis (RSSE), and the virus was isolated in 1937 from patients and Ixodes ticks. There are three TBE virus subtypes: European, Siberian, and Far Eastern, reflecting their geographic areas. TBE is known by various names, including Central European encephalitis (CEE), RSSE, and biphasic milk fever, the latter due to transmission via unpasteurized milk from infected livestock. The TBE serocomplex includes rare human neurologic disease viruses (e.g., Powassan virus, louping ill virus) and hemorrhagic fever syndrome viruses (e.g., Omsk hemorrhagic fever and Kyasanur Forest disease viruses).

**Epidemiology:** TBE virus comprises three subtypes: Far Eastern, Siberian, and European. These subtypes are geographically distributed, with Far Eastern in eastern Russia, Korea, China, and Japan; European in Scandinavia, Europe, and eastern former Soviet Union; and Siberian in western Siberia. The virus circulates between ticks and vertebrates, with humans as incidental hosts. Key vectors include *Ixodes ricinus* and *Ixodes persulcatus* ticks, thriving in humid, moderate-temperature environments such as forest edges and meadows. In central Europe, TBE cases peak between April to November, with a secondary peak in October. Rarely, transmission can also occur through consuming unpasteurized milk from infected animals or slaughtering infected animals.

**Pathogenesis:** Following a tick bite, the virus enters the bloodstream and disseminates to various organs, including the CNS. In some cases, the virus crosses the blood-brain barrier, leading to direct infection of the brain and spinal cord. This neurotropic virus then replicates within neurons and glial cells, causing inflammation and damage to the CNS. The immune response plays a crucial role in the pathogenesis of TBE. Both innate and adaptive immune mechanisms are activated to control viral replication and clear the infection. However, an excessive or dysregulated immune response can contribute to tissue damage and the development of neurological symptoms.

**Clinical presentation:** Symptoms usually start with fever, headache, and muscle pain, affecting about 1 in 250 people. Neurological issues (meningitis or paralysis) may occur in later stage of illness. Age influences prognosis, with long-term effects possible, including psychological and motor issues. Fatality rates are around 1%, with up to 60% experiencing residual effects. Lab tests show initial leukopenia, later leukocytosis, and abnormal CSF. Diagnosis is complex, especially when concurrent with Lyme disease, requiring consideration of exposure in tick-prone areas.

**Prevention:** There is no specific antiviral treatment for TBE. Treatment focuses on supportive care to manage symptoms. Vaccination is recommended for individuals living in or traveling to endemic areas, higher risk of exposure, such as forestry workers, hikers, and campers-

* **Inactivated whole virus vaccine-** This is the most commonly used TBE vaccine. It contains inactivated virus particles and is given in several doses, typically three doses over a 1-3 month period, followed by booster doses every 3-5 years.
* **Tick-borne encephalitis virus subunit vaccine-** This vaccine contains only specific proteins from the TBE virus rather than whole virus particles. It also requires multiple doses for full immunity.

**B. Tick-borne hemorrhagic fevers**

**1. Kyasanur Forest Disease (KFD)**

Kyasanur Forest Disease (KFD), also known as monkey fever, is a major public health concern in the Western Ghats of India, caused by the KFD virus (KFDV), an arbovirus in the Flaviviridae family. Humans contract KFD through tick bites and typically present with fever, which may include hemorrhagic and neurological symptoms. While 80% of patients recover without complications, around 20% experience a biphasic illness with severe symptoms. Initially confined to Karnataka State, KFD has spread to adjacent states, including Kerala, with cases reported in Wayanad and Malappuram districts in 2015. A killed KFD virus vaccine used in a small field trial showed some protective effect against the disease.

**Clinical Presentation:** The incubation period is 3-8 days. The case fatality rate is 2-10%, with higher rates in non-endemic areas due to lack of awareness and lower herd immunity. KFD typically has a biphasic presentation initially with non-specific flu like symptoms followed by neurologic and hemorrhagic manifestations.-

* **First phase-** Characterized by sudden fever, headache, body pain, conjunctival inflammation, gastrointestinal symptoms, and dehydration.
* **Intermission-**After the first phase, some patients may experience a period of apparent recovery where symptoms subside.
* **Second phase-** Fever reappears after the initial improvement. Hemorrhagic manifestations such as oral mucosal inflammation and maculopapular eruptions, often start 3-4 days after symptom onset. Neurological symptoms include drowsiness, confusion, and convulsions. Positive Kernig sign and abnormal ankle reflex are observed
* **Recovery-** Recovery usually occurs within 10-14 days, but some patients experience prolonged symptoms like muscle twitching and paraesthesia.

**Prevention:** A formalin-inactivated vaccine against KFD virus is available in endemic areas of India. It is administered to high-risk populations such as forest workers and residents of endemic regions. There is no specific antiviral treatment for KFD. Supportive care focuses on managing symptoms and complications. Insect repellents containing DEET and permethrin on clothing can be used.

**2. Omsk Hemorrhagic Fever**

Omsk hemorrhagic fever, found in Russia and Romania, is clinically similar to KFD and caused by a related virus. Dermacentor ticks serve as the vectors for this disease.

List of flaviviruses are shown below (Table 33)-

**Table 33: List of flaviviruses**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Flavivirus | Vector | Neurotropic | Disease | Geographical distribution |
| Dengue Virus (DENV) | Aedes aegypti, Aedes albopictus | No | Dengue Fever, Dengue Hemorrhagic Fever | Tropical and subtropical regions worldwide |
| Zika Virus (ZIKV) | Aedes aegypti, Aedes albopictus | Yes | Zika Fever, Congenital Zika Syndrome | |  | | --- | |  |  |  | | --- | | Africa, Americas, Asia, Pacific | |
| West Nile Virus (WNV) | Culex pipiens, Culex  quinquefasciatus | Yes | West Nile Fever, West Nile Neuroinvasive Disease | Africa, Europe, Middle East, North America, West Asia |
| Yellow Fever Virus (YFV) | Aedes aegypti, Haemagogus spp. | No | Yellow Fever | Sub-Saharan Africa, South America |
| Japanese Encephalitis Virus (JEV) | Culex tritaeniorhynchus, Culex vishnui | Yes | Japanese Encephalitis | |  | | --- | |  |   Asia, Western Pacific |
| Tick-borne Encephalitis Virus (TBEV) | Ixodes ricinus, Ixodes persulcatus | Yes | Tick-borne Encephalitis | Europe, Northern Asia |
| St. Louis Encephalitis Virus (SLEV) | Culex pipiens, Culex quinquefasciatus | Yes | St. Louis Encephalitis | North America, South America |
| Murray Valley Encephalitis Virus (MVEV) | Culex annulirostris | Yes | Murray Valley Encephalitis | Australia, Papua New Guinea |
| Alkhurma Hemorrhagic Fever Virus (AHFV) | Hyalomma dromedarii, Ornithodoros savignyi | No | Alkhurma Hemorrhagic Fever | Saudi Arabia, Egypt |
| Kyasanur Forest Disease Virus (KFDV) | Haemaphysalis spinigera | Yes | Kyasanur Forest Disease | India (Southern) |
| Omsk Hemorrhagic Fever Virus (OHFV) | Dermacentor reticulatus, Ixodes persulcatus | Yes | Omsk Hemorrhagic Fever | Russia (Western Siberia) |
| Langat Virus (LGTV) | Ixodes granulatus | No | Mild febrile illness | Southeast Asia |
| Powassan Virus (POWV) | Ixodes scapularis, Ixodes cookei | Yes | Powassan Encephalitis | North America |
| Usutu Virus (USUV) | Culex pipiens, Culex neavei | Yes | Mild febrile illness, neurological disease | Africa, Europe |
| Rocio Virus (ROCV) | Psorophora ferox | Yes | Rocio Encephalitis | Brazil |
| Ilovaisk Virus | Dermacentor marginatus | No | Mild febrile illness | |  | | --- | |  |  |  | | --- | | Russia | |
| Louping Ill Virus (LIV) | Ixodes ricinus | Yes | Louping Ill | United Kingdom, Ireland, Northern Europe |

**Laboratory diagnosis of flavivirus:** Routine diagnosis of flavivirus-associated diseases involves detecting the pathogen, its nucleic acids, or viral antigens during the acute phase, and capturing specific antibodies after one week (Figure 73).

**1. Virus isolation**- Viral isolation plays a crucial role in diagnosing diseases like YF and dengue, especially when patients are still viremic. Neurotropic flaviviruses can sometimes be isolated from blood before the onset of neurological symptoms, but is ineffective once an antibody response develops. Neurotropic viruses are typically difficult to isolate from CSF, except in the early stages of severe illness. Here are some commonly used cell lines (Table 34) for flavivirus-

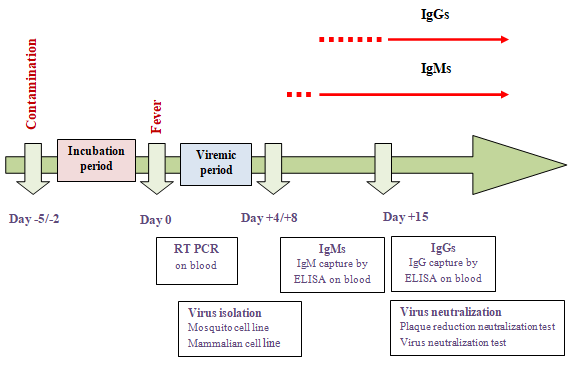
**Table 34: Some commonly used cell lines for flavivirus**

|  |  |
| --- | --- |
| Cell line | Used for |
| **Vero Cells** (African green monkey kidney cells) | Used for the cultivation and isolation of many flaviviruses, including dengue, Zika, and yellow fever viruses. |
| **BHK-21 Cells** (Baby hamster kidney cells) | Used for the propagation of flaviviruses, including West Nile virus and Japanese encephalitis virus. |
| **C6/36 Cells** (*Aedes albopictus* mosquito cells) | Adapted for the growth of arboviruses, including many flaviviruses like dengue, Zika, and yellow fever viruses. |
| **HEK 293 Cells** (Human embryonic kidney cells) | Used for transfection and expression studies involving flavivirus genetic material. |
| **Huh7 Cells** (Human hepatoma cells) | Employed for studying the replication and pathogenesis of flaviviruses (mostly HCV) |
| **PS Cells** (Porcine stable cells) | Used for flavivirus isolation and propagation |
| **Caco-2 Cells** (Human epithelial colorectal adenocarcinoma cells) | Used for studying flavivirus interactions with human epithelial cells. |

**2. Tissue sampling**- Tissue samples, ideally divided and stored properly, are essential for viral isolation and microscopy. Liver samples may be taken postmortem for YF diagnosis, but histological diagnosis alone is presumptive. Various tissues can be sampled for different viruses, with different success rates depending on illness duration and timing.

**3. Serology**- IgM detection by ELISA is preferred, though some labs use IgM and IgG detection by indirect immunofluorescence assay. Accurate testing requires timely serum collection. Both serum and CSF should be examined in flavivirus encephalitis, with positive results usually within 10 days of illness onset. Cross-reactivity between flaviviruses necessitates specific testing methods to differentiate infections. IgM antibodies are typically produced during the acute phase of infection, while IgG antibodies are produced later and can indicate past infection or immunity. Neutralization assays measure the ability of antibodies to neutralize the virus.

**4. Molecular assay-** Real-time RT-PCR is the preferred method for detecting viremia. This amplifies specific regions of the viral genome for detection. Nucleic acid amplification tests are highly sensitive and specific, making them useful for early diagnosis, especially during the acute phase of infection when the viral load is high. They are valuable for detecting viral RNA in clinical specimens such as blood, serum, or tissues.

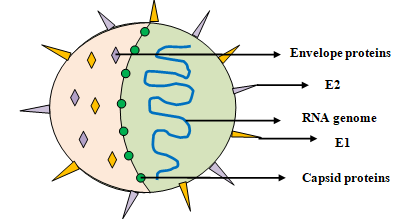
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**Figure 73: Laboratory diagnosis of flavivirus**

**Hepatitis C virus**

Approximately 170 million individuals are infected with HCV worldwide. In the late 1980s, Michael Houghton and Daniel Bradley identified the hepatitis C virus (NANB- non-A, non-B hepatitis), leading to the cloning of its genome and uncovering its link to chronic hepatitis, cirrhosis, and liver cancer.

**Structure:** HCV (Figure 74) is a roughly spherical, enveloped, positive-strand RNA virus about 55 nm in diameter, classified in the flaviviridae family but distinct enough to form its own genus, Hepacivirus. HCV often associates with low-density lipoproteins and, in some cases, with antibodies in high-density complexes, which are less infectious to chimpanzees.



**Figure 74: Structure of HCV**

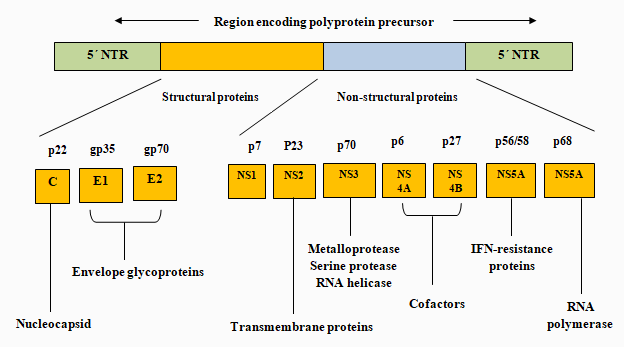
**Genome organization:** The genome encodes (Figure 75) a large polyprotein that undergoes cleavage into structural proteins (core, E1, E2) and nonstructural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B).

**a. Envelope glycoproteins (E1 and E2)-**

E1 (33-35 kDa) and E2 (70-72 kDa) form heterodimers essential for viral entry. E2, with hypervariable regions, differs significantly among HCV isolates, affecting antigen detection assays.

**b. Other nonstructural proteins-**

* **p7:** Forms ion channels critical for virus infection.
* **NS2:** Facilitates viral replication cycle completion through its transmembrane domains in ER.
* **NS3:** Possesses protease and helicase activities crucial for replication. Its interaction with NS4A enhances protease activity.
* **NS4A:** Acts as a cofactor for NS3, stabilizing its protease function.
* **NS4B:** Induces formation of the replication complex, supporting HCV replication.
* **NS5A:** Facilitates replication and is a target for antiviral therapy due to its regulatory roles.
* **NS5B:** RNA-dependent RNA polymerase essential for synthesizing new genomic RNAs.



**Figure 75: Genome organization of HCV**

**Pathogenesis:** HCV primarily targets hepatocytes in the liver, entering through receptors like CD81, SR-BI, and claudin-1. Once inside, it releases its RNA genome, which is translated into a polyprotein and cleaved into functional proteins for replication. HCV RNA appears in plasma within days of exposure, typically 1-4 weeks before liver enzyme levels rise. Viremia peaks at 8-12 weeks, then decreases but often persists, particularly in African Americans and HIV-infected individuals. A strong immune response, indicated by jaundice, reduces the likelihood of persistent infection. Co-infections like HIV and schistosomiasis, along with genetic factors, can weaken immune responses, leading to persistence.

**Immune response:** Antibodies against HCV antigens appear within months of infection.The presence of antibodies does not correlate with viral recovery, as most infections persist.Recovery has been seen in individuals with congenital agammaglobulinemia, suggesting humoral immunity alone is insufficient for clearance.Humoral responses can neutralize specific HCV variants.Persistent infection shows weaker CD4+ responses compared to CD8+ responses. Both CD4+ and CD8+ memory T cells are critical for protecting against re-infection.

**Epidemiology:** Specific areas in Japan, Taiwan, and Italy show higher infection rates, primarily among older individuals. Some urban areas also report particularly high HCV prevalence. HCV RNA can be present in body fluids such as blood, semen and saliva. It may also transmits through-

* **Blood transfusion-** HCV primarily spreads through percutaneous blood exposure. The introduction of HCV antibody testing in blood donations has substantially reduced the risk of transfusion-related HCV infections.
* **Sexual contact-** HCV is detectable in semen and saliva. Those with multiple partners have higher prevalence, but long-term partners of HCV-infected individuals generally have low transmission rates.
* **Mother to infant-** Perinatal transmission occurs in 0% to 4% of cases. HCV RNA can be detected in infants shortly after birth, but breastfeeding does not significantly increase the risk.
* **Drug use-** The primary cause of HCV infections in developed countries is the use of contaminated needles and drug paraphernalia.
* **Nosocomial transmission-** Poor infection control, particularly in hemodialysis units, can lead to transmission.
* **Occupational transmission-** About 1% to 2% of needlestick exposures in health care settings result in HCV transmission, with a risk level between that of HIV and HBV.

**Clinical presentation:** HCV infection presents a wide spectrum, ranging from asymptomatic cases to severe conditions such as liver failure requiring transplantation.

**1. Acute hepatitis C-** Acute hepatitis C is rare and typically asymptomatic. When symptoms occur, they may include anorexia, nausea, abdominal pain, and jaundice. HCV RNA can be detected in the blood within days of exposure, with liver enzyme levels rising subsequently. The incubation period for HCV ranges from 6 to 112 days.

**2. Fulminant hepatitis-** Fulminant hepatitis is rare in Western countries but more prevalent in Japan, accounting for 40-60% of fulminant NANB cases. This discrepancy may result from differences in host factors or viral strains. Fulminant liver disease is more likely if acute HAV infection occurs in individuals with chronic hepatitis C.

**3. Chronic hepatitis C-** Approximately 50-85% of acute HCV infections become chronic, leading to persistent viremia. Chronic infection often presents with non-specific symptoms such as fatigue, moderate abdominal pain, muscle pains, and malaise. While quality of life can be reduced, successful therapy can improve it. Serum ALT levels fluctuate, whereas HCV RNA levels remain stable. Chronic HCV can cause fibrosis and cirrhosis and is linked to metabolic disorders such as insulin resistance and steatosis.

**4. Liver cirrhosis-** Chronic hepatitis C can progress to cirrhosis, characterized by liver scarring. Symptoms include jaundice, ascites, peripheral edema, variceal bleeding, and hepatic encephalopathy.

**5. Hepatocellular Carcinoma (HCC)-** HCC is a late complication of chronic HCV, usually occurring in patients with cirrhosis. Symptoms include a sudden worsening of cirrhosis symptoms, right upper quadrant pain, and elevated serum α-fetoprotein levels. Diagnosis often requires a liver biopsy.

**6. Extrahepatic manifestations of HCV-** HCV is strongly associated with several conditions, including essential mixed cryoglobulinemia, membranoproliferative glomerulonephritis, and porphyria cutanea tarda. Up to 50% of HCV-infected individuals have circulating cryoglobulins, but only a few develop vasculitic syndrome. HCV-related renal disease may stabilize with treatment. HCV is also linked to B-cell lymphoproliferative disorders, Mooren corneal ulcers, Sjögren’s syndrome, lichen planus, and idiopathic pulmonary fibrosis. Chronic HCV can cause thyroid issues, particularly in women.

**Laboratory diagnosis:** Diagnosing HCV infection (Table 35; Figure 76) is crucial for identifying and treating infected individuals, preventing disease progression, and limiting viral spread.

**A. Indirect tests-** Indirect tests detect antibodies produced in response to viral infection:

* **IgM**: Indicates recent infection.
* **IgG**: Indicates recent or past infection.

**1. Antibody detection-**

**a. Screening tests**

* **EIA (Enzyme Immunoassays)**: The third-generation EIA, which detects antibodies against HCV core, NS3, NS4, and NS5 regions, is commonly used. It is highly sensitive and specific but not recommended for infants under 18 months due to possible maternal antibody interference. Fourth-generation EIAs are now available and can detect antibodies earlier.
* **Rapid, Point-of-Care Tests (POCTs)**: These are used at patient care sites, offering quick results with high sensitivity and specificity. The OraQuick HCV Rapid Antibody Test, FDA-approved, is one example.

**b. Confirmatory tests**:

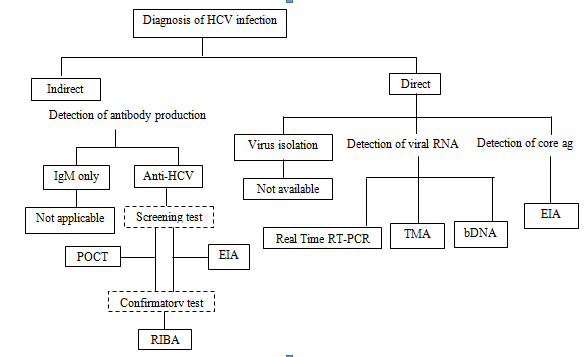
* **Recombinant Immunoblot Assays (RIBA)**: Used to confirm anti-HCV antibodies detected by EIAs. Due to the high sensitivity and specificity of EIAs, RIBA is less commonly needed, with nucleic acid tests (NATs) now preferred for confirmation.

**B. Direct detection**

**1. Viral RNA detection-**

* **Qualitative detection-** Qualitative assays, like RT-PCR and TMA, detect the presence of HCV RNA, indicating viremia.
* **Quantitative detection-** Quantitative assays measure HCV RNA levels, crucial for monitoring viral load before and during treatment. Real-time RT-PCR is commonly used for its sensitivity and broad quantification range.

**2. Core antigen detection:** HCV core antigen can also be detected via EIA, with the antigen titer correlating closely with HCV RNA levels, though this method is not widely used in practice.



**Figure 76: Laboratory diagnosis of HCV**

**C. Genotyping for therapy:** Determining the HCV genotype is critical for predicting therapy response. Commercial assays for genotyping include direct evaluation of the 5′ noncoding sequence and hybridization to genotype-specific probes. These assays are generally accurate for clinical decision-making, though occasionally they may misclassify subtypes. A serologic method for genotyping also exists but is not widely available.

**Table 35: Interpretation of diagnostic results**

|  |  |
| --- | --- |
| Diagnostic results | Interpretation |
| Presence of HCV RNA without anti-HCV antibodies | Suggests acute infection or immunosuppression. |
| Positive antibody test and negative HCV RNA | Indicates resolved infection or acute infection with low-level viremia. |
| Persistence of HCV RNA for more than six months. | Chronic HCV Infection |

**Treatment:** Treatments include (Table 36)-

**Table 36: Virologic response of HCV infection**

|  |  |
| --- | --- |
| Virologic responses | HCV RNA |
| Rapid Virologic Response (RVR) | HCV RNA undetectable (<50 IU/mL) 4 weeks after starting treatment. |
| Early Virologic Response (EVR) | HCV RNA level drops by at least 2 logs or becomes undetectable 12 weeks after starting treatment. |
| End of Treatment Response (ETR) | Undetectable virus at the end of therapy. |
| Sustained Virologic Response (SVR) | Absence of HCV RNA in serum at the end of treatment and 6 months later. |
| Nonresponders | HCV RNA levels fail to decline by at least two logs by 24 weeks. |
| Partial Responders | HCV RNA levels decline but never become undetectable. |
| Relapse | HCV RNA is detected again in persons who achieved an ETR. |

1. **Direct-Acting Antivirals (DAAs)-** Common DAAs include sofosbuvir, ledipasvir, velpatasvir, and glecaprevir/pibrentasvir. They are often used in combination to target different viral proteins (NS3/4A protease, NS5A protein, and NS5B polymerase). Treatment duration ranges from 8-12 weeks and has revolutionized with high cure rates (sustained virologic response, SVR).
2. **Ribavirin-** Used in combination with DAAs in certain cases, especially for patients with advanced liver disease or those who have failed previous treatments. Ribavirin can cause significant side effects, including hemolytic anemia .
3. **Interferon-based therapy-** Previously the standard of care before the advent of DAAs, involving pegylated interferon and ribavirin. This regimen had lower cure rates and more severe side effects. It is now rarely used.

**Prevention:**

**A. Pre-exposure-**

* **Screening and diagnosis-** Regular screening for HCV antibodies and surrogate markers, especially for high-risk groups (e.g., people who inject drugs, recipients of blood transfusion), helps in early detection and treatment, reducing transmission.
* **Safe injection practices-** Ensuring the use of sterile needles and syringes in healthcare and community settings.
* **Blood supply safety-** Rigorous screening of blood donors and blood products for HCV has significantly reduced the risk of transmission through transfusions.
* **Public awareness and education-** Campaigns to educate the public about HCV transmission, risk factors, and the importance of testing and treatment.

**B. Post-Exposure Prophylaxis (PEP)-** There is no standard PEP regimen for HCV. Post-exposure management involves monitoring for HCV RNA and starting treatment early if infection is confirmed.

**Prophylaxis:** Currently, there is no effective vaccine for HCV due to genetic diversity and poor immunological response.

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**Arenaviridae**

Arenaviruses are significant human pathogens, causing hemorrhagic fever (HF) in endemic regions and posing a global health threat. The first arenavirus, LCMV, was identified in 1933 and linked to aseptic meningitis in humans. By the late 1960s, the Arenaviridae family was established, including viruses that cause chronic infections in rodents. Notable pathogenic arenaviruses include:

* **Junín virus (JUNV)**- Causes Argentinian hemorrhagic fever.
* **Machupo virus (MACV)**- Causes Bolivian hemorrhagic fever.
* **Lassa virus (LASV)**- Causes Lassa fever.
* **Other pathogenic arenaviruses**- Guanarito (GTOV), Sabiá (SBAV), Chapare (CHAPV), and Lujo (LUJV) viruses.

**Structure:** Arenaviruses are round, oval, or pleomorphic particles, typically 110 to 130 nm in diameter but ranging from 50 to 300 nm. They possess a viral envelope formed by budding from the host cell's plasma membrane. The surface is covered with 6- to 10-nm spikes, and the interior contains dense granules (20 to 25 nm) identified as host cell ribosomes, giving the virus a "sandy" appearance, hence the name Arenaviridae. Arenaviruses have a segmented RNA genome consisting of S (small) and L (large) strands.

**Epidemiology:** Transmission occurs both vertically and horizontally among rodents, maintaining the virus within populations. Human infections typically result from exposure to rodent excreta, contaminated food, or inhalation of aerosolized virus particles (Table 37).

**Table 37: Epidemiology of arenaviridae**

|  |  |
| --- | --- |
| Virus | Epidemiology |
| Lassa Fever (LF) | Endemic in parts of West Africa; mostly asymptomatic; primary reservoir is Natal multimammate mouse. Human infection occurs through direct contact with rodent excreta or by virus inhalation and by person-to-person transmission |
| Lujo Hemorrhagic Fever (LHF) | Identified in 2008; symptoms similar to LF but includes disseminated intravascular coagulation (DIC); natural reservoir is unknown. |
| Lymphocytic Choriomeningitis (LCM) | Primary host is house mouse; transmission to humans occurs via close contact with infected rodents or their secretions, and through organ transplantation or congenital infection. |
| South American hemorrhagic fevers (SAHFs) | **Argentinian Hemorrhagic Fever (AHF)-** Endemic to Argentina's Pampas region; primary reservoir is drylands *Calomys musculinus* rodent. Human infection peaks during corn harvesting season and is primarily due to inhalation of aerosolized virus (Junin virus) from contaminated soil or rodent excreta. |
| **Bolivian Hemorrhagic Fever (BHF)-** Mostly seen among young children and the elderly; caused by Machupo virus ; primary reservoir is *Calomys callosus* rodent; human infections occur mainly during agricultural activities, through exposure to aerosolized virus from rodent excreta. |
| **Venezuelan Hemorrhagic Fever (VeHF)-** The disease is focal to specific regions in Venezuela and primarily affects agricultural workers; caused by Guanarito virus; short-tailed *Zygodontomys brevicauda* rodent is the primary reservoir. Infections are presumed to occur outdoors in rodent-infested areas. |
| **Brazilian hemorrhagic fever-** Caused by Sabia virus; transmission and epidemiology are less understood compared to AHF and BHF. |

**Pathogenesis:** The viruses typically enter the human body through the lungs and initially replicate in antigen-presenting cells, spreading to other organs. Pathological findings vary, with multifocal hepatocellular necrosis common in LF but insufficient to cause liver failure. Hemorrhagic manifestations are more pronounced in diseases like AHF. LCMV exhibits a strong tropism for the fetal brain, causing severe congenital abnormalities. The degree of vascular damage and coagulopathy differs between arenaviruses, with minimal histological lesions seen in LF and AHF but impaired endothelial function contributing to disease severity.

**Clinical presentation:** Arenavirus infections present with similar initial symptoms, including fever, headache, myalgia, and malaise. Relative bradycardia and skin hyperesthesia are common. The diseases diverge in their progression (Table 38).

**Table 38: Clinical presentations of arenaviridae**

|  |  |
| --- | --- |
| Virus | Clinical presentation |
| Lassa Fever (LF) | Mild or asymptomatic (80% cases); Onset within 10-14 days after exposure; includes headache, myalgia, arthralgia, gastrointestinal issues; advances respiratory distress; recovery after 8-10 days. |
| Lujo Hemorrhagic Fever (LHF) | Patients often experience a sudden onset of fever, general malaise, fatigue, and muscle aches (myalgia). As the disease progresses, patients may develop hemorrhagic manifestations (bleeding gums) and neurological manifestations (confusion). LHF can lead to death due to multiple organ failure |
| Lymphocytic Choriomeningitis (LCM) | Often asymptomatic or mild, featuring febrile illness with gastrointestinal symptoms and mild CNS involvement; meningoencephalitis with CSF pleocytosis (complication). |
| South American Hemorrhagic Fevers (SAHF) | Incubation period of 1–2 weeks; patients experience fever, malaise, headache, myalgia, epigastric pain, and anorexia; symptoms intensify after 2–4 days, including prostration and gas trointestinal disturbances. |
| Intrauterine Infection | Arenaviruses readily infect fetuses, causing high mortality and abortion in pregnant women with Lassa fever. Similar outcomes occur in South American hemorrhagic fevers. LCM virus infection during pregnancy can result in fetal abnormalities such as hydrocephalus and chorioretinitis. |

**Laboratory detection:**

**1. Lassa Fever (LF)-**

* **LASV isolation**- LASV can be isolated from blood during the febrile phase and from autopsy tissue samples.
* **Antibody detection**-LASV-specific antibodies can be detected using immunofluorescence (IF) and ELISA. ELISA IgM titers appear earlier and persist longer. Virus-specific IgG detected by ELISA remains for long periods, while IF antibodies wane below detectable levels over time.
* **RT-PCR**- Reverse transcriptase-PCR can detect virus RNA in blood with high sensitivity.
* **Nanopore sequencing**- In 2018, nanopore sequencing using the MiniON device was used to genetically characterize LASV isolates during an LF case upsurge in Nigeria. This technology's ability to genetically characterize in situ RNA viral samples in real-time is a major breakthrough in LF epidemiology.

**2. Lymphocytic Choriomeningitis (LCM)-**

* **LCMV isolation**- LCMV can be isolated from blood during the febrile phase and during meningitis symptoms, with CSF showing higher viral loads.
* **PCR tests**- PCR-based tests using CSF samples have been effective.
* **Antibody detection**- LCMV-specific IgM antibodies are detectable by ELISA and IF in serum and CSF in acute cases, though neutralizing antibodies appear late and have limited diagnostic value.

**New World Mammarenaviral Hemorrhagic Fevers (HFs)-**

* **Virus isolation**- During the acute febrile phase, the virus can be isolated from blood samples by- (i) inoculating newborn hamsters or mice, (ii) cocultivation of patient peripheral blood mononuclear cells and (iii)vero cells offering greater sensitivity.
* **Autopsy**- The virus can be isolated from autopsy tissues, except the brain.
* **Antigen detection**- Viral antigens in blood and tissues from viral infections can be detected by antigen-capture ELISA.
* **Serologic diagnosis**- Serologic diagnosis of (AHF) and (BHF) is typically conducted using IF and CF, though these tests have limited specificity and sensitivity.
* **ELISA**- ELISA is the most practical for rapid detection of IgM and IgG antibodies in clinical settings and sero-epidemiologic surveys.
* **Plaque neutralization test**- This test is valuable for evaluating convalescent plasma units intended for therapeutic use in AHF.

**Treatment and prevention:**

1. **Supportive therapy-** Supportive care includes hydration, pain management, and interventions to control bleeding, such as platelet transfusions and factor replacement. **Contagion and Precautions-** Patients can excrete the virus in urine or semen for weeks post-recovery, necessitating monitoring of body fluids for infectivity before discharge. Counseling on protection of sexual partners and disinfection practices is essential. Parenteral exposure can be mitigated by staff training and using respiratory protection against aerosols.
2. **Antiviral drugs-**

* **Ribavirin (Rib)-** Ribavirin has shown efficacy against mammarenaviruses in cell culture and animal models, reducing morbidity and mortality in Lassa fever (LF
* **Drug developmen-** Combining direct-acting antivirals (DAAs) and host-targeting agents (HTAs) can prevent drug resistance.

1. **Antibody therapy-** Convalescent plasma therapy has been effective in reducing AHF mortality, though some patients may experience transient neurological symptoms.
2. **Vaccines-**

* **JUNV Candid #1-** The JUNV live-attenuated Candid #1 strain vaccine has shown safety and efficacy in preclinical studies and human vaccination campaigns, leading to its licensing in Argentina. However, stability concerns have arisen due to varying virulence among clonal isolates.
* **LASV vaccines-** Effective LF vaccines should elicit strong CD4+ and CD8+ T cell responses. Promising vaccine platforms include live-attenuated vaccines, recombinant VSV and MV vectors, DNA-based vaccines, and the reassortant ML29. The ML29 candidate has shown cross-protective immune responses and safety in animal models.

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**Coronaviridae**

Coronaviridae is a family of viruses known for causing a spectrum of illnesses in various animals, including severe respiratory infections in humans. They are named for the crown-like spikes that protrude from their surfaces, resembling a solar corona when viewed under an electron microscope.

The first case reported in India was on 21st January 2020 in a 20-year-old female from Kerala, Thrissur, who had returned from Wuhan city on 23rd January 2020.

**Classification:** Coronaviruses belong to the family Coronaviridae and are classified into several genera based on their genetic and serological properties (Figure 77).

**Genus**

Arterivirus

Okariviridae

α-mesonivirus

Alpha

Beta

Gamma

Delta

Bafinivirus

Torovirus

**Family**

Arteriviridae

Roniviridae

Mesoniviridae

Coronaviridae

**Order**

Nidovirales

**Order**

MARS-CoV

SARS-CoV

SARS-CoV-2

**Subfamily**

Coronavirinae

Torovirinae

**Figure 77 : Classification of coronaviruses**

1. **Alphacoronavirus**- Includes several human coronaviruses and those that infect other mammals, such as bats and pigs.
2. **Betacoronavirus**- Includes coronaviruses that infect mammals, including bats and humans.
3. **Gammacoronavirus**- Includes coronaviruses primarily found in birds but may occasionally infect mammals.
4. **Deltacoronavirus**- Includes coronaviruses that infect both birds and mammals. They are less studied compared to Alphacoronaviruses and Betacoronaviruses.

Seven human coronaviruses have been identified. The alpha coronaviruses include HCoV-229E and HCoV-NL63, while the beta coronaviruses include HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2. HCoV-OC43 and HCoV-229E are associated with the common cold, HCoV-NL63 and HCoV-HKU1 cause mild respiratory infections, MERS-CoV causes Middle East Respiratory Syndrome (MERS), SARS-CoV causes Severe Acute Respiratory Syndrome (SARS), and SARS-CoV-2 causes Coronavirus Disease 2019 (COVID-19).

**Structure:** Coronaviruses (Figure 78) are enveloped viruses with a single-stranded RNA genome. The envelope is studded with spike proteins that give them their characteristic crown-like appearance. Their RNA genome is the largest among all known RNA viruses, ranging from approximately 26 to 32 kilobases in size. Inside the virion's envelope is a helically symmetrical nucleocapsid, a structure more typical of negative-sense RNA viruses.

Spike protein (S)

Envelope protein (E)

Nucleoprotein (N)

RNA genome

Membrane protein (M)

Hemagglutinin-esterase (HE)

**Figure 78: Structure of coronaviridae**

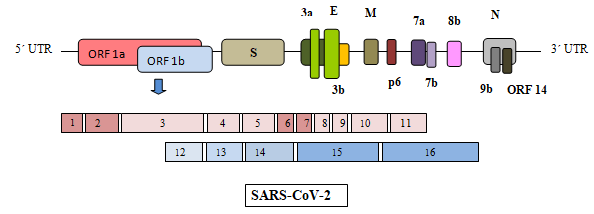
**Genome organization:** Coronaviruses (Figure 79) have a non-segmented, positive-sense RNA genome of about 30 kb. This genome features a 5′ cap structure and a 3′ poly (A) tail, enabling it to function as mRNA for translating replicase polyproteins. The genome (Table 39 and 40)) is organized as 5′-leader-UTR-replicase-S (Spike)-E (Envelope)-M (Membrane)-N (Nucleocapsid)-3′ UTR-poly (A) tail, with accessory genes interspersed among structural genes at the 3′ end. Open Reading Frame 1a/1b (ORF1a/1b) encodes two large polyproteins, pp1a and pp1ab, which are processed into 16 non-structural proteins (nsps). The accessory proteins are encoded by ORFs scattered throughout the genome (e.g., ORF3, ORF6, ORF7a, ORF7b, ORF8, ORF9b).

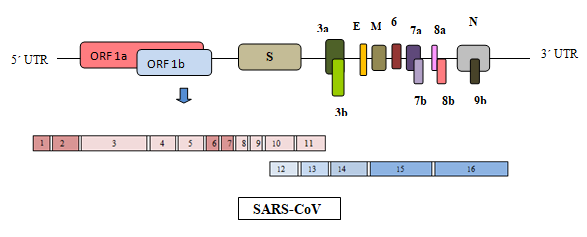
**Table 39: Structural proteins of coronaviridae**

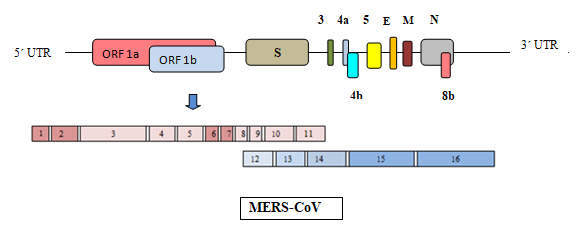
|  |  |
| --- | --- |
| Structural protein | Function |
| Spike (S) Protein | Forms the distinctive spikes on the virus surface; consists of an N-terminal signal sequence and is heavily glycosylated; functions as a class I fusion protein, mediating attachment to host receptors; typically cleaved by host proteases into S1 (receptor-binding domain) and S2 (stalk). |
| Membrane (M) Protein | Most abundant structural protein, small (25–30 kDa) with three transmembrane domains; gives the virion its shape and exists as a dimer; has a glycosylated ectodomain and a larger endodomain extending into the viral particle. |
| Envelope (E) Protein | Small (8–12 kDa) and present in small quantities; involved in viral assembly and release; functions as an ion channel and is crucial for pathogenesis in some coronaviruses. |
| Nucleocapsid (N) Protein | Sole protein in the nucleocapsid, with N-terminal and C-terminal domains that bind RNA; heavily phosphorylated, which enhances its RNA binding specificity; binds viral RNA and interacts with the M protein and nsp3, facilitating genome packaging. |
| Hemagglutinin-Esterase (HE) | Some of the β-coronaviruses possess this protein that binds sialic acids on surface glycoproteins and has acetyl-esterase activity. It Enhances S protein-mediated cell entry and virus spread. |

**Table 40: Non-structural proteins of coronaviridae**

|  |  |  |
| --- | --- | --- |
| Non-structural protein | Abbreviation | Function |
| nsp1 |  | Suppresses host gene expression and immune responses. |
| nsp2 |  | Involved in host cell modification; specific functions not well understood. |
| nsp3 | PLpro | Papain-like protease, processes viral polyprotein, helps in evading host immune response. |
| nsp4 |  | Involved in membrane rearrangement within infected cells. |
| nsp5 | 3CLpro | Main protease (3C-like protease) that processes the viral polyprotein. |
| nsp6 |  | Involved in autophagosome formation, contributing to virus replication. |
| nsp7 |  | Forms a complex with nsp8, acting as a primase for viral RNA synthesis. |
| nsp8 |  | Works with nsp7 in viral RNA synthesis; may act as a cofactor. |
| nsp9 |  | RNA-binding protein involved in viral replication. |
| nsp10 |  | Cofactor for nsp14 and nsp16, involved in RNA synthesis and modification. |
| nsp11 |  | Short peptide with unclear function; believed to play a role in the regulation of viral RNA synthesis. |
| nsp12 | RdRp | RNA-dependent RNA polymerase, essential for viral RNA replication. |
| nsp13 |  | Helicase that unwinds RNA, also possesses NTPase activity. |
| nsp14 | ExoN | Exoribonuclease involved in RNA proofreading, ensuring replication fidelity; also involved in mRNA cap methylation. |
| nsp15 |  | Endoribonuclease involved in evading the host immune response. |
| nsp16 | 2´-O-MTase | 2'-O-methyltransferase involved in mRNA capping and immune evasion. |



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**Figure 79: Genome structure of SARS-CoV-2, SARS-CoV and MERS-CoV-2**

**Virus replication:**

**1. Attachment and entry-** Coronaviruses initiate infection by the spike (S) protein binding to specific cellular receptors. The receptor binding domains (RBD) on the S protein vary by virus type, such as the C-terminal RBD on SARS-CoV's S1 region. This interaction dictates the virus's host range and tissue specificity. Proteolytic cleavage of the S protein reveals a fusion peptide, facilitating membrane fusion in acidified endosomes, though some coronaviruses can fuse at the plasma membrane.

**2. Replicase protein expression-** The viral RNA genome translates into two polyproteins, pp1a and pp1ab, through ribosomal frame shifting. These polyproteins are cleaved by viral proteases like PLpro and Mpro into non-structural proteins (nsps), with pp1a containing nsps 1-11 and pp1ab containing nsps 12-16. These nsps form the replicase-transcriptase complex (RTC), essential for RNA replication and sub-genomic RNA transcription. Some nsps also modulate host immune responses and ensure replication fidelity.

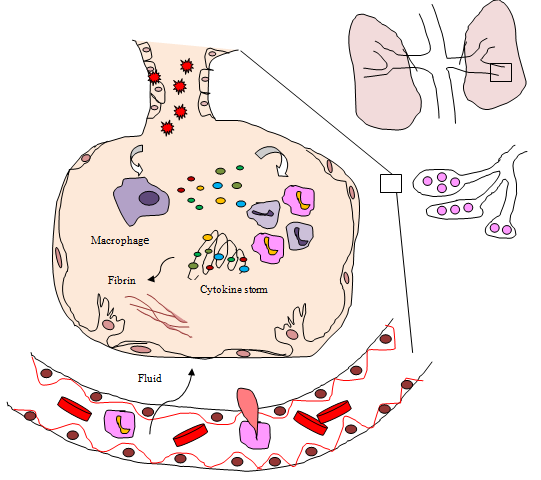
**3. Replication and transcription-** Replicase complexes synthesize both genomic and sub-genomic RNAs, with sub-genomic RNAs acting as mRNAs for structural and accessory proteins. Cis-acting sequences and transcription-regulating sequences (TRS) guide this process. Coronaviruses exhibit high recombination rates, aiding their evolution and adaptability.

**4. Assembly and release-** After replication, structural proteins (S, E, M) are translated, inserted into the endoplasmic reticulum (ER), and move to the ER-Golgi intermediate compartment (ERGIC). Viral genomes are encapsidated by the nucleocapsid (N) protein, budding into ERGIC membranes to form mature virions. The M protein is crucial for assembly, assisted by the E protein, while the S protein is incorporated but not essential for assembly. The interaction between M and N proteins completes virion formation. Virions are then transported to the cell surface and released by exocytosis. The S protein also enables cell-cell fusion, facilitating virus spread without detection.

**Pathogenesis:**

**A. Animals-** Coronaviruses cause various diseases in animals, prompting significant research due to their impact on livestock and pets like pigs, cows, chickens, dogs, and cats. For instance, Transmissible Gastroenteritis Virus (TGEV) and Porcine Epidemic Diarrhea Virus (PEDV) cause severe gastroenteritis in young piglets, leading to high morbidity, mortality, and economic losses. Feline enteric coronavirus (FCoV) usually causes mild or asymptomatic infections in cats but can mutate into Feline Infectious Peritonitis Virus (FIPV), causing the lethal disease feline infectious peritonitis (FIP). FIP, which has wet and dry forms, is believed to result from abnormal cytokine and chemokine expression and lymphocyte depletion (Figure 80).

**B. Human-** When SARS-CoV-2 enters the body through the mouth or nose, it travels to the lungs and uses its spike proteins to infect alveolar cells. The immune system responds by attacking the infected cells, but this also damages healthy alveolar cells. This damage leads to reduced surfactant production from alveolar epithelial type II (pneumocytes II) cells and causes fluid build up in the alveoli, impairing gas exchange. If the immune response becomes uncontrolled, it can trigger a cytokine storm, where excessive cytokine release further damages the body's cells and organs, resulting in severe disease and organ failure, as seen in severe COVID-19 cases.



**Figure 80: Pathogenesis of coronaviridae (COVID-19)**

**Epidemiology:**

**A. Respiratory coronaviruses**- Respiratory coronavirus infections occur worldwide, peaking in winter and spring in temperate climates and contributing up to 35% of upper respiratory illnesses during peak activity. About 15% of adult colds are caused by coronaviruses. In the U.S., HCoV-OC43 and 229E cause epidemics every 2-3 years, while data on NL63 and HKU1 show variable incidence. Reinfection is common due to rapidly declining antibody levels. These infections affect all ages but are most frequent in children, with both symptomatic and asymptomatic cases detectable by molecular methods.

**Severe Acute Respiratory Syndrome (SARS)**- The SARS epidemic began in Guangdong Province, China, in November 2002 and gained global attention in March 2003. It spread in hospitals, among families, and occasionally in hotels and markets, with the largest outbreaks in China, Hong Kong, Taiwan, Singapore, and Toronto. Case-fatality rates ranged from 7% to 17%, reaching 50% in those over 65 or with underlying conditions. WHO's control measures, including isolation, quarantine, and travel advisories, halted the global spread by July 2003. SARS spread primarily through droplets, direct or fomite contacts. The epidemic likely started with transmission from palm civets or other animals in Chinese wild game markets to humans, with the virus mutating for human transmission. Horseshoe bats are now considered the ultimate source of SARS-like viruses.

**B. Middle East Respiratory Syndrome (MERS**)- MERS was first identified in Saudi Arabia in 2012. It is caused by the MERS-CoV and primarily affects the respiratory system. The virus is zoonotic, originating in bats and transmitted to humans through dromedary camels, which are considered the primary source of human infection. MERS-CoV spreads through close contact with infected individuals, particularly in healthcare settings. Human-to-human transmission has been limited but significant in hospital environments. Most MERS cases have occurred in the Arabian Peninsula, particularly Saudi Arabia. However, travel-associated cases have been reported in several countries outside the Middle East, including South Korea, where a significant outbreak occurred in 2015. MERS has a high case-fatality rate of about 35%.

**C. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)-** SARS-CoV-2, the virus causing COVID-19, emerged in December 2019 in Wuhan, China. The virus rapidly spread worldwide, leading to a global pandemic declared by the World Health Organization in March 2020. The first case reported in India was on 21st January 2020 in a 20-year-old female from Kerala, Thrissur. SARS-CoV-2 primarily spreads through respiratory droplets from infected individuals. It can also spread through contact with contaminated surfaces and, less commonly, through airborne transmission in enclosed spaces. The pandemic has led to multiple waves of infection, with the extent and impact differing across regions due to factors like healthcare capacity, public health measures, and vaccination rates.

* **COVID-19 pandemic in India -** The COVID-19 pandemic in India experienced several distinct waves, each characterized by varying levels of infection rates, hospitalizations, and mortalities. The key waves (Table 41) and their impacts are-

**Table 41: Characteristics of COVID-19 waves in India**

|  |  |
| --- | --- |
| COVID-19 waves | Characteristics |
| First Wave (Early 2020 - Mid 2020) | The first confirmed cases in India were reported in late January 2020, with initial cases primarily among travelers from affected regions. The first wave peaked around September 2020. This wave (Wuhan strain) saw a steady rise in cases, with significant spread in urban areas. The healthcare system experienced stress but managed the load reasonably well. The government also launched awareness campaigns about hand hygiene mask-wearing and massive lockdown (March 25 to April 14, 2020). |
| Second Wave (Early 2021 - Mid 2021) | The second wave began in March 2021 and the peak occurred in April and May 2021. This wave was far more severe, driven by the Delta variant. There was a rapid and massive surge in cases, overwhelming hospitals, leading to shortages of oxygen, beds, and critical supplies. The death toll was significantly higher compared to the first wave. The government re-imposed lockdowns (April-May 2021) in various states, ramped up testing, and accelerated the vaccination drive. Efforts were made to increase medical oxygen supply and enhance healthcare infrastructure. |
| Third Wave (Late 2021 - Early 2022) | The third wave of COVID-19 in India began around December 2021, peaking in January 2022. Driven primarily by the Omicron variant, this wave saw high infection rates but generally lower severity compared to the second wave, with fewer hospitalizations and deaths. Efforts during this period emphasized vaccination, including booster doses, and implemented localized restrictions and curfews. Public health campaigns highlighted the importance of wearing masks and avoiding large gatherings. |

* **COVID-19 variants-** The World Health Organization (WHO) classifies SARS-CoV-2 variants into several categories based on their potential impact on public health (Table 42).

**Table 42: WHO classifications of COVID-19 variants**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Category |  | Variant | Lineage | First identified |
| Variants of Concern (VOC) |  | Alpha | B.1.1.7 | United Kingdom, Sep 2020 |
| Beta | B.1.351 | South Africa, May 2020 |
| Gamma | P.1 | Brazil, Nov 202 |
| Delta | B.1.617.2 | India in Maharashtra, Oct 2020, named on 31st May 2021 |
| Omicron | B.1.1.529 | South Africa on 24th November 2021 and named by WHO on 26th December 2021 |
| Variants of Interest (VOI) |  | Eta | B.1.525 | Multiple countries, Dec 2020 |
| Iota | B.1.526 | USA, Nov 2020 |
| Kappa | B.1.617.1 | India, Oct 2020 |
| Lambda | C.37 | Peru, Dec 2020 |
| Mu | B.1.621 | Colombia, Jan 2021 |
| Variants Under Monitoring (VUM) |  | Epsilon | B.1.427/B.1.429 | USA, Mar 2020 |
| Zeta | P.2 | Brazil, Apr 2020 |
| Theta | P.3 | Philippines, Jan 2021 |

**D. Gastrointestinal coronaviruses**- Enteric coronaviruses are commonly associated with gastrointestinal diseases in neonates and infants under 12 months. They have been found in the stools of adults with AIDS and are often shed asymptomatically, particularly in tropical climates and areas with poor hygiene. These viruses can be detected for extended periods without a clear seasonal pattern.

**Immune response:**

**1. SARS-CoV-**

* **Innate response:** Strong interferon response early in infection.
* **Adaptive response:** High levels of neutralizing antibodies and robust T cell responses, which are crucial for clearing the virus and preventing severe disease.

**2. MERS-CoV-**

* **Innate response:** Delayed and weaker interferon response compared to SARS-CoV.
* **Adaptive response:** Effective neutralizing antibody response, but T cell responses are critical for controlling the infection and preventing severe outcomes.

**3. SARS-CoV-2 (COVID-19)-**

* **Innate response:** Similar to SARS-CoV, but some patients show dysregulated interferon response leading to severe disease.
* **Adaptive response:** Strong humoral response with high levels of neutralizing antibodies, especially following vaccination. T cell responses also play a significant role in controlling the virus and reducing disease severity.

**4. Immune evasion and pathogenesis-**

* **Cytokine storm-** Severe cases of COVID-19 and SARS have been associated with a "cytokine storm," where an excessive and uncontrolled release of pro-inflammatory cytokines leads to tissue damage and severe respiratory distress.
* **Viral mutations-** Variants of SARS-CoV-2, like Delta and Omicron, have mutations in the spike protein that can partially evade immune responses, reducing vaccine effectiveness but still generally protecting against severe disease.

**5. Vaccination and immune response-**

* **Vaccine-induced immunity-** Vaccines elicit strong humoral and cellular immune responses, providing protection against infection and severe disease. Booster doses enhance immunity, especially against emerging variants.
* **Herd immunity-** Achieved when a significant portion of the population is immune, either through infection or vaccination, reducing the spread of the virus.

**Clinical presentation:**

**1. Respiratory coronaviruses-** The incubation period for these colds is longer, and their duration is somewhat shorter compared to rhinovirus colds. These infections can lead to serious respiratory illnesses such as pneumonia, bronchiolitis, otitis, asthma exacerbations, chronic bronchitis, and pneumonia, particularly in the elderly and immunocompromised. In children, NL63 may lead to croup more frequently than other respiratory coronaviruses.

* **SARS-** Fever, headache, malaise, myalgia, nonproductive cough, dyspnea, diarrhea (in 25% of cases). Severe cases may progress to ARDS, especially in older adults or those with pre-existing conditions. The disease can be severe during pregnancy, with high mortality for both mother and fetus.
* **MERS-** MERS typically presents with fever, cough, and shortness of breath, often accompanied by diarrhea. Severe cases can quickly escalate to pneumonia and ARDS, necessitating intensive care.
* .SARS-CoV-2- COVID-19 presents a broad range of symptoms from mild to severe, including fever, dry cough, and fatigue, with some patients experiencing sore throat, headache, muscle aches, diarrhea, and notably, loss of taste or smell. Severe cases can develop into pneumonia, ARDS, multi-organ failure, and thromboembolic complications.

**2. Gastrointestinal coronaviruses and toroviruses-** Enteric coronavirus infections in infants are associated with gastroenteritis. Studies indicate significant associations with symptoms like water-loss stools, bloody stools, abdominal distention, and bilious gastric aspirates.. Coronaviruses have also been linked to outbreaks of necrotizing enterocolitis in newborns.

Toroviruses are found in feces of symptomatic and asymptomatic individuals, with a higher presence in symptomatic cases, suggesting a pathogenic role in diarrheal disease. Studies have found significant associations with acute and chronic diarrhea, with symptomatic torovirus infections more common in older and immunocompromised children.

**3. Neurologic syndromes-** Coronaviruses have been investigated as potential etiologic agents in multiple sclerosis (MS). HCoV-OC43 and HCoV-229E have been detected in brain tissue from MS patients.

**Diagnosis:** Virus RNA is detectable in upper and lower respiratory tract, blood, stool, and urine samples, with higher yields when multiple specimen types are tested.

1. **Blood screening tests-** Complete blood count of SARS-CoV-2 infected individuals shows normal white blood cell count or leukopenia and lymphopenia. Liver enzymes, D-dimer and fibrinogen are decreased.
2. **Rapid antigen tests-** Useful for point-of-care testing with quicker results, though generally less sensitive than PCR. There are two techniques used by the widely available SARS-CoV-2 antigen kits: a) the immunochromatographic (ICT) assay, which employs colloid gold attached antibodies to produce visible coloured bands that signal positive results; b) the automated immunofluorescence reader is used to obtain results from the fluorescence immunochromatographic assay (FIA). CoviEasy, OmiSure (for omicron, approved by ICMR), Abbott BinaxNOW are some examples of COVID-19 rapid test kits.
3. **Antibody detection-** Antibody tests using cultured virus and methods like immunofluorescence or ELISA, detect IgM early in infection and IgG approximately 10 days after fever onset, becoming nearly universal after 4 weeks.
4. **Molecular testing-** RT-PCR utilizing various primers and detectors are widely employed. A cycle threshold (Ct) value of below 40 for target genes are regarded as positive tests Viral nucleocapsid gene (N1 and N2), RNase P  and RNA-dependent RNA polymerase (RdRp) can be the targets. Generic tests for respiratory CoVs have been developed but show slightly lower sensitivity compared to specific assays.
5. **Chest imaging-** Shows patterns of viral pneumonia (scattered air-space opacification) for SARS, severe lung involvement, often with bilateral infiltrates for MERS and ground-glass opacities and consolidation for SARS-CoV-2.
6. **Virus isolation and culture-** Isolation used for research and less commonly used in routine diagnostics. The cell lines used are shown in beloe table (Table 43).
7. **Next-Generation Sequencing (NGS)-** Used for detailed genomic analysis and tracking variants and mutations

**Table 43: Cell lines for coronaviridae culture**

|  |  |
| --- | --- |
| Virus | Cell line |
| SARS-CoV | * Vero- Derived from African green monkey (vervet) kidney (verda reno, which means 'green kidney') * Vero CCL81- Another strain of Vero cells * FRhK4- Fetal rhesus monkey kidney cells * Huh-7- Human liver carcinoma cells * CaCo-2- Human colorectal adenocarcinoma cells, (from Cancer coli, "colon cancer") |
| MERS-CoV | * Vero B4- Derived from African green monkey (vervet) kidney * Huh-7- Human liver carcinoma cells * Calu-3- Cultured Human Airway Epithelial Cells * LLC-MK2- Rhesus monkey (Macaca mulatto) kidney epithelial cells * MRC-5- Human lung fibroblast cells |
| SARS-CoV-2 | * Vero E6- Derived from African green monkey (vervet) kidney , used for virus isolation and propagation * Vero CCL81- Another strain of Vero cells, used for primary virus culture. * Huh-7- Human liver carcinoma cells, Used for studying virus replication and screening antivirals. * Calu-3- Cultured Human Airway Epithelial Cells * A549- Adenocarcinomic human alveolar basal epithelial cells * Caco-2- Human colorectal adenocarcinoma cells (from Cancer coli, "colon cancer") |
| HCoV-229E | * MRC-5 * Huh-7 * Caco-2 |
| HCoV-OC43 | * BSC-1- African green monkey kidney cell * HRT-18- Human rectal tumor cells |
| HCoV-NL63 | * LLC-MK2- Rhesus monkey kidney cells * CaCo-2 |
| HCoV-HKU1 | * HRT-18 * CaCo-2 |

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**Treatment:** Despite the severity of SARS, empirical treatments with corticosteroids and ribavirin were widely used, though ribavirin proved ineffective in vitro and showed no clear benefits in treating SARS. Anecdotal and partially controlled evidence suggested some benefit from corticosteroid or interferon-α treatment. Protease inhibitors like lopinavir/ritonavir demonstrated in vitro activity against SARS-CoV, but their efficacy remains debated. A critical review found nearly all studies inconclusive, with no treatments during the epidemic showing definitive beneficial effects

**Prevention:** Strict adherence to hospital infection control procedures, particularly those focusing on contact and droplet transmission, significantly reduced the spread of SARS-CoV. Similarly, during the COVID-19 pandemic, governments worldwide implemented various precautionary measures, including (i) the use of facemasks, (ii) hand sanitizers, (iii) lockdowns, (iv) increased testing, (v) contact tracing, and (vi)the introduction of vaccines, to control the virus's spread.

**Prophylaxis:** Vaccines for animal coronaviruses have been developed with mixed success, some, like the feline infectious peritonitis vaccine, have sometimes worsened the disease upon natural infection.

* **SARS-CoV vaccines**- Research has resumed intermittently, especially during outbreaks, focusing on similar technologies as those used for SARS-CoV-2.
* **MERS-CoV vaccines**- Still in experimental stages with various approaches including viral vector, DNA, and protein subunit vaccines.
* **SARS-CoV-2-** WHO approved vaccines (Table 44I are-

**Table 44: WHO-EUA qualified COVID-19 vaccines**

|  |  |  |  |
| --- | --- | --- | --- |
| Vaccine | Type | Approved Schedule | Manufactured by |
| BNT162b2 | mRNA | 2 dosage, 21-28 days | Pfizer-BioNTech |
| mRNA-1273 | mRNA | Two doses, 28 days apart | Moderna |
| AZD1222 Vaxzevria | Adenovirus (CHAdOx1) vector | 2 doses, 4-12 weeks apart | AstraZeneca |
| COVISHIELD | Adenovirus (CHAdOx1) vector | 2 doses, 4-12 weeks apart | Serum Institute of India |
| Ad26.COV2.5 | Adenovirus type 26 vector | One dose | Janssen (Johnson & Johnson) |
| Covilo / BBIBPCorV | Whole inactivated Coronavirus | Two doses, 21-28 days apart | SinoPharm / Beijing Institute of Biological Products |
| CoronaVac | Whole inactivated virus | Two doses, 14-28 days apart | Sinovac |
| COVAXIN | Whole inactivated virus | Two doses, 28 days apart | Bharat Biotech, India |
| NVX-CoV2373 / Nuvaxovid | Protein subunit | 2 doses, 21-28 days apart | Novavax |
| NVX-CoV2373 / Covovax | Protein subunit | Two doses, 21-28 days apart | Serum Institute of India |
| Ad5-nCoV | Adenovirus Type 5 vector | One dose | CanSinoBio |

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**Retroviridae**

The Retroviridae family comprises seven genera (Table 45), primarily infecting mammals and causing a range of symptoms including cancers, neurological disorders, immunodeficiency diseases, and asymptomatic infections

**Table 45: Classification of retroviridae**

|  |  |  |
| --- | --- | --- |
| Genus | Morphology | Examples |
| Alpharetrovrus | C type | Avian leukosis virus (ALV)  Rous sarcoma virus (RSV) |
| Betaretrovirus | B and D type | Mouse mammary tumor virus (MMTV) Mason-Pfizer monkey virus (MPMV) Jaagsiekte sheep retrovirus (JSRV) |
| Gammaretrovirus | C type | Murine leukemia viruses (MuLV)  Feline leukemia virus (FeLV)  Gibbon ape leukemia virus (GaLV) Reticuloendotheliosis virus (RevT) |
| Deltaretrovirus | **-** | Human T-cell lymphotropic virus (HTLV)–1,–2 Bovine leukemia virus (BLV)  Simian T-cell lymphotropic virus (STLV)–1,–2,–3 |
| Epsilonretrovirus | **-** | Walleye dermal sarcoma virus Walleye epidermal hyperplasia virus 1 |
| Lentivirus | Rod/cone core | Human immunodeficiency virus type 1 (HIV-1)  Human immunodeficiency virus type 2 Simian Immunodeficiency viruses (SIV) Equine infectious encephalitis virus (EIAV) Feline immunodeficiency virus (FIV)  Caprine arthritis encephalitis virus (CAEV) Visna/maedi virus |
| Spumavirus | Immature | Human foamy virus (HFV) |

**Alpha retroviruses**

Alpha retroviruses belong to the Retroviridae family and are known for their ability to induce tumors in various avian species. These viruses are oncogenic and primarily infect birds, although they can also affect other vertebrates. Upon infecting a host cell, alpha retroviruses utilize a single-stranded RNA genome that is reverse transcribed into DNA. This DNA integrates into the host cell genome, potentially leading to the expression of viral genes and causing oncogenic transformations.

In poultry, alpha retroviruses have been extensively researched due to their association with diseases such as avian leukosis and sarcomas. Transmission of these viruses can occur horizontally through contact with contaminated materials or vertically from infected parents to offspring. Studies on alpha retroviruses have significantly contributed to our understanding of retroviral oncogenesis and have been pivotal in the development of vaccines and control strategies in poultry farming.

**Betaretroviruses**

Betaretroviruses, infect a range of mammalian species with their single-stranded RNA genome. Upon entering host cells, this RNA is reverse transcribed into double-stranded DNA, which integrates into the host genome. This integration can result in latent infection or the production of viral proteins.

Historically, betaretroviruses have been linked to various animal diseases, such as mammary tumors in mice and cancers in other mammals. A notable example is the mouse mammary tumor virus (MMTV), extensively studied to understand retroviral replication, cancer development, and immune responses.

In humans, betaretroviruses have been associated with certain diseases, although their role is less clearly defined compared to Lentivirus families like HIV. Ongoing research aims to elucidate betaretrovirus pathogenesis mechanisms and their potential implications for human health.

**Gamma-retroviruses**

Gamma-retroviruses, belonging to the Retroviridae family, are known for their ability to infect a wide range of vertebrate species. These viruses have a single-stranded RNA genome that undergoes reverse transcription upon entering host cells, producing double-stranded DNA. This DNA integrates into the host cell genome, where it can persist and lead to the expression of viral genes.

In animal research, gammaretroviruses, such as the prototype Murine Leukemia Virus (MLV), have been extensively studied due to their association with diseases like leukemia and lymphoma in mice. MLV serves as a crucial model for investigating how retroviruses cause cancer and trigger immune responses in hosts. In humans, gammaretroviruses have also been linked to certain diseases, although their specific impact is less well-defined compared to Lentivirus.

**Deltaretrovirus**

Delta retroviruses, are a subgroup within the Retroviridae family characterized by their delta-shaped appearance. These viruses possess a single-stranded RNA genome that undergoes reverse transcription upon infecting a host cell, converting RNA into DNA which integrates into the host cell's genetic material.

**Genome structure:** Delta retroviruses have an RNA genome that is reverse transcribed into DNA upon infection, subsequently integrating into the host genome.

**Taxonomy:** Classified in the genus Deltaretrovirus, examples include:

* **Human T-cell lymphotropic virus type 1 (HTLV-1)-** Associated with diseases like adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).
* **Human T-cell lymphotropic virus type 2 (HTLV-2)-** Similar to HTLV-1 but generally less pathogenic.
* **Bovine leukemia virus (BLV)-**Causes bovine leukemia in cattle.

**Epidemiology:** Delta retroviruses are transmitted primarily through bodily fluids such as blood, semen, and breast milk. HTLV-1 spreads through sexual contact, blood transfusions, and from mother to child through breastfeeding. HTLV-1 is endemic in regions like Japan, the Caribbean, Central Africa, and South America, posing significant public health challenges.

**Clinical presentation:**

* HTLV-1 can lead to chronic infections and diseases such as ATLL and HAM/TSP.
* HTLV-2 is less pathogenic but can cause neurological disorders and T-cell lymphomas.
* BLV affects cattle globally, causing bovine leukemia.
* **Oncogenic potential-** Particularly HTLV-1 can transform infected cells, potentially leading to cancers like ATLL.

**Diagnosis:** Typically involves serological tests to detect antibodies against delta retroviruses or PCR-based methods to detect viral RNA/DNA.

**Treatment:** No specific antiviral therapy exists for HTLV infections; management focuses on treating associated diseases and complications.

**Epsilonretroviruses**

Epsilonretroviruses are part of the Retroviridae family and infect fish, especially teleost fish. They have a single-stranded RNA genome that is converted into DNA upon infecting host cells, integrating into the host genome and potentially leading to viral gene expression. Historically studied in fish diseases, such as walleye dermal sarcoma virus (WDSV) causing skin tumors in walleye fish, these viruses spread horizontally among fish populations through contaminated water or infected fish contact. Research focuses on their replication, oncogenic mechanisms, and immune responses in fish to develop strategies for disease control in aquaculture. Epsilonretroviruses contribute to our understanding of retroviral biology in aquatic environments.

**Lentiviruses**

Lentiviruses, part of the Retroviridae family, infect various mammalian species including humans, causing persistent infections. They possess a single-stranded RNA genome that is converted to double-stranded DNA upon infecting host cells, integrating into the host genome. HIV, a notable lentivirus, leads to AIDS by depleting CD4+ T cells, weakening immunity and causing opportunistic infections. Other lentiviruses like FIV in cats and SIV in non-human primates also cause immunodeficiency diseases. Transmission occurs through bodily fluids, prompting antiretroviral therapies for treatment. Research focuses on understanding pathogenesis, developing therapies, and addressing drug resistance, highlighting the global health impact of lentiviruses.

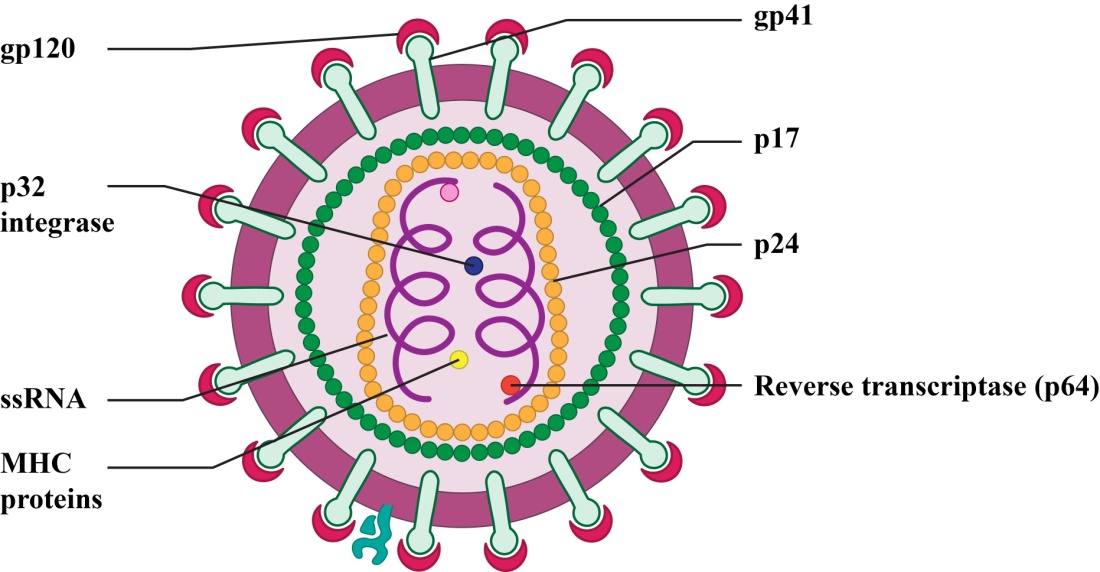
**Human immunodeficiency virus type 1 (HIV-1)**

The first documented case of HIV-1 infection in humans dates back to 1959, identified from a blood sample taken from a man in Kinshasa, Democratic Republic of Congo. In 1983, the virus was isolated in Paris using interleukin-2 and anti-interferon serum to culture T lymphocytes obtained from a lymph node biopsy specimen. This discovery was independently made by Robert Gallo and Luc Montagnier. HIV-2 was later identified in 1986. The reverse transcriptase enzyme essential for HIV replication was originally discovered in 1970 by Temin and Baltimore.

**Table 46: Major differences between HIV-1 and HIV-2**

|  |  |  |
| --- | --- | --- |
| Feature | HIV-1 | HIV-2 |
| Discovery | Discovered earlier (1983) | Discovered later (1986) |
| Source | Zoonosis, from chimpanzee | Sooty Mangabey Monkey from West Africa |
| Global distribution | Widespread | Predominantly found in West Africa and parts of Central Africa |
| Genotype | Groups M, N and O; M has subtypes of A-K except I | None |
| Phenotype | Syncytium Inducing (use CXCR4, called as X4 virus), Non-syncytium inducing (use CCR5, called as R5 virus) | None |
| Viral load | Higher viral load | Lower viral load |
| Envelope protein | Contains Vpu | Contains Vpx |
| Drug susceptibility | Generally sensitive to standard antiretrovirals | Less sensitive to some antiretrovirals |

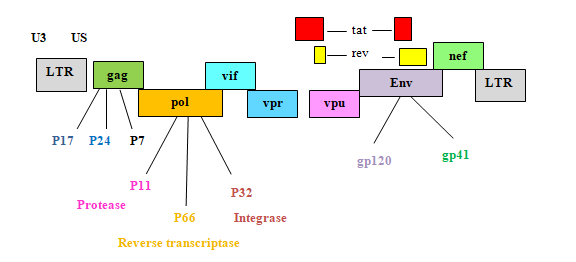
**Structure:** HIV is composed of an outer lipid bilayer envelope with glycoproteins (Table 47) gp120 and gp41, surrounding an inner protein core made up of p15, p17, and p24 proteins. Within this structure (Figure 81) is the viral RNA complexed with reverse transcriptase. The HIV genome, about 10 kilobases in size, is larger than that of HTLV. It contains structural genes (Table 47) gag, pol, and env, as well as regulatory genes tat (similar to HTLV's tax) and rev (similar to HTLV's rex). Additionally, HIV-1 includes regulatory genes (Table 47) nef, vif, vpu, and vpr, whereas HIV-2 lacks vpu but has vpx, a gene also present in simian immunodeficiency virus (Figure 82).



**Figure 81: Structure of HIV**

**Table 47: Viral proteins of HIV**

|  |  |  |
| --- | --- | --- |
| Category | Protein | Function |
| Structural proteins | gag | Core structural proteins, components are- precursor p55 (cleaved into three proteins- p15, p18 and p24 capsid), p17 (matrix), p7 (Nucleocapsid) and p6 (late domain) |
| pol | Encodes for-   * **polymerase reverse transcriptase-** conversion of ssRNA to dsDNA) * **protease-** cleaves the viral polyproteins produced by the viral genome into individual functional proteins * **Integrase-** facilitates the integration of the viral DNA into the host cell genome. |
| env | The HIV genome encodes the envelope protein gp140, which can be cleaved into two subunits: gp120, the primary envelope antigen that facilitates viral attachment to host cells, and gp41, which plays a key role in the fusion of viral and cellular membranes during infection. |
| Non-structural proteins | tat | A protein that binds to RNA and activates transcription, Tat stimulates the production of TNF-β and TGF-β. |
| rev | Essential for the shift in HIV gene expression from early to late stages of infection. |
| nef | Enhances virus replication and immune evasion |
| vif | Facilitates viral infectivity by counteracting host antiviral factors |
| vpr | Regulates nuclear import of the viral pre-integration complex |
| vpu | Enhances virion release from infected cells |
| vpx (HIV-2/SIV) | Facilitates nuclear import of viral DNA |

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**Figure 82: Genome structure of HIV**

**Antigenic diversity of HIV-1:** HIV-1, the most widespread and pathogenic strain of the Human Immunodeficiency Virus, is classified into different groups, subtypes, and circulating recombinant forms (CRFs). Here’s an overview of its classification (Table 48)-

**Table 48: Antigenic diversity of HIV-1**

|  |  |
| --- | --- |
| Groups | Characteristics |
| **Group M (Major)** | This is the main group responsible for the global HIV pandemic. Group M itself is further divided into subtypes (A-D, F-H, J, and K), each with distinct genetic characteristics and geographic distributions. Subtype B is predominant in North America and Western Europe, while subtype C is the most prevalent globally, particularly in Southern Africa.  There are at least nine distinct genetic clades of HIV-1-  **Subtype A-** Predominant in West and Central Africa, linked to the Russian epidemic.  **Subtype B-** Most prevalent in Europe, America, Japan, and Australia, especially among men who have sex with men (MSM) in Europe.  **Subtype C-** Dominant in Southern and East Africa, India, and Nepal, responsible for a global epidemic.  **Subtype D-** Confined to East and Central Africa.  **Subtype E-** Found primarily in Southeast Asia, originating from Central Africa.  **Subtype F-** Identified in Central Africa, South America, and Eastern Europe.  **Subtype G-** Observed in West and East Africa, as well as Central Europe.  **Subtype H-** Only detected in Central Africa.  **Subtype J-** Found in Central America.  **Subtype K-** Found exclusively in the Democratic Republic of Congo and Cameroon. |
| Group O (Outlier) | Less common and primarily found in West and Central Africa. Group O viruses show significant genetic differences from Group M viruses and may require specific diagnostic tests and treatments |
| Group N | This group is extremely rare and has only been identified in a few individuals from Cameroon. |
| **Circulating recombinant forms (CRFs** | CRFs are novel hybrid viruses formed through genetic recombination between different HIV subtypes within an infected person.. They often possess distinct biological characteristics and can hold significant epidemiological relevance in specific geographic areas. Examples include Subtype A/B, Subtype A/G (West and Central Africa), Subtype A/E (Southeast Asia), and CRF A/G (West and East Africa). |

**Resistance:** HIV is sensitive to heat, being inactivated within 10 minutes at 60°C and in seconds at 100°C. At room temperature (20-25°C), it can survive in dried blood for up to seven days. The virus has been isolated from various tissues up to 16 days after death during autopsies. While HIV can withstand lyophilisation, it can be inactivated in lyophilised blood products by heating at 68°C for 72 hours and in liquid plasma by heating at 60°C for 10 hours.

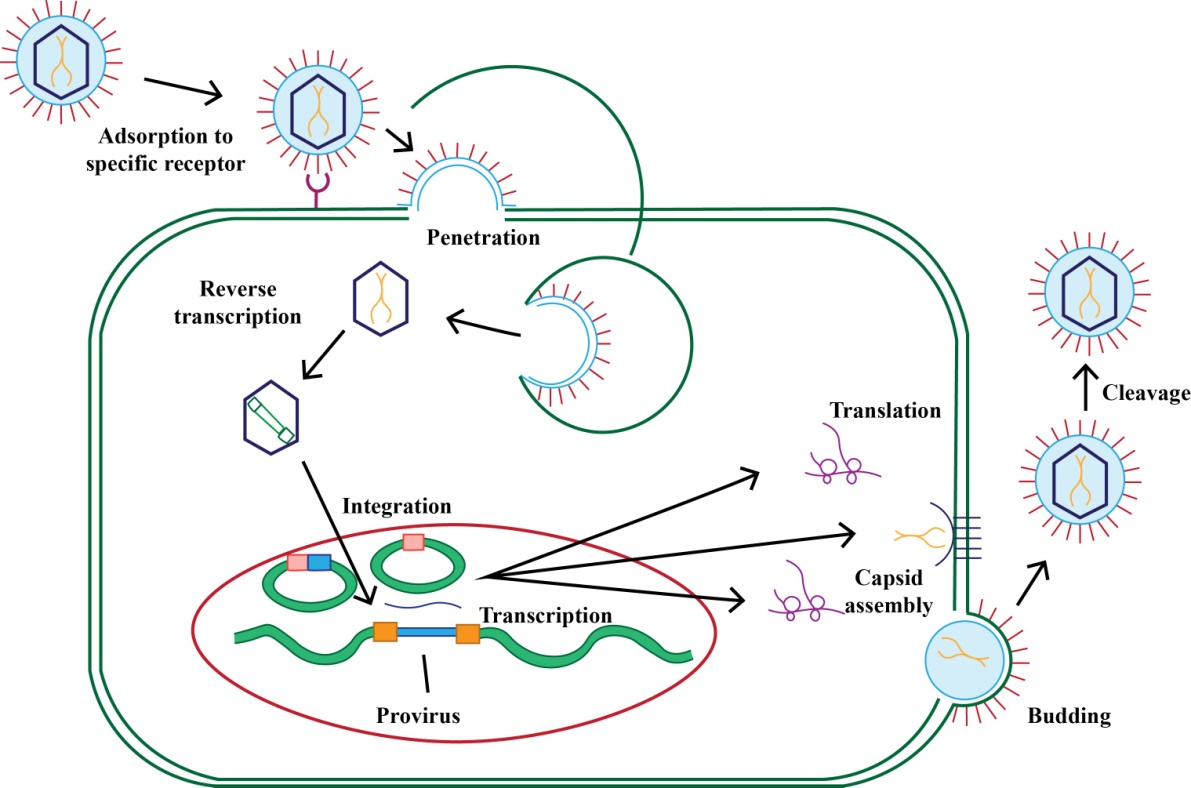
**Replication:** The retroviral infection cycle (Figure 83) consists of several distinct phases:

**Early Phase-**

1. **Recognition and adsorption**- The virus attaches to the host cell membrane through membrane protein complexes. Different retroviruses use various cellular receptors, which can be multiple- or single-transmembrane proteins with diverse functions in uninfected cells. For HIV-1, the primary receptor is CD4, with co-receptors CXCR4 and CCR5, while FeLV-B uses the multiple membrane protein Pit2 as its receptor.
2. **Fusion and entry**- The virus fuses with the host cell membrane and enters through a fusion pore. This is followed by the uncoating of viral RNA, releasing it into the cytoplasm.
3. **Reverse transcription**- The viral RNA genome is transcribed into viral DNA by reverse transcriptase. The viral DNA associates with the preintegration complex (including integrase, matrix protein, reverse transcriptase, and in HIV, vpr and cellular HMG-I(Y)). The complex is transported to the nucleus where viral integrase integrates the proviral DNA into the host genome.

**Late Phase-**

1. **Transcription and translation**- The infection cycle proceeds with the transcription of regulatory genes such as rev and tat, along with accessory genes. The Tat protein enhances transcription elongation of viral proteins by indirectly phosphorylating RNA polymerase II. The gp160 protein is translated in the endoplasmic reticulum (ER) and undergoes glycosylation and cleavage, forming a trimer of gp120 and gp41, which is transported to the cell surface via Golgi vesicles.
2. **Virus assembly and budding**- The gag polyprotein is translated from unspliced mRNA and binds to Gag/Pol fusion proteins. The virus assembles and buds from the cellular membrane, or as recent findings suggest, into endosomes within infected cells.
3. **Maturation**- Final maturation of the infectious viral particle occurs outside the host cell.



**Figure 83: HIV replication**

**Pathogenesis:** HIV infection occurs when the virus enters the blood or tissues and contacts CD4 lymphocytes. Cells bearing the CD4 antigen, including CD4+ T lymphocytes, B lymphocytes, monocytes, macrophages, glial cells, microglia, and follicular dendritic cells, can be infected. The virus binds to the CD4 receptor via the envelope glycoprotein gp120, with cell fusion mediated by gp41, and requires co-receptors CXCR4 and CCR5 for attachment.Inside the cell, the viral genome integrates into the host genome through integrase, leading to latent infection and contributing to the long and variable incubation period of HIV. The virus can be isolated from various body fluids, including blood, semen, and saliva.

The primary pathogenic mechanism of HIV is the damage to CD4+ T lymphocytes, resulting in their depletion and reversal of the T4: T8 (helper:suppressor) cell ratio. Infected T4 cells release fewer interleukins and other lymphokines, suppressing cell-mediated immunity. Humoral immunity is also affected as helper T cells are crucial for B cell function. HIV-infected individuals cannot respond to new antigens, and polyclonal activation of B lymphocytes leads to hypergammaglobulinemia, causing allergic reactions. Monocyte-macrophage function is impaired, affecting chemotaxis, antigen presentation, and intracellular killing, and reducing NK and cytotoxic T lymphocyte activity.

Clinical manifestations of HIV arise from immune response failure, making patients vulnerable to opportunistic infections and malignancies. Dementia and neurological issues result from the virus's impact on CNS cells. During asymptomatic periods, HIV remains latent in lymph node T4 cells and monocytes-macrophages. Although HIV does not directly cause cancer, the immunosuppression in AIDS patients leads to tumors such as Kaposi sarcoma, driven by factors promoting cellular proliferation. HIV proviral DNA is absent in these tumors.

**Immune response:** Throughout all stages of HIV infection, a wide range of immune responses is observed. Antibodies against HIV's structural and regulatory proteins generally appear weeks to months after initial infection. As HIV progresses, antibodies to the p24 core antigen decrease, while antibodies to the envelope protein remain constant. Virus-neutralizing antibodies are produced by HIV-infected individuals but decline in the late stages of AIDS. Antibodies mediating antibody-dependent cellular cytotoxicity (ADCC) are also present in AIDS patients.

Cell-mediated responses include T-cell proliferation and cytotoxicity by T-cells and NK cells. Interferon is found in some AIDS patients, possibly due to coinfecting opportunistic viruses. Since HIV causes a slowly progressive, persistent infection, the role of immune responses in preventing disease remains unclear. The emergence of HIV genetic variants may help the virus evade both humoral and cellular immune response

**Epidemiology:** HIV-1 infection is widespread globally, affecting both developed and developing countries. In the United States and other developed nations, HIV is mainly found among homosexual and bisexual men, intravenous drug users, hemophiliacs, transfusion recipients, sexual partners of infected individuals, and infants born to infected mothers (Figure 84). In Africa and the Caribbean, HIV-1 is predominantly seen in heterosexuals, transfusion recipients, and infants of infected mothers. HIV-2 primarily affects individuals in West Africa, Portugal, and Brazil. Both HIV-1 and HIV-2 are transmitted through sexual contact, exposure to contaminated blood or blood products, and perinatally from mother to child. As of 1990, an estimated 800,000 to 1.3 million people in the United States were infected with HIV, though the proportion who will develop AIDS is unknown. The median incubation period for the virus is about 10 years.

**HIV transmission**

**Mother-to-child transmission, including peripartum and breastfeeding (15%-30%)**

**Injecting drug users [IDUs] (1.7%)**

**Unprotected sex**

**(primary mode; 91.5%)**

**Infected blood and blood products (1.0%)**

**Occupational exposures [needle-stick injuries] (0.4%)**

**Heterosexual (87.4%)**

**Bisexual (11.3%)**

**Homosexual (1.3%)**

**Figure 84: Transmission of HIV virus**

**Clinical presentation:** In this section we will be discussing both CDC and WHO (Table 49) classifications of clinical presentation of the disease.

**A. WHO classifications-**

**Table 49: WHO classifications of HIV disease progression**

|  |  |
| --- | --- |
| Clinical stage | Clinical condition |
| Stage 1: Primary HIV infection  [CD4 count ≥500 cells/mm³] | **A. Asymptomatic-** No symptoms present.  **B. Persistent Generalized Lymphadenopathy (PGL)-** Swelling of lymph nodes in two or more non-contiguous sites, excluding the inguinal nodes. |
| Stage 2: Clinical stage 2 (Mild symptoms)  [CD4 count 350-499 cells/mm³] | In early HIV infection, patients may exhibit various clinical symptoms. In stage 2, the mildly symptomatic stage, these include unexplained weight loss of less than 10% of body weight and recurrent respiratory infections like sinusitis, bronchitis, otitis media, and pharyngitis.  Dermatological conditions also appear, such as herpes zoster flares, angular cheilitis, recurrent oral ulcers, papular pruritic eruptions, seborrheic dermatitis, and fungal nail infections. |
| Stage 3: Clinical stage 3 (Advanced symptoms)  [CD4 count 200-349 cells/mm³] | As HIV disease progresses, or the moderately symptomatic stage, additional clinical manifestations may emerge. These include weight loss exceeding 10% of body weight, prolonged unexplained diarrhea lasting more than one month, and pulmonary tuberculosis.  Severe systemic bacterial infections such as pneumonia, pyelonephritis, empyema, pyomyositis, meningitis, bone and joint infections, and bacteremia may also develop. Mucocutaneous conditions, including recurrent oral candidiasis, oral hairy leukoplakia, and acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis, are also common at this stage. |
| Stage 4: Clinical stage 4 (Severe symptoms, AIDS)  [CD4 count <200- cells/mm³] | The severely symptomatic stage, includes all AIDS-defining illnesses.  Key clinical signs are- HIV wasting syndrome, Pneumocystis pneumonia (PCP), recurrent severe bacterial pneumonia, extrapulmonary tuberculosis, HIV encephalopathy, CNS toxoplasmosis, chronic or orolabial herpes simplex infection lasting more than one month, esophageal candidiasis, and Kaposi’s sarcoma.  Additional conditions include CMV infections, extrapulmonary cryptococcosis, disseminated endemic mycoses, cryptosporidiosis, isosporiasis, disseminated non-tuberculous mycobacterial infection, tracheal, bronchial, or pulmonary candida infection, visceral herpes simplex infection, acquired HIV-associated rectal fistula, cerebral or B cell non-Hodgkin lymphoma, progressive multifocal leukoencephalopathy (PML), and HIV-associated cardiomyopathy or nephropathy. These conditions should prompt confirmatory testing if not accompanied by typical AIDS-defining illnesses. |

**B. CDC classifications of HIV disease progression-**

**Group I: Acute HIV infection**- Occurs within 3-6 weeks after infection. Symptoms include low-grade fever, malaise, headache, lymphadenopathy, and occasionally rash and arthropathy resembling glandular fever. Some individuals may experience acute encephalopathy. This stage is also known as seroconversion illness or acute retroviral syndrome. Symptoms typically resolve spontaneously within weeks.

**Group II: Asymptomatic or latent infection**- This phase is characterized by symptomless infection (clinical latency) that can last for several years. Individuals are positive for HIV antibodies and can transmit the virus to others. During this stage, there is gradual progression through various levels of CD4 lymphocyte depletion, minor opportunistic infections, and persistent generalized lymphadenopathy (PGL). The median time from primary HIV infection to AIDS development is approximately 10 years.

**Group III: Persistent Generalized Lymphadenopathy (PGL)**- Defined by the presence of enlarged lymph nodes in two or more non-contiguous sites persisting for at least three months. PGL may progress to ARC or AIDS.

**Group IV: AIDS-Related Complex (ARC)**- Characterized by constitutional symptoms such as fatigue, unexplained fever, persistent diarrhea, and significant weight loss (>10% body weight-wasting syndrome). Common opportunistic infections include

* **Subgroup A. Constitutional disease**- Includes patients with persistent fever (>1 month), involuntary weight loss (>10% baseline), or prolonged diarrhea (>1 month) without other concurrent illnesses to explain these symptoms.
* **Subgroup B. Neurologic disease**- Involves patients exhibiting dementia, myelopathy, or peripheral neuropathy, with no concurrent illnesses explaining these neurological findings.
* **Subgroup C. Secondary infectious diseases**: Divided into:
  + **Category C-1-** Symptomatic or invasive diseases from specific infections indicative of cell-mediated immunity defects, such as Pneumocystis pneumonia, cryptococcosis, and cytomegalovirus infection.
  + **Category C-2**- Symptomatic diseases from other specified infections like oral hairy leukoplakia, tuberculosis, and recurrent Salmonella bacteremia.
* **Subgroup D. Secondary cancers**- Includes Kaposi's sarcoma, non-Hodgkin's lymphoma, and primary brain lymphoma, all strongly associated with HTLV-III/LAV infection and indicative of immune deficiency.
* **Subgroup E. Other conditions**- Encompasses diverse clinical findings potentially linked to HTLV-III/LAV infection, including chronic lymphoid interstitial pneumonitis and conditions complicating disease management or course, such as unexplained constitutional symptoms or atypical infectious diseases.

CDC classifications of CD4+ are as follows (Table 50)-

**Table 50: CDC classification of CD4+**

|  |  |  |  |
| --- | --- | --- | --- |
| CD4+ cell count (cells/mm3) | Clinical categories | | |
| A | B | C |
| Asymptomatic, acute (primary) HIV, persistent generalized lymphadenopathy (PGL) | Symptomatic, not A or C | AIDS indicator |
| ≥500 | A1 | B1 | C1 |
| 200-499 | A2 | B2 | C2 |
| <200 | A3 | B3 | C3 |

**C. AIDS (End-stage disease)**- Represents the irreversible breakdown of the immune system, leaving individuals highly vulnerable to progressive opportunistic infections and malignancies. Clinical severity varies depending on the specific infections or cancers present. AIDS is associated with conditions such as Pneumocystis pneumonia, Kaposi’s sarcoma, CNS opportunistic infections, and other AIDS-defining illnesses. Chronic complications include neurological impairment (dementia) and distinct pediatric manifestations that differ significantly from adults.

**D. AIDS associated dementia-** HIV can directly damage the central nervous system, leading to encephalopathy and subsequent dementia by crossing the blood-brain barrier.

**E. Pediatric HIV-** Pediatric AIDS affects a significant portion of infants born to infected mothers, with transmission often occurring perinatally. Many infected children do not survive their first year, and transmission can also happen through blood products or transfusions. Key differences between adult and pediatric AIDS include early onset of humoral immunodeficiency in children, resulting in recurrent bacterial infections and failure to thrive. Chronic diarrhea and pronounced lymphadenopathy are common, along with frequent tuberculosis and other opportunistic bacterial infections. Children also experience lymphocytic interstitial pneumonia more frequently, whereas Kaposi's sarcoma, toxoplasmosis, and cryptococcosis are less prevalent compared to adults

**Laboratory diagnosis:** Laboratory confirmation (Figure 86) of HIV/AIDS involves several methods used for diagnosis, treatment monitoring, blood screening, antenatal screening, and screening high-risk groups. Methods vary depending on the disease stage and primarily include viral isolation, antibody detection, and detection of viral DNA, RNA, or antigens.

**A. Immunological tests-** Immunological tests include assessments such as total leukocyte and lymphocyte counts, T cell subset assays, platelet counts, and measurements of elevated IgG and IgA levels.

**B. Specific tests for HIV infection encompass several methods-**

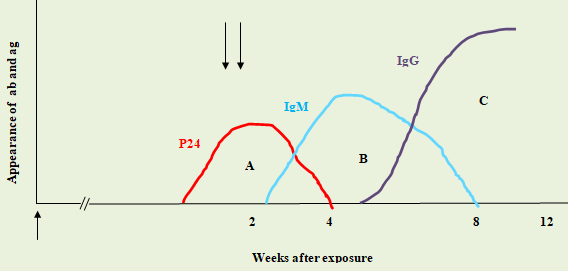
1. **Antigen detection**- Virus antigens, particularly p24, can be detected in blood approximately two weeks after infection. The p24 antigen capture assay (ELISA), utilizing anti-p24 antibody as the solid phase, is utilized for this purpose.
2. **Virus isolation**- HIV is present in various parts of the body and can be isolated from peripheral lymphocytes. Isolation involves co-cultivating patient’s lymphocytes with uninfected lymphocytes in the presence of interleukin-2. Viral replication can be detected through reverse transcriptase activity and antigen presence.
3. **PCR**-

* **Qualitative PCR**- This method amplifies viral nucleic acids for detection in patient specimens, offering high specificity and sensitivity. It is particularly useful in diagnosing mother-to-baby transmission of HIV. This test is highly sensitive and specific when performed with proper controls, capable of detecting HIV proviral DNA at a frequency of one copy per 10,000 cells.
* **Quantitative RNA PCR and genotyping**- Quantitative RNA PCR is used to monitor viremia levels in HIV-positive individuals before or during antiretroviral therapy, and to assess therapy response. Genotyping is employed to monitor drug resistance development or presence in patients before or during therapy. Viral loads exceeding 1,000 copies/mL are typically analyzed for drug resistance testing.
* **bDNA assay**- This involves sequential oligonucleotide hybridization steps to amplify viral RNA.

**Dried Blood Spots (DBS) on filter paper can be used as an alternative to plasma, facilitating specimen transport to molecular testing facilities in remote areas especially in the case of Early Infant Diagnosis (EID) and Integrated Bio-Behavioural Surveillance IBBS)- HIV sentinel surveillance in High Risk Group (HRP).**

**C. Antibody detection-**

* It takes 2-8 weeks to months for antibodies to appear after HIV infection (Figure 85). During this time, individuals may be highly infectious, known as the seronegative infective stage or window period. In infants, until after 18 months of age antibody test is not diagnostic of infection due to presence of maternal antibodies that may cross react.



**Figure 85: Appearance of p24 antigen and antibodies after a massive HIV infection**

**D. Serological tests for anti-HIV antibodies are categorized into two types-**

1. **Screening tests**- These assays have high sensitivity and broad reactivity. The ELISA test, a direct solid-phase antiglobulin enzyme-linked immunosorbent assay, is commonly used. It is simple and relatively inexpensive, although false positives may occur, especially with sera containing rheumatoid factor, anti-lymphocyte antibodies, or other autoantibodies.
2. **Confirmatory tests**- This immunoblotting method (western blot) characterizes antibodies against specific HIV proteins. Nitrocellulose strips containing separated HIV proteins are reacted with test sera, producing distinct color bands. It is a crucial confirmatory test despite its subjective interpretation, often requiring experienced personnel.

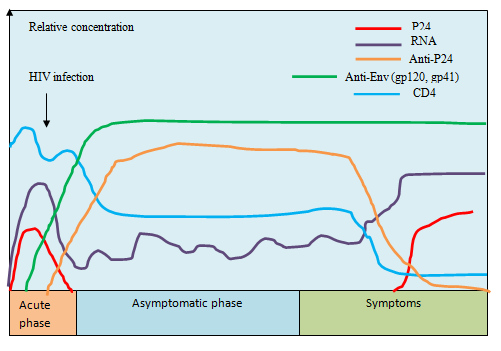
**E. HIV rapid tests-** HIV rapid tests are designed for quick and easy detection of HIV antibodies, making them ideal for screening in various settings. These tests are highly sensitive and can produce results in a short time, typically within 20-30 minutes.

**Types of HIV rapid tests-**

1. **Cassette ELISA**: This format uses a small cassette where the test sample is applied. The test detects HIV antibodies and produces a visible result within minutes.
2. **Immunochromatographic Tests (ICT)**: These tests use a strip of paper or other material that changes color if HIV antibodies are present in the sample. They are simple to use and require minimal training.
3. **Coated particle agglutination**: This method involves mixing the test sample with coated particles. If HIV antibodies are present, they cause the particles to clump together, indicating a positive result.
4. **Immunoperoxidase or Dip-stick tests**: These tests involve dipping a stick into the test sample. A color change on the stick indicates the presence of HIV antibodies.
5. **Saliva and urine tests**: These tests use saliva or urine samples instead of blood. They are less invasive and more convenient for certain populations.

**National AIDS Control Organization (NACO) approved rapid tests (blood samples) used for routine diagnosis are**-

1. **The Tridot HIV test,** manufactured by J. Mitra & Co., is a dot immunoassay used for screening HIV-1 and HIV-2 antibodies. The results are available in 10-20 minutes. A blood sample from a finger prick or venous blood is applied to a nitrocellulose membrane strip containing HIV-1 and HIV-2 antigens. A color change indicates the presence of HIV antibodies. This test is quick, easy to perform, and can be used in various settings, including remote areas.
2. **The Meriscreen HIV 1-2 WB,** produced by Meril Diagnostics Pvt. Ltd., is an immunochromatographic assay for screening HIV-1 and HIV-2 antibodies. It provides results in 5-15 minutes. A blood sample is added to the test cassette with pre-coated HIV antigens. The appearance of a colored line indicates the presence of HIV antibodies. This test is rapid and simple, making it suitable for field use.
3. **The SD Bioline HIV-1/2 3.0,** manufactured by Standard Diagnostics, Inc. (now part of Abbott), is another immunochromatographic assay used for screening HIV-1 and HIV-2 antibodies, delivering results in 5-20 minutes. A blood sample is added to the sample pad on the test device, and the development of a colored line indicates the presence of HIV antibodies. This test is known for its high sensitivity and specificity, with easy-to-interpret results and a clear visual readout.



**Figure 86: Laboratory diagnosis of HIV**

**Treatment:** The details of treatments are given below (Table 51)-

**Table 51: HIV antivirals**

|  |  |
| --- | --- |
| HIV antivirals | Characteristics |
| Nucleoside Reverse Transcriptase Inhibitors (NRTIs) | Acts as DNA chain terminators, blocking the reverse transcription of the viral RNA genome into DNA.  AZT- Zidovudine  ddl- Didanosine  ddC- Zalcitabine  d4T- Stavudine  3TC- Lamivudine |
| Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) | Bind to and inhibit the reverse transcriptase enzyme.  Delaviridine  Efavirenz  Nevirapine |
| Protease Inhibitors | Target the protease enzyme, which is essential for cleaving precursor proteins (gag and gag-pol).  Saquinavir  Ritonavir  Indinavir  Nelfinavir |
| Integrase Inhibitors | Block the action of integrase, the enzyme that inserts the viral genome into the host cell’s DNA.  Raltegravir  Elvitegravir  Dolutegravir  Bictegravir |

**WHO recommendation-** TLD (Tenofovir disoproxil fumarate + Lamivudine + Dolutegravir) is recommended as a first-line treatment for HIV by many health authorities, including the World Health Organization (WHO). It is also used as a switch option for patients who are stable on other regimens but could benefit from the simplicity and effectiveness of TLD (Figure 87).

**ART for mother to child transmission-** The World Health Organization (WHO) recommends a combination regimen that typically includes drugs like Tenofovir, Lamivudine, and Efavirenz. Newborns should receive antiretroviral prophylaxis to further reduce the risk of HIV transmission. This usually involves a short course of antiretroviral drugs like Nevirapine or Zidovudine, starting within hours after birth. Exclusive breastfeeding is recommended for the first six months of life, combined with maternal ART, to minimize the risk of HIV transmission.

HIV-infected with viral load

>5000-10000 or CD4 <500/µl

First line treatment

ddl

AZT+ddl

AZT+3TC

AZT+ddC

Intolerence to regimen

Progression of disease or viral load does not decrease by ≥ 0.5 log with initiation of treatment or increase of viral load by ≥ 0.5 log while on treatment

Change to alternative first-line treatment

d4t+ddl

Saquinavir + one or more nucleoside analogs

Ritonavir + one or more nucleoside analogs

Indinavir + one or more nucleoside analogs

d4t+3TC

Second line treatment

Progression of disease or viral load criteria as above

Third line treatment

Nelfinavir + one or more nucleoside analogs

Nevirapine + AZT + ddl

Saquinavir + Indinavir + one or more nucleoside analogs

**Figure 87: Treatment of HIV**

**Prophylaxis:** HIV prophylaxis refers to the measures taken to prevent HIV infection in individuals who are at risk. There are two primary types of HIV prophylaxis: Pre-Exposure Prophylaxis (PrEP) and Post-Exposure Prophylaxis (PEP).

**I. Pre-Exposure Prophylaxis (PrEP)-** PrEP involves taking a daily medication to prevent HIV infection in people who are HIV-negative but at high risk of contracting the virus. The most commonly used medication for PrEP is a combination of tenofovir and emtricitabine. Another medication, Descovy, which combines tenofovir alafenamide with emtricitabine, is also approved for PrEP but is not recommended for individuals assigned female at birth who engage in vaginal sex, as its efficacy has not been studied in this population.

PrEP is highly effective when taken consistently and is recommended for individuals who:

* Have a sexual partner who is HIV-positive.
* Have multiple sexual partners or engage in sexual activity without consistent condom use.
* Have been diagnosed with a sexually transmitted infection (STI) in the past six months.
* Use injection drugs and share needles or other drug paraphernalia.
* Are involved in commercial sex work.

Regular follow-up with a healthcare provider is essential for individuals on PrEP to monitor for side effects and ensure ongoing HIV testing and adherence to the medication.

**II. Post-Exposure Prophylaxis (PEP)-** PEP is an emergency treatment started after potential exposure to HIV to prevent the virus from taking hold in the body. It must be initiated within 2 hours but not less than 72 hours of exposure and involves taking antiretroviral medications for 28 days. The sooner PEP is started, the more effective it is.

PEP is recommended for:

* Individuals who have had unprotected sex with someone known to be HIV-positive or whose HIV status is unknown.
* Healthcare workers or others who have had occupational exposure to HIV, such as needlestick injuries.
* Individuals who have shared needles or other injection drug equipment.
* People who have been sexually assaulted.

PEP typically consists of a combination of three antiretroviral drugs, such as tenofovir, emtricitabine, and either raltegravir or dolutegravir. Follow-up HIV testing is necessary after completing PEP to ensure that HIV infection has not occurred.

**Vaccine:** HIV vaccine development is challenging due to high mutation rate, latent reservoirs, immune evasion and diversity of strains.

**Spumaviruses**

Spumaviruses, also known as foamy viruses, belong to the retrovirus family and are characterized by their unique replication strategy and ability to establish persistent infections. They are named for their foamy appearance in infected cells under electron microscopy. Spumaviruses have a broad host range and infect various mammalian species, including non-human primates and humans.

Unlike other retroviruses, spumaviruses exhibit a distinct replication cycle. They replicate through a unique strategy of reverse transcription, where the virus synthesizes a DNA intermediate from its RNA genome in the cytoplasm before integrating into the host cell genome. This process involves the production of a full-length DNA copy that is subsequently translocated into the nucleus for integration.

Spumaviruses are generally considered non-pathogenic in their natural hosts but can cause disease under certain conditions, particularly in immunocompromised individuals. In humans, spumaviruses have been detected in various tissues and fluids, including blood, saliva, and urine. They are not associated with any specific disease, but their potential to integrate into the host genome raises concerns regarding long-term effects, although no disease has been definitively linked to spumavirus infection in humans

**References:**

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**Oncogenic viruses**

Oncoviruses constitute a significant subset of infectious agents linked to human cancers, contributing to approximately 12% of all cancer cases globally. Despite widespread infection among the human population, the development of cancer as a result of these viruses is relatively rare. This phenomenon underscores the intricate interplay between viral factors and host immunity, along with additional environmental and genetic influences, that collectively create an environment conducive to oncogenesis.

**Viral oncogenes (V-onc)-** Viral oncogenes, also known as "cancer genes," encode proteins that can trigger the transformation of normal cells into cancer cells. These oncogenes are not essential for viral replication, and mutant viruses lacking them can still replicate normally without being oncogenic.

**Cellular and proto-oncogenes-**

* **Cellular oncogenes (C-onc)**- Oncogenes found in cancer cells.
* **Proto-oncogenes**- Proto-oncogenes are highly conserved across vertebrates and metazoans, indicating they serve essential roles in normal cell functions such as growth and differentiation. For example, the oncogene src is related to tyrosine-specific protein kinases, sis to platelet-derived growth factor, and myc to DNA-binding proteins, all of which are involved in regulating normal cell growth and differentiation.

**Transfection-** Transfection is a method used to study oncogenes. Certain cell lines, like NIH 3T3, can take up foreign DNA, incorporate it into their genome, and express it. This technique has demonstrated that DNA from human tumor cells can transform 3T3 cells, confirming the presence of cellular oncogenes.

**Anti-oncogenes-** There are also tumor suppressor genes, known as anti-oncogenes, which help regulate cell growth. For example, the loss of the retinoblastoma (Rb) gene is associated with retinoblastoma in children, and the p53 gene is a tumor suppressor with a wide range of effects. Specific chromosomal deletions associated with certain types of human cancers may indicate the loss of such tumor suppressor genes.

**Mechanisms of viral oncogenesis-**

**Oncogenic DNA viruses-**

* Viral DNA, or parts of it, integrate into the host cell genome, leading to malignant transformation without producing infectious virus. The transformed cell is similar to a bacterium lysogenized by a defective phage, where the cell is not destroyed and no virus is produced.

**Oncogenic RNA viruses-**

* Retroviruses induce tumors by either:
  1. Introducing a new transforming gene (oncogene) into the cellular genome.
  2. Altering the expression of pre-existing cellular genes, potentially converting benign proto-oncogenes to cancer genes through mechanisms like overexpression, recombination, promoter insertion, chromosomal translocation, gene amplification, and mutation.

**Factors influencing oncogenesis by oncoviruses**

* Oncogenesis often requires additional factors like chronic inflammation or immunosuppression.
* Directly linked to specific cancers such as Kaposi sarcoma and cervical cancer.
* Classified as direct (viral oncogenes) or indirect (chronic inflammation) carcinogens.

**Common pathways and mechanisms of oncogenesis**

* Inhibition of tumor suppressors (e.g., p53, Rb).
* Activation of cell survival pathways (e.g., PI3K-AKT-mTOR).
* Modulation of immune responses critical for cellular integrity.

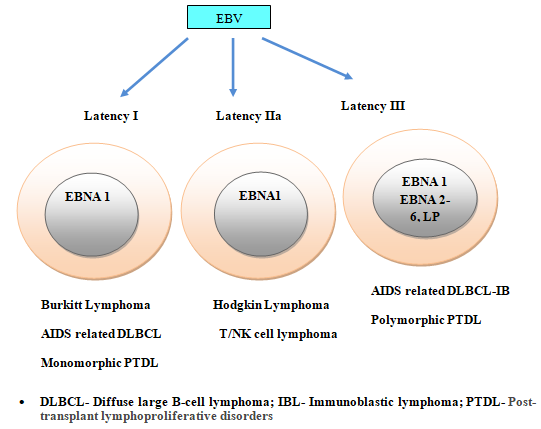
**Oncogenic viruses-**

**1. Epstein-Barr Virus (EBV)**- EBV is linked to various cancers such as Burkitt lymphoma, Hodgkin lymphoma, and nasopharyngeal carcinoma (Figure 88), primarily infecting B cells and establishing latency within them. This virus manipulates host signaling pathways to drive cell proliferation and evade immune detection. EBV exhibits three distinct latency programs in normal B cells: latency 0-I, II, and III. Latency III is marked by unrestricted gene expression of a wide range of latency proteins, typically observed in immunosuppressed individuals due to the high immunogenicity of these proteins. Latency II involves restricted expression of EBNA-1, EBERs, and LMP. Latency 0 and I are characterized by minimal gene expression, with latency I specifically expressing EBNA-1 during the cell cycle.

**2. Human Papillomavirus (HPV)**- Causes cervical cancer and other anogenital cancers, as well as a significant portion of oropharyngeal cancers. HPV oncoproteins E6 and E7 disrupt cell cycle regulation and tumor suppressor pathways, facilitating malignant transformation.

HPV is implicated in various cancers, including:

* **Cervical cancer**- Most commonly associated with HPV infection, particularly HR types like HPV 16 and 18. Screening and vaccination have significantly impacted cervical cancer rates.

****

**Figure 88: EBV latent gene expression**

* **Oropharyngeal cancer**- Increasingly linked to HPV infection, particularly HPV 16, affecting the tonsils and base of the tongue. Improved understanding has influenced treatment strategies.
* **Penile cancer**- Rare but associated with HPV infection, particularly in cases of squamous cell carcinoma.
* **Anal, vulvar, and vaginal cancers**- HPV is also linked to these cancers, with varying degrees of prevalence and impact.

3. **Hepatitis B and C viruses (HBV and HCV)**- HBV is a major cause of hepatocellular carcinoma (liver cancer), while HCV is also implicated in liver cancer development through chronic inflammation and direct oncogenic effects.

**4. Human Herpesvirus-8 (HHV-8)**- Associated with Kaposi's sarcoma, particularly in immunocompromised individuals. HHV-8 encodes viral proteins that disrupt host cell signaling and promote cell proliferation.

**5. Merkel Cell Polyomavirus (MCPyV)**- Identified in Merkel cell carcinoma, a rare but aggressive skin cancer. MCPyV integrates into host DNA and expresses viral proteins that contribute to cellular transformation.

**6. Human T-cell Lymphotropic Virus-1 (HTLV-1)**- Linked to adult T-cell leukemia/lymphoma (ATL), HTLV-1 alters T-cell function and promotes genomic instability, contributing to lymphomagenesis.

**Host specificity:** Retroviruses typically infect only one host species, determined primarily by the presence of specific viral receptors on the host cell surface. Based on their ability to infect cells from different species, retroviruses are categorized as:

1. **Ecotropic**- Infect and multiply only in cells of their native host species.
2. **Amphotropic**- Infect and multiply in cells of both native and foreign species.
3. **Xenotropic**- Infect and multiply only in cells of foreign species, not in cells of the native host species.

**Virus transmission:** Retroviruses transmit in two ways:

* **Exogenous retroviruses**- Spread horizontally between individuals. Most oncogenic retroviruses fall into this category.
* **Endogenous retroviruses**- Transmitted vertically from parent to offspring via the provirus integrated into the germline cell genome. These behave like cellular genes under host cell regulatory control and are usually inactive, not causing disease or cell transformation. They can be detected through activation by radiation or chemicals, or via nucleic acid hybridization techniques.

**Resistance:** Retroviruses are labile and can be inactivated by- heat at 56°C within 30 minutes, mild acids**,** etherand formalin**.** They remain stable when frozen at -30°C.

**Antigens:** Retroviruses contain two types of antigens:

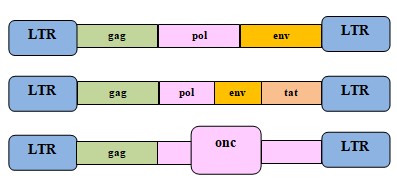
1. **Type-specific glycoprotein antigens**- Located on the envelope.
2. **Group-specific nucleoprotein antigens**- Found in the virion core.

Cross-reactions do not occur between the surface antigens of retroviruses from different host species.

**Genomic structure:** Retroviruses have a relatively simple genomic structure, consisting of three main genes necessary for viral replication, arranged sequentially from the 5' to the 3' end:

* **gag gene**- Codes for nucleocapsid core proteins, which are group-specific antigens.
* **pol gene**- Encodes RNA-dependent DNA polymerase.
* **env gene**- Encodes envelope glycoproteins.

Additionally, long terminal repeat (LTR) sequences (Figure 89) are present at both ends of the provirus and are directly linked to the host DNA, regulating provirus gene functions. Some retroviruses, like HTLV and HIV, carry an additional fourth gene (tex or tat) after the env gene. This transactivating gene regulates the function of viral genes.



**Figure 89: Provirus genomic structure of retroviruses**

**Virus transformation:**

**Slow transforming viruses**- These standard oncogenic retroviruses, such as chronic leukemia viruses, have low oncogenic potential and induce malignancy, usually in blood cells, after a long latent period.They do not transform cultured cells but can replicate normally.

**Acute transforming viruses-** Highly oncogenic and cause malignancy after a short latent period of weeks or months. They can induce various malignancies, including sarcoma, carcinoma, and leukemia, and can transform cells in culture.

1. **Replication defective**- Most acute transforming viruses cannot replicate normally because they carry an additional viral oncogene (V-onc gene), which replaces some essential replication genes. These viruses can only replicate if co-infected with a standard helper retrovirus.
2. **Replication competent**- An example is the Rous sarcoma virus, which carries the oncogenic src gene. It can replicate normally as it contains the full set of gag, pol, and env genes.

Brief description for all these viruses are already covered in DNA and RNA viruses units.

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**Orthomyxoviridae**

Orthomyxoviridae is a family of segmented single-stranded RNA viruses comprising five genera: Influenzavirus A, Influenzavirus B, Influenzavirus C, Isavirus, and Thogotovirus. The family name indicates their ability to attach to mucous proteins on cell surfaces ("myxo" means mucous in Greek).

**Thogotovirus**

The Thogotovirus genus is unique among orthomyxoviruses because its species are transmitted by arthropods, typically ticks. Thogoto virus, the type species, can infect humans and causes disease in livestock and other mammals. Another species, Dhori virus, can also infect humans but is not a significant human pathogen due to the rarity of infections.

**Isavirus**

Isavirus, represented by Infectious salmon anemia virus, is a pathogen of salmon. It does not affect humans and is transmitted via water, posing significant concerns for aquaculture but not for public health.

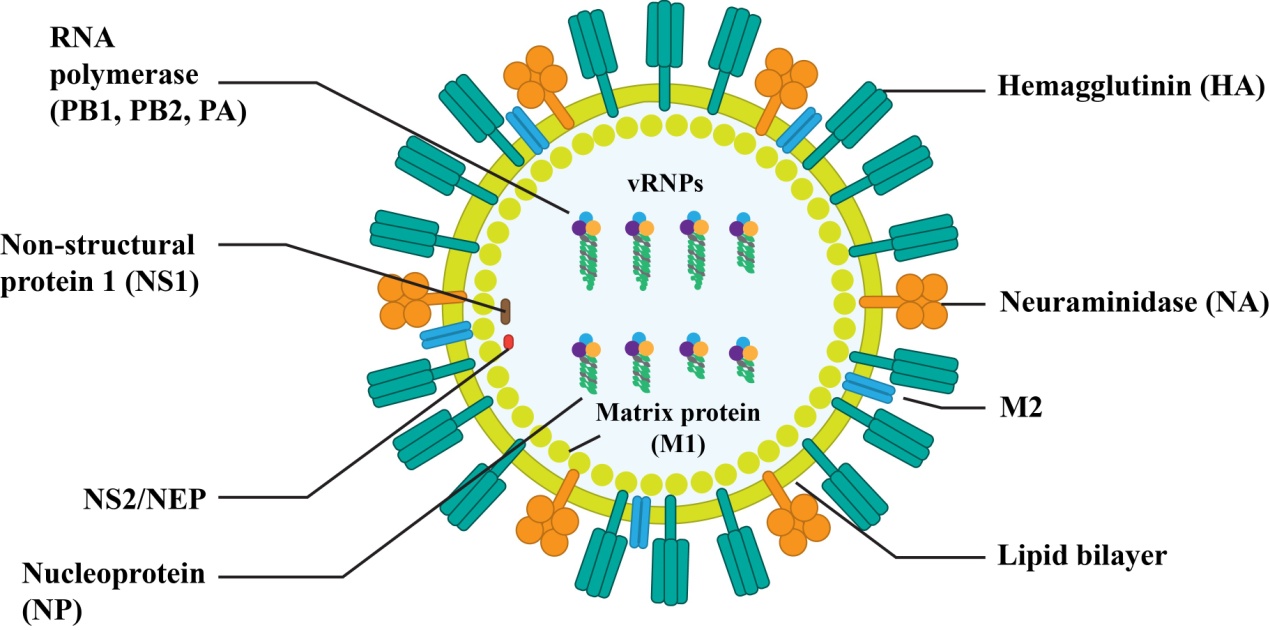
**Influenzavirus**

Influenza A, B, and C viruses are the most relevant orthomyxoviruses for human health. Influenza A is the most significant due to its frequent role in almost-annual epidemics of respiratory illness, alongside Influenza B. In contrast, Influenza C generally causes mild or asymptomatic infections and does not lead to epidemics.

Influenza is an acute respiratory disease that appears sporadically, in epidemics, and occasionally as pandemics. Its history began with the mistaken identification of Haemophilus influenzae as the cause during the 1889-90 pandemic. The devastating 1918-19 pandemic clarified that H. influenzae was secondary, prompting the isolation of the influenza virus in 1933 by Smith, Andrewes, and Laidlaw, a pivotal moment in virology. They demonstrated its ability to cause disease in ferrets using filtered patient nasopharyngeal secretions.

Further insights came with Hirst, McClelland, and Hare's 1941 discovery that influenza viruses could agglutinate fowl erythrocytes, a feature shared by many viruses. Avian influenza virus was recognized in 1901, but its linkage to human influenza was affirmed in 1955 by Schaefer, who highlighted antigenic similarities between fowl plague virus and type A influenza virus. Shope's 1931 isolation of swine influenza virus underscored clinical and epidemiological parallels with human influenza, suggesting interspecies transmission during the 1918 pandemic.

**Structure**: Orthomyxoviruses (Figure 90) are spherical or pleomorphic, with diameters ranging from 80 to 120 nm. Their capsids exhibit tubular helical symmetry and contain segmented negative polarity RNA. The orthomyxovirus genome consists of ribonucleoproteins surrounded by a membrane embedded with viral glycoproteins and nonglycosylated proteins.



**Figure 90: Structure of orthomyxovirus**

**Genomic organization**: Influenza A and B viruses have eight genome segments of ssRNA(-), while Influenza C virus has seven. RNA segments 1, 2, and 3 code for PB2, PB1, and PB-A respectively, which comprise the viral polymerase complex (P). Key surface proteins include hemagglutinin (HA), which mediates host cell binding and virus entry, neuraminidase (NA), responsible for virus budding and release, and matrix protein 2 (M2), an ion channel involved in viral genome replication. Within the virion, RNA genome segments are bound to nucleoprotein (NP) along with several viral proteins that form the viral RNA polymerase complex (RNA polymerase acidic, RNA polymerase basic 1, and RNA polymerase basic 2). A layer of matrix protein 1 (M1) surrounds the RNP and is involved in nuclear transport of the RNP. Nonstructural proteins 1 and 2 (NS1 and NS2) regulate viral protein expression and replication, with NS2 present in very low amounts in the virion.

**Genetic diversity:** Influenza virus genomes evolve continuously due to the error-prone RNA-dependent RNA polymerase (RdRp), leading to gradual genetic changes known as antigenic drift. Orthomyxoviruses can also undergo genome reassortment because of their segmented genomes. This occurs when genomic segments from different strains mix in a co-infected host cell, creating new genetic strains through a process called antigenic shift. Antigenic shift can result in new viral strains with no existing herd immunity, potentially causing deadly pandemics. It also enables some influenza strains to adapt to new species, such as avian influenza adapting to humans.

The below table shows difference between orthomyxovirus and paramyxovirus (Table 52)-

**Table 52: Difference between orthomyxovirus and paramyxovirus**

|  |  |  |
| --- | --- | --- |
| Property | Orthomyxovirus | Paramyxovirus |
| Size of virion | 80-120 nm | 100-300 nm |
| Shape | Spherical; filaments in fresh isolates | Pleomorphic |
| Genome | Segmented; eight pieces of RNA | Single linear molecule of RNA |
| Diameter of nucleocapsid | 9 nm | 18 nm |
| Site of synthesis of ribonucleoprotein | Nucleus | Cytoplasm |
| Genetic reassortment | Common | Absent |
| Dependent RNA synthesis | Required for multiplication | Not required |
| Effect of Actinomycin D | Inhibits multiplication | Does not inhibit |
| Antigenic stability | Variable | Stable |
| Hemolysin | Absent | Present |

**Strain classification:** Influenza strains are classified by the antigenic properties of the hemagglutinin (HA) and neuraminidase (NA) proteins on their membranes. Type A viruses are divided into subtypes based on these proteins, with HA subtypes ranging from H1 to H16 and NA subtypes from N1 to N9. The subtype nomenclature includes the host organism, geographical location of the first isolation, strain number, year of isolation, and the antigenic properties of HA and NA. For instance, the last pandemic virus is labeled A/California/04/09 (H1N1). Human viruses typically omit the host organism in their names, unlike those originating from animals, such as A/Swine/Iowa/15/30 (H1N1).

**Host range and distribution:** Influenza viruses A, B, and C can infect humans, but only A and B cause annual epidemics, with only type A linked to pandemics. Types B and C mainly infect humans, but type B is also found in seals and sea lions, and type C in dogs and pigs. Type A viruses have a broader host range, infecting land mammals like pigs, horses, and minks, as well as marine mammals like seals and whales. They primarily infect birds, where the virus multiplies in the digestive tract, typically causing asymptomatic infections. Wild aquatic birds, which host all type A viral subtypes (16 H and 9 N), are considered the main reservoirs of type A viral genetic diversity.

**Resistance:** The virus is susceptible to heat, being inactivated at 50°C for 30 minutes. It can survive for about a week at temperatures between 0-4°C and can be preserved for years at -70°C or through freeze-drying. Slow drying allows the virus to remain viable on surfaces like blankets for up to two weeks. Chemical disinfectants such as ether, formaldehyde, phenol, salts of heavy metals, and many others effectively destroy its infectivity, with iodine being particularly potent. The virus's hemagglutinating, enzymatic, and complement-fixing activities are more stable than its ability to remain infectious.

**Replication:**

**1. Viral entry and fusion**- Influenza A virus initiates infection by binding to host cell receptors, primarily N-acetylneuraminic acid (NeuAc or sialic acid), with human viruses preferring α2,6-linked NeuAc and avian viruses favoring α2,3-linked NeuAc. This interaction is mediated by the viral hemagglutinin (HA). The virus enters the host cell through receptor-mediated endocytosis facilitated by the HA1 domain of HA.

**2. Endosomal release and cytoplasmic entry**- Within the endosome, the acidic environment triggers a conformational change in the HA2 domain of HA, facilitating fusion of the viral envelope with the endosomal membrane. This fusion releases the viral ribonucleoprotein (RNP) complex into the host cell cytoplasm.

**3. Nuclear entry and RNA synthesis**- Once in the nucleus, the viral RNP, guided by nuclear localization signals in the viral NP protein, initiates replication. The process begins with the synthesis of complementary RNA (cRNA) in positive sense, which serves as a template for the production of new viral RNA (vRNA). Concurrently, messenger RNAs (mRNAs) for viral protein synthesis are transcribed. The virus utilizes a process called cap-snatching, facilitated by its PA subunit, to steal caps from host cell RNAs for initiating viral mRNA synthesis.

**4. Viral protein synthesis and assembly**- Essential viral proteins involved in replication and transcription include the PB1, PB2, and PA subunits of the transcription complex, along with NP. These proteins interact sequentially with cellular components to facilitate vRNA and cRNA synthesis.

**5. Virion assembly and budding**- Assembly of new viral particles occurs at the apical surface of infected epithelial cells. The M1 protein plays a crucial role in viral particle assembly by interacting with internal viral components and RNPs. Despite ongoing research, the mechanism by which all eight RNA segments are packaged into each viral particle remains unclear.

**6. Release and spread**- Neuraminidase (NA), a viral surface protein, assists in the release of newly formed viral particles from the host cell surface by cleaving the bond between hemagglutinin and NeuAc, preventing viral aggregation. This step facilitates the spread of the virus to infect new host cells.

**7. Induction of apoptosis**- Viral proteins NA, NS1, and PB1 also contribute to the induction of apoptosis in infected cells, which aids in viral pathogenesis and progression of infection.

**Epidemiology**: Influenza, a respiratory disease caused by influenza viruses, manifests in various forms: sporadic cases, epidemics, and periodic pandemics. Understanding its epidemiology involves examining its transmission, historical outbreaks, surveillance efforts, and the dynamics of viral evolution.

* **Transmission and source of infection**- Influenza viruses primarily spread through respiratory secretions from infected individuals. The virus is shed shortly before symptoms begin and continues for about 3-4 days thereafter. Subclinical infections, where individuals show no symptoms, are common but contribute to viral spread.
* Influenza type C circulates globally, causing mild or unnoticed infections. Type B strains lead to sporadic cases and occasional epidemics. In contrast, type A viruses have a broader impact, capable of causing both epidemics and pandemics due to their antigenic variability.
* **Pandemics**- The history of influenza pandemics (Table 53) highlights significant global health crises. The 1889-90 pandemic, initially attributed to Haemophilus influenzae, marked the beginning of modern influenza study. The catastrophic 1918-19 "Spanish flu" pandemic underscored the virus's potential for high mortality, particularly affecting young adults. Subsequent pandemics in 1957 ("Asian flu") and 1968 ("Hong Kong flu") varied in severity but spread globally, causing considerable illness.
* **Surveillance and monitoring**- The World Health Organization (WHO) coordinates global surveillance efforts to track influenza viruses and their antigenic changes. This monitoring is crucial for detecting shifts and drifts in viral strains that could lead to epidemics or pandemics. Surveillance helps in predicting seasonal trends and preparing vaccines.
* **Antigenic drift and shift**- Influenza viruses constantly evolve through antigenic drift, caused by mutations in viral genes. This gradual change influences annual epidemics. Antigenic shift, on the other hand, occurs when different influenza viruses reassort their genetic material, potentially creating new strains to which humans have little immunity, thus leading to pandemics.
* **Avian influenza and zoonotic risks**- Avian influenza viruses, carried asymptomatically by wild birds, pose a persistent pandemic threat. Occasionally, these viruses cross into humans, as seen with the H5N1 strain in Hong Kong in 1997. Such events highlight the zoonotic potential of influenza viruses and the importance of surveillance in preventing human outbreaks.
* **Evolutionary patterns and recombination**- Influenza viruses exhibit cyclical patterns where dominant subtypes replace older strains. However, exceptions occur, challenging previous assumptions about strain displacement. Recombination between human and animal strains, especially in intermediate hosts like pigs, can lead to the emergence of novel influenza viruses with pandemic potential.

**Table 53: Influenza outbreaks**

|  |  |  |
| --- | --- | --- |
| Date | Antigenic subtypes | Epidemiology |
| 1889-1900 | H2N8 | Pandemic and epidemics |
| 1900-1910 | H3N8 | Extensive epidemics |
| 1918-1933 | H1N1 (former Hsw N1) | 'Spanish flu'. The most severe pandemic recorded; heavy mortality |
| 1933-1946 | H1N1 (former H0N1) | Discovery of influenza virus (WS strain-1933); epidemics of 'AO' strains |
| 1946-1957 | H1N1 | Epidemics of 'Al' strains |
| 1957-1968 | H2N2 | Extensive pandemics of 'Asian flu' (formerly called A2 (Asian) strain), low mortality |
| 1968 to present | H3N2 | Moderate pandemic of 'Hong Kong flu' (formerly called A2 time (Hong Kong) strains), very low mortality |
| 1977 to present | H1N1 | Re-emergence of former Al strains. First appeared in Russia and China ('Red flu'); mild pandemic, very low mortality |

**Immune response:** Influenza infection offers immunity that typically lasts for one to two years, but this protection is limited due to the virus's frequent antigenic changes. Following infection or vaccination, antibodies develop against various viral antigens, particularly hemagglutinin (HA) and neuraminidase (NA), which are crucial for protecting the respiratory tract. Local concentrations of IgA antibodies play a significant role in this defense.

During subsequent infections with different strains of influenza A virus, individuals produce antibodies not only against each infecting strain but also against the initial strain encountered. This phenomenon, termed 'original antigenic sin,' results in a dominant antibody response that primarily targets the first encountered strain.

Influenza virus infection also triggers cell-mediated immunity, although its precise role in providing protection is not fully elucidated. Understanding these immune responses is vital for developing effective vaccination strategies and improving public health measures against influenza.

**Pathogenesis:** Influenza enters the body through the respiratory tract. Even small doses, such as about three viable particles administered as aerosols in experimental infections, can initiate infection. Higher doses are required when the virus is introduced via intranasal instillation. The viral neuraminidase plays a critical role by reducing the viscosity of the respiratory tract's mucus film, which exposes cell surface receptors necessary for virus attachment.

Once attached, the virus primarily infects the ciliated cells lining the respiratory tract. These cells are damaged and shed, exposing the underlying basal cells in the trachea and bronchi. This renders the respiratory tract vulnerable to bacterial invasion, particularly in severe cases where viral pneumonia occurs. Viral pneumonia is characterized by increased blood flow (hyperemia), thickening of alveolar walls, infiltration of white blood cells into the interstitial spaces, capillary blockages (thrombosis), and leakage of white blood cells (leucocytic exudation).

In severe cases, a hyaline membrane may form, obstructing the alveolar ducts and alveoli. During later stages, macrophages infiltrate the affected areas, engulfing and removing dead alveolar cells. Typically, influenza remains localized in the respiratory tract, though during the 1957 pandemic, isolated cases showed viral presence in organs like the spleen, liver, kidneys, and others, albeit very rarely.

**Clinical presentation:** The incubation period of influenza ranges from 1 to 3 days. The disease severity can vary widely, from mild symptoms resembling a common cold to severe cases of rapidly progressing pneumonia that can be fatal. Many infections are asymptomatic or mild. In typical clinical cases, the illness begins suddenly with symptoms like fever, headache, and widespread muscle pain (myalgia). Respiratory symptoms are prominent, and patients often experience significant fatigue. Children infected with type B influenza may also develop abdominal pain and vomiting, sometimes mimicking acute abdominal emergencies.

Typically, uncomplicated cases of influenza resolve within approximately seven days. The most serious complication is pneumonia, primarily caused by secondary bacterial infections, although viral pneumonia (caused by the influenza virus itself) can also occur rarely. Other complications may include cardiac issues like congestive heart failure or myocarditis, as well as neurological problems such as encephalitis, though these are infrequent.

Influenza, especially type B infections, have been linked to Reye syndrome, a condition primarily affecting young children. Reye syndrome involves acute degenerative changes in the brain, liver, and kidneys. Additionally, type B infections can occasionally manifest with gastrointestinal symptoms, often referred to as "gastric flu."

**Laboratory diagnosis:** Influenza virus detection involves several methods to identify and characterize the virus.

**1. Immunofluorescence for virus antigen detection**- Rapid diagnosis of influenza involves detecting virus antigens on nasopharyngeal cells using immunofluorescence.

**2. Virus isolation**- Throat garglings are collected using saline or buffered salt solution, stored at 4°C, or at -70°C for prolonged storage to maintain virus viability. Virus is inoculated into 11-13-day-old eggs' amniotic cavity. After incubation, fluids are harvested and tested for hemagglutination using guinea pig and fowl cells. Subtype identification is performed via hemagglutination inhibition test. For cell culture method, inoculation into monkey or baboon kidney cell cultures and incubation at 33°C with trypsinfor increase sensitivity.

**3. Serological diagnosis-**

* **Hemagglutination inhibition (HI) Test:** Detects and quantifies antibodies specific to influenza virus hemagglutinin. Requires paired sera to show antibody titer rise. Treatment with RDE or trypsin removes non-specific inhibitors.
* **Complement fixation (CF) test:** Uses RNP antigen to detect infection-specific antibodies; less commonly used due to complexity.
* **Radial immunodiffusion:** Screens for antibodies against RNP, hemagglutinin, and neuraminidase; more useful for screening than routine diagnosis.

**4. PCR-based diagnosis-**

* **Multiplex PCR assay:** Uses subtype-specific primers to identify influenza virus in clinical specimens with high specificity.

**Treatment:** Amantadine and rimantadine are effective treatments for influenza, shortening the illness duration and improving symptoms without affecting virus shedding or antibody response. However, resistance to these drugs develops quickly. Newer drugs like zanamivir and oseltamivir, which block viral neuraminidase, are also effective in treating and preventing influenza when administered via nasal spray.

**Prophylaxis:** Influenza vaccines have been pivotal in preventing the disease for decades, but they face challenges due to the virus's frequent antigenic changes. The main difficulty lies in the rapid evolution of influenza viruses, necessitating frequent updates of vaccine formulations to match circulating strains. This dynamic nature means vaccines cannot be stockpiled for future outbreaks. During pandemics, timely vaccine production is crucial to curb rapid virus spread before it infects large populations. Delays in vaccine availability can lead to widespread outbreaks.

1. **Inactivated vaccines-**
   * Traditional vaccines use formalin-inactivated virus grown in eggs, posing risks of allergic reactions due to egg proteins.
   * Modern subunit vaccines use purified hemagglutinin and neuraminidase antigens to reduce adverse effects.
   * Recombinant vaccines combine growth characteristics of established strains with surface antigens from new variants, facilitating efficient vaccine production.
2. **Live attenuated vaccines-**
   * Administered intranasally, these vaccines induce local immunity but can occasionally cause clinical symptoms, particularly in children.
   * Temperature-sensitive mutants replicate in nasal mucosa but not lungs, ensuring safety while providing effective protection.
3. **Chemoprophylaxis-**
   * Antiviral drugs like amantadine and rimantadine block the viral M2 protein channel, effective against type A influenza but ineffective against type B due to different viral components.

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**Paramyxoviridae**

Paramyxoviruses are enveloped, single-stranded, negative-sense RNA viruses that replicate in the cytoplasm. The incidence of serious illness due to paramyxoviruses has significantly decreased with the development and use of vaccines. However, cases have increased even in developed countries due to inconsistent vaccination uptake. Effective vaccines are still under development for some species within the Paramyxoviridae family.

The family Paramyxoviridae is divided into two subfamilies relevant to humans: Pneumovirinae and Paramyxovirinae.

* **Pneumovirinae** includes the genus Pneumovirus, which contains the respiratory syncytial virus (RSV).
* **Paramyxovirinae** includes several genera:
  + **Morbillivirus** (measles virus/rubeola)
  + **Respirovirus** (parainfluenza viruses 1 and 3)
  + **Rubulavirus** (mumps virus and parainfluenza viruses 2 and 4)

The World Health Organization (WHO) targets the elimination of measles and rubella in five regions by 2020. Paramyxoviridae members are significant pathogens, especially in infants and children, causing acute respiratory infections (such as those from RSV and parainfluenza viruses) and highly contagious childhood diseases (measles and mumps). Infections, though less common, can also occur in adults.

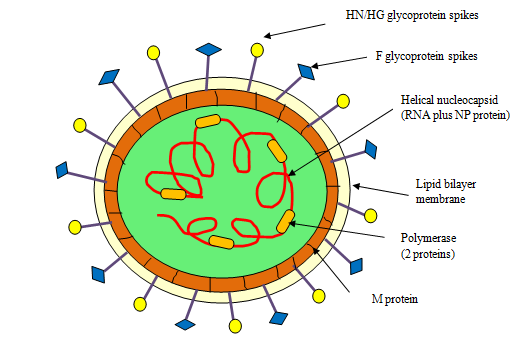
**Structure:** All paramyxoviruses are enveloped particles ranging from 150 to 300 nm in diameter (Figure 91). These viruses have a tubular, helically symmetrical nucleocapsid that contains a single-stranded, negative-sense RNA genome and an RNA-directed RNA polymerase. This nucleocapsid is associated with the matrix protein (M) and is encased in a double-layered lipid envelope. The envelope has spikes that consist of two glycoproteins: a viral attachment protein and a fusion protein.

Paramyxoviruses (Table 54) can be distinguished by the gene order of their viral proteins and the biochemical properties of their viral attachment proteins. For example, in parainfluenza viruses, the protein spikes exhibit both hemagglutinating and neuraminidase activities (HN). In contrast, respiratory syncytial virus lacks these activities, while the measles virus lacks neuraminidase but has hemagglutinating activity.

**Resistance:** All paramyxoviruses are highly labile and can be rapidly inactivated by heat, organic solvents, detergents, ultraviolet or visible light, and low pH.

**Table 54: Difference between different paramyxoviruses**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Property | Measles | Mumps | Respiratory Syncytial Virus | Parainfluenza 1-4 |
| Genus | Morbillivirus | Rubulavirus | Pneumovirus | Human Parainfluenza Virus |
| Surface Glycoproteins | Hemagglutinin (H) and Fusion (F) | Hemagglutinin-Neuraminidase (HN) and Fusion (F) | Fusion (F) and Glycoprotein (G) | Hemagglutinin-Neuraminidase (HN) and Fusion (F) |
| Attachment Protein (G/HN) | Hemagglutinin (H) | Hemagglutinin-Neuraminidase (HN) | Glycoprotein (G) | Hemagglutinin-Neuraminidase (HN) |
| Intracellular Inclusions | Cytoplasm (C) | Cytoplasm (C) | Nucleus (N) and Cytoplasm (C) | Cytoplasm (C) |
| Diameter of Nucleocapsid (nm) | 18 | 18 | 18 | 13 |
| Hemolysin | + | + | + | - |



**Figure 91: Structure of paramyxovirus**

**Measles virus**

Measles virus (MV) belongs to the Morbillivirus genus within the Paramyxoviridae family. It shares antigens with other related viruses such as canine distemper and rinderpest. The virus has been recognized for centuries, with Peter Panum's study of a Faroe Islands epidemic in 1846 providing foundational insights. The viral origin was confirmed in 1911 by Goldberger and Anderson, and isolated in 1954 by Enders and Peebles, leading to vaccine development

**Antigenic composition:** Measles encodes at least eight structural proteins, including nucleoprotein (N), phosphoprotein (P), and matrix protein (M). Glycoproteins H and F facilitate attachment and spread, with CD46 receptors aiding viral entry into cells.

**Host range:** Propagation of wild virus in vitro is challenging due to slow growth. Humans are the natural host, while monkeys can also be infected with milder symptoms. Laboratory animals are generally not susceptible, except through vaccine strains.

**Epidemiology:** The measles virus remains infectious in airborne droplets for longer periods, especially in low humidity, leading to a winter surge in cases. Infection sources are virus-containing respiratory secretions, either airborne or via fomites. The contagious period lasts about 6 days, starting with prodromal symptoms and continuing until 2 days after the rash appears, when antibodies first develop. The viral RNA is detectable in respiratory secretions up to 48 hours post-rash. Factors affecting virus spread include universal susceptibility, high contagiousness, population density, and living standards

Pre-vaccination, measles epidemics happened every 2 to 5 years. Vaccination drastically reduced incidence, though sporadic outbreaks still occur due to importation or immunization gaps, particularly among unvaccinated populations. In developed societies, measles usually affects children aged 4 to 7, while in underdeveloped regions, it occurs before age 4. By ages 7 to 12, most children have had measles and developed specific antibodies. The risk from the live vaccine is one-tenth that of natural infection. Recent studies suggest a link between early measles infection or vaccination and Crohn's disease.

**Replication:** The virus multiplies through a series of steps-

* **Attachment and fusion**- The virus attaches to the cell surface and fuses its envelope with the cytoplasmic membrane, allowing the nucleocapsid to penetrate the cytoplasm.
* **RNA transcription**- The negative-sense RNA is transcribed by nucleocapsid-associated P and L proteins.
* **Gene order and function**- The viral genome follows the sequence N, P, M, F, H, and L. This RNA serves as a template for mRNA production and for replicating intact RNA via a positive-stranded intermediate.
* **Maturation**- Genomic RNA and structural proteins accumulate in the cell cytoplasm. The virus matures by budding from the cell, with the cell membrane modified by N-linked carbohydrate chains before the appearance of viral transmembrane proteins on the surface.

Viral particle release varies from a few hours to indefinitely in chronic infections. Chronic CNS diseases, such as subacute sclerosing panencephalitis, can result from mutations that prevent viral budding, reduce envelope protein expression, and allow ribonucleoprotein spread through the CNS despite a strong immune response.

**Pathogenesis:** The measles virus causes a systemic infection that spreads via viremia, affecting the lymphatic and respiratory systems, skin, and occasionally the brain. The virus typically enters the host through the oropharynx and possibly the conjunctiva. After local multiplication in the respiratory tract and regional lymph nodes, primary viremia disseminates the virus to the reticuloendothelial system for extensive replication. A secondary viremia follows 5 to 7 days later, spreading the virus to the respiratory, gastrointestinal, and urinary tracts, skin, and central nervous system. Virus-infected cells contain viral antigens and inclusions, and may form giant cells (inclusion bodies in nucleus and cytoplasm, i.e., Warthin- Finkeldey cell). In SSPE, noninfectious viral ribonucleoprotein (RNP) inclusions appear in various cell types in the brain, causing inflammation and some demyelination.

**Immune response:** Measles virus infection typically encounters minimal natural resistance in hosts. Early in infection, nonspecific substances like interferon play a role in limiting viral spread until virus-specific antibodies develop. Interferon remains detectable until the appearance of virus-specific antibodies. During primary infection, the humoral immune response aids in clearing extracellular virus and prevents systemic spread upon reinfection. Maternal IgG antibodies provide complete protection to infants for the first 6 months of life. Between 6 to 12 months, infants may experience subclinical infection or modified disease as maternal antibodies wane. In patients with SSPE, there are notably high titers of measles oligoclonal antibodies (IgG) present in both serum and cerebrospinal fluid. Cell-mediated immunity is crucial for recovery from primary measles infection and provides resistance against reinfection at the entry site.

**Clinical presentation:**

* **Prodromal stage/pre-eruptive stage**- After a 9 to 12 day incubation period, symptoms include malaise, fever, coryza, cough, and conjunctivitis, followed by the appearance of Koplik spots. The Koplika’s spot (bluish white with erythematous halo) appears on the buccal mucosa opposite the first and second upper molars, a day or 2 before the rash can be observed.
* **Eruptive phase**- The rash starts on the head and spreads downward, initially erythematous and maculopapular, later becoming confluent. The illness typically lasts 7 to 10 days.

**Complications-**

* **Common complications**- Otitis media from bacterial superinfection is frequent. Pneumonia, either viral or bacterial, is the most common cause of hospitalization and death.
* **Rare complications**- These include myocarditis, pericarditis, hepatitis, appendicitis, mesenteric lymphadenitis, and ileocolitis.
* **Mild measles**- Occurs in children with low levels of maternally derived or injected antibodies.
* **Pregnancy**- Measles infection during pregnancy can lead to spontaneous abortion, stillbirth, or preterm delivery.
* **Other complications**- Croup, bronchiolitis, and fatal giant-cell pneumonia can occur, especially in immunocompromised children.

**Atypical and severe presentations-**

* **Atypical measles syndrome**- This severe form features high fever, atypical pneumonia, and a urticarial, purpuric rash, mainly in inadequately immunized individuals.
* **Postinfectious encephalitis**- With a frequency of 0.1 to 0.2 percent, this severe complication has a 20 percent mortality rate and can result in permanent neurological sequelae.

**Laboratory diagnosis:** In typical cases, measles diagnosis is straightforward based on clinical presentation. However, in atypical cases or for differentiation from rubella, laboratory tests are valuable.Nasal secretions, throat swabs, conjunctival samples, and blood are suitable specimens. CSF is collected in suspected cases of SSPE.

1. **Direct microscopy**- Early diagnosis can be made by identifying multinucleated giant cells in Giemsa-stained smears of nasal secretions, even before the rash appears.
2. **Immunofluorescence assay (IFA)**- Measles virus antigen in nasal secretions can be detected using immunofluorescence, providing rapid diagnostic confirmation.
3. **Virus isolation**- The virus can be isolated from nasal swabs, throat swabs, conjunctival swabs, and blood during the prodromal phase and up to two days after rash onset. Virus may also be isolated from urine for a few additional days. Primary human or monkey kidney cells are commonly used, and cytopathic changes indicative of viral growth may take up to a week to develop.
4. **Serological diagnosis**- Various serological tests such as specific neutralization, hemagglutination inhibition (HAI), and complement-fixing antibody tests (CFT) are employed. Diagnosis is confirmed by a fourfold rise in antibody titer between acute and convalescent phase sera collected 10-21 days apart. Detection of measles-specific IgM antibodies in a single serum specimen collected 1-2 weeks after rash onset is confirmatory. Timing is critical, as false negatives may occur if serum is collected too early (before one week after rash onset) or too late (after two weeks).
5. **PCR (Polymerase chain reaction)**- Reverse transcriptase PCR is highly sensitive and specific for detecting measles virus RNA, providing a molecular diagnostic tool.

**Treatment and prophylaxis:** Management focuses on symptomatic relief, while bacterial superinfections should be treated with appropriate antibiotics, avoiding prophylactic use.

**A. Immunoglobulin administration (passive immunization)-** Administering measles immunoglobulin within 3 days of exposure effectively prevents measles by halting viremia. When given between days 5 and 9 post-exposure, it modifies disease progression and aids immune response, albeit with reduced efficacy. The protection provided by immunoglobulin typically lasts about 4 weeks.

**B. Active immunization with MMR vaccine**- A safe and effective live attenuated measles vaccine is available, originally derived from the Edmonston strain, which underwent multiple passages through human kidney, amnion, and chick embryo cultures. However, due to its propensity to cause febrile rash (vaccination measles), further attenuation was required. The Schwartz and Moraten strains were subsequently developed, proving safer but effective only in children older than 15 months.

In tropical regions, where measles is prevalent and severe in children under 12 months, the Edmonston-Zagreb strain, attenuated through human diploid cell passages, is preferred. This strain induces seroconversion even in infants aged 4-6 months. Developing countries now recommend measles vaccination at nine months, while advanced nations maintain the age at 15 months.

Healthy children aged 12 to 18 months should receive the measles-mumps-rubella (MMR) live-virus vaccine. This vaccine induces antibodies in approximately 94% of initially seronegative recipients and offers immunity that typically persists for over 18 years. Revaccination strategies vary by country to address primary vaccine failures and boost antibody levels, with emphasis on adolescents entering college.

**MR vaccination campaign:** India and other countries in the WHO South East Asia Region are working towards eliminating measles and controlling rubella and congenital rubella syndrome (CRS) by 2020. The Ministry of Health & Family Welfare has launched a nationwide measles-rubella (MR) vaccination campaign targeting children aged 9 months to less than 15 years. The campaign is rolled out in phases across the country with the aim of vaccinating approximately 410 million children. The recommended schedule includes two doses of the MR vaccine, ideally at 9-12 months and 16-24 months of age. Children who miss these doses can still receive the vaccine up to 5 years of age.

**Mumps**

The etymology of "mumps" remains unclear, potentially derived from the English noun "mump" meaning a lump, or the verb "to mump," describing a sulky expression—a characteristic seen in affected individuals. Alternatively, the term may relate to the mumbling speech pattern associated with the disease. Historically known as "epidemic parotitis," the mumps virus was later successfully used in killed virus vaccines in the 1950s, albeit with limited success. A breakthrough came in 1966 when Buynak and Hilleman developed an effective live virus vaccine, marking a significant milestone in preventing mumps infections.

**Replication:** Mumps virus initiates infection by attaching its HN protein to sialic acid on cell-surface glycolipids, aiding in fusion with the host cell plasma membrane via the F protein. Following uncoating, viral RNA is transcribed and translated into viral proteins necessary for replication. Viral assembly occurs in the cytoplasm, followed by maturation through budding.

**Pathogenesis**: Pathogenesis involves systemic infection via viremia, targeting glandular and nervous tissues. Initial infection likely occurs via the pharynx or conjunctiva, followed by local viral replication in epithelial cells. This leads to primary viremia, followed by secondary viremia and dissemination to various organs, including salivary glands, testes, ovaries, pancreas, and brain.

**Immune response:** Following infection, rapid interferon production limits virus spread, followed by specific cellular and humoral immune responses. IgM antibodies to mumps antigens develop within days of symptom onset, followed by lifelong protective IgG antibodies. Vaccination has significantly reduced mumps incidence and complications, contributing to herd immunity in populations where vaccination rates are high.

**Epidemiology:** Mumps is endemic worldwide, with peak incidence in urban areas from January to May. Outbreaks occur in settings with dense populations of children and young adults, such as schools, boarding schools, and military camps. Transmission primarily occurs through direct person-to-person contact via salivary gland secretions, particularly before and shortly after symptom onset. Asymptomatic infections also contribute to transmission.

**Clinical presentation:** Mumps infection typically spreads via inhalation and possibly through the conjunctiva. The virus replicates initially in the upper respiratory tract and cervical lymph nodes before disseminating through the bloodstream to various organs. The incubation period ranges from 12 to 25 days. The primary clinical feature is swelling of the parotid glands, which usually appears unilateral initially but can become bilateral. This swelling is accompanied by fever, local pain, and tenderness. The skin over the gland is not warm or erythematous. Parotitis is non-suppurative and typically resolves within about a week. However, the virus can also affect extraparotid sites, sometimes without parotitis.

**Complications-**

* **Epididymo-orchitis**- About one-third of postpubertal males with mumps develop epididymo-orchitis, characterized by acute swelling and pain in the testis, along with fever and chills. Orchitis is usually unilateral but bilateral cases can lead to testicular atrophy, sterility, or low sperm counts.
* **CNS involvement-** Approximately 60% of mumps cases involve the central nervous system, evident by pleocytosis in the CSF, although only around 10% exhibit symptoms of meningitis. Mumps is responsible for 10-15% of cases of aseptic meningitis. Mumps meningitis and meningoencephalitis typically resolve without lasting effects, but deafness can occasionally occur. Mumps meningitis may occur even without parotitis, relying solely on laboratory evidence. The virus can be easily cultured from the CSF during early meningitis.
* **Others-** Less common complications include arthritis, oophoritis, nephritis, pancreatitis, thyroiditis, and myocarditis. These complications highlight the systemic nature of mumps infection beyond its primary presentation in the parotid glands.

**Laboratory diagnosis:** Laboratory confirmation of mumps is generally unnecessary in typical cases but becomes crucial in atypical presentations or when systemic involvement like meningitis is the primary manifestation. Mumps virus can be isolated from saliva (within 4-5 days of symptom onset), urine (up to two weeks), or cerebrospinal fluid (CSF, 8-9 days after onset).

**1. Virus isolation**-

* **Cell culture-** Specimens should be inoculated promptly as the virus is unstable. Monkey kidney cell cultures, human amnion, or HeLa cells are suitable. Virus growth is detected by hemadsorption and identified using hemadsorption inhibition with specific antiserum. Cytopathic changes are not always reliable. Isolation typically takes 1-2 weeks, but immunofluorescence testing of infected cell cultures can yield results as early as 2-3 days.
* **Virus inculation-** Inoculation into chick embryos via the amniotic route and testing amniotic fluid after 5-6 days can also confirm virus presence through hemagglutination inhibition with specific antisera, though this method is less sensitive than cell cultures.

**2. Direct antigen detection**- Early diagnosis is aided by direct antigen detection using immunofluorescence assays (IFA).

**3. Serology**- Serological diagnosis involves demonstrating a rise in antibody titers in paired serum samples. Complement fixation (CF) and hemagglutination inhibition (HI) tests are commonly used, although cross-reactions with parainfluenza viruses can complicate interpretation. IgM-ELISA is particularly useful as it detects IgM antibodies, which indicate recent infection without interference from cross-reacting IgG antibodies. A positive CF test for antibodies against the S antigen in acute-phase serum is indicative of current infection.

**4. PCR**- Molecular diagnosis using reverse transcriptase PCR (RT-PCR) offers rapid and sensitive detection of mumps virus RNA, aiding in early diagnosis and surveillance.

**Prophylaxis:** Mumps challenges in containment due to prolonged shedding and high subclinical infection rates. Risk groups are passively immunized with mumps immunoglobulin. Active immunization with MMR vaccine (Jeryl Lynn strain) recommended at 12-18 months, effective but doesn't prevent reinfection. Two doses reduce mumps cases by 95% in the USA; second dose recommended at 6 or 12-13 years to maintain immunity.

**Respiratory syncytial virus**

Respiratory syncytial virus (RSV) is classified under the Pneumovirus genus due to unique surface features, nucleocapsid size, molecular characteristics of N and P proteins, absence of hemagglutinin and neuraminidase functions, and genetic arrangement. RSV is categorized into two subgroups, A and B, differentiated by the G protein antigen. RSV was initially discovered in 1956 in chimpanzees showing symptoms of coryza, and it was initially referred to as the 'chimpanzee coryza agent' (CCA).

**Multiplication**: Upon entering host cells and shedding its envelope, the respiratory syncytial virus (RSV) genome undergoes replication, generating 10 mRNA species and a complementary RNA (cRNA). These mRNA species direct the synthesis of viral proteins, while the cRNA serves as a template for the production of new viral RNA. Within 10 to 24 hours post-infection, viral proteins appear on the cell surface, and new virions bud out, incorporating host cell membrane components into their envelope.

**Pathogenesis**: RSV typically initiates infections in the upper respiratory tract, infecting ciliated mucosal epithelial cells. It can spread both through extracellular means and by cell-to-cell fusion, forming syncytia. This method of spread evades humoral antibodies that do not penetrate cells, allowing the virus to persist in respiratory secretions for up to 3 weeks. Clinical manifestations include bronchiolitis and pneumonia in infants, croup and tracheobronchitis in young children, and similar respiratory illnesses in the elderly. Additionally, secondary infections may lead to conjunctivitis, otitis media, and skin rashes.

**Immune response:** Nonspecific defenses such as secretory substances in mucosal secretions contribute to resistance against RSV. Specific immunity involves both cell-mediated and antibody responses, though reinfections can occur shortly after recovery due to incomplete protection. Immunity is primarily mediated by antibodies targeting the F and G proteins, with neutralizing antibodies on mucosal surfaces playing a crucial role in preventing reinfection.

**Epidemiology**: RSV is globally distributed and causes seasonal epidemics primarily affecting infants and young children, with outbreaks typically occurring in winter months. Both RSV subgroups A and B circulate during these epidemics, infecting approximately half of susceptible infants annually. Reinfection rates are high in childhood but decrease in adulthood. Transmission occurs through direct contact and airborne droplets, often introduced into households by schoolchildren. In institutional settings, mildly symptomatic adults can also transmit the virus. Age, exposure opportunity, and socioeconomic factors influence infection outcomes.

**Clinical presentation**: Respiratory syncytial virus (RSV) infections vary widely in severity, ranging from mild upper respiratory symptoms to severe lower respiratory illnesses like bronchiolitis and pneumonia, especially in infants and young children. RSV is a leading cause of serious lower respiratory tract infections in this age group and is also associated with otitis media, either through direct infection of the middle ear or by increasing susceptibility to bacterial infections. In older children and adults, symptoms typically resemble those of a common cold. In elderly individuals, RSV can once again cause significant lower respiratory tract infections. The most severe morbidity and mortality occur in infants younger than 6 months, premature infants with underlying lung or heart conditions, and immunocompromised children. These groups are at highest risk for severe complications from RSV infections.

**Laboratory diagnosis:** Nasopharyngeal swabs or nasal washings are recommended.

* **Virus isolation-** Clinical samples should be promptly inoculated into cell cultures such as HeLa or HEp-2 to avoid virus degradation. RSV in cell cultures causes distinctive giant cell and syncytial formation, though cytopathic effects may take up to 10 days to appear. Immunofluorescence tests can detect viral growth earlier. Rapid diagnosis can be achieved by immunofluorescence testing of nasopharyngeal swab smears.
* **Serology-** Diagnosis relies on detecting increasing antibody titers in paired serum samples using ELISA, complement fixation (CF), neutralization assays, or immunofluorescence tests.
* **PCR-** Molecular diagnosis using reverse transcriptase PCR (RT-PCR) is highly sensitive and provides rapid results.

**Treatment and prophylaxis:** RSV-enriched polyclonal immunoglobulin (RSVIG) shows promise, administered monthly in high doses to maintain high-titer neutralizing antibodies, effectively reducing the severity and incidence of RSV illness in high-risk children. Ribavirin, the sole approved antiviral for RSV in the USA since 1986, is controversial in terms of safety and efficacy. Continuous aerosol ribavirin administration in hospitalized patients has shown benefits by shortening illness duration and reducing virus shedding. Vaccine development has been challenging, with formalinized vaccines abandoned due to causing more severe illness upon subsequent exposure.

**Parainfluenza**

Human parainfluenza viruses are categorized into four types within the Paramyxoviridae family, specifically belonging to the genera Respirovirus (parainfluenza types 1 and 3) and Rubulavirus (parainfluenza types 2, 4A, and 4B).

* **Parainfluenza virus type 1-** The Sendai virus, first identified in Japan in 1952, was initially found in mice, causing asymptomatic infections. It was later recognized globally in human sera, leading to the identification of a similar virus in children with acute respiratory infections in 1958, designated as HA-2. Both viruses are now classified under parainfluenza virus type 1, with Sendai representing the murine strain and HA-2 the human strain.
* **Parainfluenza virus type 2-** It was isolated in 1955 from children with croup, also known as the "croup-associated" (CA) virus. It exhibits syncytial cytopathic effects in monkey kidney cell cultures and shares similarities with simian viruses infecting monkeys.
* **Parainfluenza virus type 3**- Discovered in 1958 from children with respiratory infections, was initially termed hemadsorption virus type 1 (HA-1). A related virus, SF-4, causes respiratory disease in cattle known as "shipping fever."
* **Parainfluenza virus type 4-** It is isolated in 1960 from children with mild respiratory infections, has two subtypes, A and B.

**Replication:** Parainfluenza viruses enter host cells by binding hemagglutinin to neuraminic acid receptors on the cell surface, followed by fusion mediated by glycopeptides F1 and F2. Their single-stranded negative-sense RNA genome serves as a template for transcription by virion transcriptase, yielding positive-sense messenger RNA. These direct viral protein synthesis and are subsequently copied into negative-sense RNA for incorporation into new virions. Viral glycoproteins accumulate in the cell membrane for envelopment, and assembly completes with budding of the nucleocapsid through the membrane studded with glycoproteins.

**Pathogenesis**: Parainfluenza viruses typically cause localized infections in the respiratory tract without systemic spread, though viremia can occur. Immunity develops after primary infection but does not prevent reinfection, providing partial protection against disease. Initial infection targets ciliated epithelial cells in the nose and throat, possibly extending to sinuses, middle ear, and occasionally lower respiratory tract. Progeny viruses spread via extracellular and intracellular routes, with shedding in respiratory secretions lasting 3 to 16 days post-primary infection and 1 to 4 days post-reinfection. Pathologically, inflammation in mucous membranes' superficial layers is prominent.

**Immune response:** Nonspecific defenses like interferon aid against parainfluenza viruses. After infection, short-lived IgA antibodies provide limited protection; reinfection can occur. Maternal antibodies affect disease severity but don't prevent infection completely.

**Epidemiology**: Parainfluenza viruses are ubiquitous, with type 3 infections common in infants under one year. Types 1 and 2 affect preschool children, with type 3 being more endemic and types 1 and 2 causing periodic epidemics. Initial infections are typically more severe than reinfections, which are common. Infected children shed the virus for about a week, primarily through respiratory secretions or contaminated hands. Nosocomial spread is also observed. Currently, no vaccine is available.

**Clinical presentation:** Parainfluenza viruses cause a spectrum of illnesses ranging from mild upper respiratory infections in adults to severe diseases such as croup (types 1 and 2), bronchitis, bronchiolitis, and pneumonia (type 3). Type 4 typically causes minor respiratory illnesses and occasionally parotitis. Unlike mumps, parainfluenza viruses primarily affect the respiratory tract without systemic involvement.

**Laboratory diagnosis:** Diagnosis involves collecting throat and nasal swabs for virus isolation in monkey kidney cell cultures. Typing is performed using immunofluorescence, hemadsorption inhibition, or hemagglutination inhibition methods due to antigenic cross-reactions. Serological tests detect rising antibody titers in paired sera, while molecular diagnosis via reverse transcriptase PCR is increasingly utilized for its sensitivity and rapidity.

**Prophylaxis:** Cross-infection between parainfluenza virus types 1 and 3 is common in hospitals and day care centers, preventable by strict isolation and rapid diagnostic methods. Active immunization against parainfluenza viruses is currently unavailable; experimental killed vaccines are ineffective. A live attenuated bovine parainfluenza virus type 3 vaccine shows promise in safety and efficacy for infants and children. Passive prophylaxis with human immunoglobulin is not recommended due to potential interference with active serum antibody response. Ribavirin, while potentially effective, carries significant toxicities.

**The Newcastle disease virus**

The Newcastle disease virus (NDV), also known as avian paramyxovirus type 1, is a significant pathogen affecting poultry, often leading to severe outbreaks of pneumoencephalitis or 'avian influenza' with high mortality rates. In India, it is referred to as the Ranikhet virus. Control strategies primarily involve vaccination programs and the culling of infected birds. Human infections with NDV are limited to mild conjunctivitis, typically observed in poultry workers and individuals in direct contact with infected birds.

**Henipavirus**

In the 1990s, the discovery of the Henipavirus genus introduced two zoonotic paramyxoviruses associated with outbreaks in Australia and Malaysia, where fruit bats serve as natural hosts.

**1. Nipah virus-** Nipah virus, a member of the Henipavirus genus, emerged during an outbreak in Malaysia in 1998-1999. Nipah virus is naturally hosted by fruit bats (***Pteropus spp*.)**, specifically species of the Pteropus genus. These bats are asymptomatic carriers of the virus and can shed it in their saliva, urine, and excreta.

**Transmission:**

* **Direct contact**- Transmission to humans occurs primarily through direct contact with infected bats, their excreta, or consumption of fruits partially eaten by infected bats.
* **Intermediate hosts**- During the Malaysian outbreak, pigs served as intermediate hosts. Bats contaminated pigs through their excreta, and the virus spread from pigs to humans through close contact.

**Clinical presentation**:

* **Severe encephalitis**- Infection with Nipah virus can cause severe encephalitis (inflammation of the brain), characterized by fever, headache, drowsiness, disorientation, and confusion.
* **Respiratory symptoms**- Some patients also experience respiratory symptoms such as cough, sore throat, and difficulty breathing.

**Epidemiology**: Outbreaks of Nipah virus have shown significant mortality rates ranging from 40% to 75%. Survivors of Nipah virus infection may suffer from long-term neurological effects, including persistent convulsions and personality changes. The outbreaks are described below-

* **Malaysia and Singapore (1998-1999)**- The initial outbreak affected pig farmers in Malaysia and subsequently spread to Singapore, resulting in several fatalities.
* **Bangladesh and India**- Subsequent outbreaks in Bangladesh and India have primarily affected individuals in close contact with infected bats or contaminated fruits.
* **Siliguri, West Bengal (2001)-** The first outbreak of Nipah virus in India occurred in Siliguri, West Bengal, in 2001. The outbreak affected both humans and animals, with a significant number of fatalities reported.
* **Nadia district, West Bengal (2007)-** The second outbreak occurred in Nadia district, West Bengal, in 2007. This outbreak also resulted in fatalities and led to extensive public health measures to control the spread of the virus.
* **Kozhikode and Malappuram districts, Kerala (2018)-** The third and most recent outbreak of Nipah virus in India was reported in Kozhikode and Malappuram districts of Kerala on May 19, 2018. This outbreak resulted in several deaths and prompted a coordinated response from both state and central government agencies.
* **Ernakulam district, Kerala (2019)-** A small outbreak of Nipah virus was reported in Ernakulam district of Kerala in 2019. Prompt public health measures helped contain the spread of the virus, and no fatalities were reported during this outbreak.

**Treatment and prevention**: There is no specific antiviral treatment for Nipah virus infection. Supportive care and intensive care for severe cases are crucial. Prevention involves avoiding direct contact with bats and their excreta, practicing strict hygiene, and refraining from consuming fruits that may have been contaminated by bats.

**2. Hendra virus**

Initially identified in Australia, it primarily affected horses and led to fatalities in humans as well. Both viruses are categorized as Biosafety level 4 agents due to their high mortality rates.

**Human metapneumovirus**

Human metapneumovirus, identified in 2001 through molecular techniques, primarily affects the respiratory system. It was initially discovered during investigations into respiratory illnesses resembling RSV infections in children. This virus also poses risks to adults with lymphomas or leukemias, as well as the elderly.

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**Bunyaviridae**

Globally, emerging viral infections pose significant public health challenges, with Bunyaviridae, a vast family encompassing over 350 arthropod-borne viruses, being particularly noteworthy. These viruses cause a spectrum of diseases ranging from mild febrile illnesses to severe conditions such as encephalitis, hemorrhagic fever, and acute respiratory illness. In India, several Bunyaviruses like Ingwavuma virus, Thottapalayam virus, and others have been serologically identified, highlighting the need for deeper molecular understanding to elucidate their natural disease profiles.

**Structure:** Named after Bunyamwera, Uganda, where it was first discovered in mosquitoes, Bunyaviridae particles are spherical, 80-120 nm in diameter, and characterized by a genome consisting of three segmented negative-stranded RNA (S, M, and L segments).

**Classification:** The Bunyaviridae family (Table 55) is classified into five genera based on antigenic, genetic, and ecological characteristics:

1. **Orthobunyavirus**- Includes viruses primarily transmitted by mosquitoes and midges, causing diseases like California encephalitis and La Crosse virus encephalitis in humans.
2. **Hantavirus**- Known for causing hemorrhagic fevers with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) in humans, transmitted mainly by rodents.
3. **Nairovirus**- Includes tick-borne viruses causing diseases such as Crimean-Congo hemorrhagic fever (CCHF) in humans, transmitted by ticks.
4. **Phlebovirus**- Spread by sandflies and associated with diseases like Rift Valley fever and sandfly fever in humans.
5. **Tospovirus**- Pathogenic to plants, transmitted by thrips, and not known to cause disease in humans.

**Replication:** Replication of bunyaviruses occurs in the cytoplasm of the host cell. Most bunyaviruses have an antisense RNA genome, while some phleboviruses have an ambisense small RNA segment, meaning one part is complementary to viral sense and the other part is in viral sense. Due to the segmented nature of their RNA, genetic reassortment can occur during infection, leading to genetic diversity. Virus particles bud into the Golgi apparatus and are released from the cell through plasma membrane disruption and fusion of intracellular vacuoles with the plasma membrane

**Table 55: Different bunyaviruses**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genus/group | Virus | Vector/Reservoir | Geographic origin | Characteristics |
| Orthobunyavirus | Umbre virus (UMBV) | Culex vishnui mosquitoes | Maharashtra, India | Orthobunyavirus  Part of Turlock serogroup; cross-reactivity with Barmah Forest virus; distinct lineages in G2 gene |
| Simbu group | Kaikalur virus | Culex tritaeniorhynchus | Andhra Pradesh, India | Related to Shuni and Aino viruses; broader distribution pattern |
| Thimiri Virus (1962) | Birds, midges | Tamil Nadu, India | Distinct complex within Simbu group; found in South India, Egypt, Australia |
| Sathuperi virus (SATV) | Culex vishnui mosquitoes | Tamil Nadu, India | Found in cattle and biting midges in Japan; similar to Schmallenberg virus |
| Ingwavuma virus (INGV) | Birds, mosquitoes | South Africa, India | Found across Africa and Asia |
| Nairovirus | Ganjam virus (GANV) | Ticks, mosquitoes, sheep | Odisha, Karnataka, Tamil Nadu, India | Related to Nairobi sheep disease virus; causes febrile illness in humans, antibodies in livestock. Causes febrile illness in humans and livestock |
| Crimean-Congo Haemorrhagic Fever (CCHF) virus | Ixodid ticks, livestock | India | Causes severe haemorrhagic fever; transstadial and transovarial transmission. High fatality rate in humans |
| Phlebovirus | Malsoor virus (MV) | Rousettus bats | Maharashtra, India | First Phlebovirus from these bats; infects various mammalian and insect cell lines; heterogeneous morphology. No human infections reported. |
| Rift Valley Fever Virus (RVFV) | Aedes and Culex genera | Sub-Saharan Africa, | In humans, RVF can cause a range of symptoms, from mild flu-like illness to severe hemorrhagic fever and even death. It can also affect livestock, causing high mortality rates in young animals and abortions in pregnant animals. |
| Sandfly fever | Sandflies (Phlebotomus spp.) typically active during dusk and dawn. | Mediterranean, Middle East, and parts of Central Asia. | Sandfly fever typically presents as a self-limiting febrile illness characterized by sudden onset of fever, headache, body aches, and malaise. Severe complications are rare, and most cases resolve on their own without specific treatment. |
| Hantavirus | Hantavirus | Rodents |  | Causes haemorrhagic fever with renal syndrome and cardiopulmonary syndrome. Potential infections suggested in India. |
| Thottapalayam virus (TPMV) | Asian house shrew | Tamil Nadu, India | First Hantavirus isolated in cell culture; distinct from rodent-borne Hantaviruses |
| Arbovirus | Kaisodi virus | Bird ectoparasites | Karnataka, India | Cross-reactivity with Malayan viruses; confirmed as Arbovirus. No human disease reported |

**Pathogenesis:** Except for hantaviruses, bunyaviruses replicate in arthropods. Initially, the virus infects the gut of the vector, and after a few days or weeks, it appears in the saliva, rendering the arthropod infective for life without causing illness. When the vector bites a human or other vertebrate host, the infective saliva enters the small capillaries or lymphatics. The primary site of replication in humans is unknown, potentially occurring in the vascular endothelium, skin, or regional lymph nodes. After an incubation period of a few days, the host develops viremia. Typically, the infection is asymptomatic, but it can cause fever and more severe symptoms characteristic of the virus. Viremia subsides with the appearance of humoral antibodies, and the host usually recovers unless a specific target organ is affected. Damage to specific organs, such as the liver in Rift Valley fever, the brain in La Crosse encephalitis, and the lungs in hantavirus pulmonary syndrome, results in distinct diseases. While most damage is thought to result from direct viral invasion rather than a host-mediated immune response, the pathogenesis of bunyaviruses in vertebrate hosts is not well-studied. Infections like hemorrhagic fever with renal syndrome and Rift Valley fever show organ damage even after humoral antibodies form, suggesting possible host reactions.

**Immune response:** The initial response to bunyavirus infection involves the production of interferon, to which bunyaviruses are sensitive, potentially playing a protective role. Humoral antibody is also protective, as its appearance, either naturally or through passive administration, correlates with the disappearance of the virus from the blood. The role of cell-mediated immunity has not been fully evaluated.

**Epidemiology:** Except for hantaviruses, the life cycle of bunyaviruses involves alternating replication in arthropods (such as mosquitoes, ticks, Culicoides midges, or phlebotomine sand flies) and vertebrate hosts, usually small mammals. Humans can become ill from infection but rarely contribute to the natural cycle by infecting biting arthropods, making them typically dead-end hosts. Some bunyaviruses, particularly those in the California and phlebotomus fever groups, can be transmitted transovarially in arthropods, allowing them to overwinter in eggs and infect humans when adult arthropods emerge in late spring or early summer. Bunyaviruses have a global presence, but each serotype has a limited geographic distribution due to reliance on specific arthropod species to maintain their natural cycle. In contrast, hantaviruses are maintained in rodent reservoirs and are not arthropod-borne, with human transmission believed to occur through inhalation of virus particles excreted in rodent urine and other body fluids.

**Diagnosis:**

* **Virus isolation**- The virus can be isolated from blood or postmortem from brain, liver, and other organs during the viremic phase, typically not beyond the third day of fever. Virus propagation is done in baby mice, mosquitoes, or vertebrate/invertebrate tissue cultures.
* **RNA detection**- RNA is detected by RT-PCR, with RNA found in lung tissue from hantavirus pulmonary syndrome cases postmortem.
* **Serologic tests**- These include enzyme-linked immunosorbent assay (ELISA), complement fixation, fluorescent antibody, neutralization, and hemagglutination inhibition tests. ELISA, complement fixation, and fluorescent antibody tests are often group-reactive, while neutralization and hemagglutination inhibition tests are type-specific. IgM assessments are useful for early diagnosis.
* **Virus identification**- Once isolated, the virus is identified using reference immune serum with the same tests.

**Treatment and prophylaxis:** Bunyavirus transmission is controlled by managing the arthropod vector or vertebrate reservoir. Personal measures, such as protective clothing, repellents, bed nets, and house screens, are effective but often overlooked Rodenticides are used during outbreaks of hemorrhagic fever with renal syndrome.

Rift Valley fever vaccines are used in Africa to immunize sheep and cattle, thereby preventing transmission to humans. Human vaccines for Crimean-Congo hemorrhagic fever are used in the former Soviet Union and Bulgaria, and in Asia to prevent hemorrhagic fever with renal syndrome. Treating hemorrhagic fever with renal syndrome cases with ribavirin during the first week of illness has proven effective.

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https://doi.org/10.4103/ijmr.IJMR\_1871\_15

**Bornaviridae**

Bornaviruses are enveloped viruses with a single, negative-strand RNA genome, belonging to the family Bornaviridae in the order Mononegavirales. These viruses establish persistent infections with minimal cell damage. It is first identified in horses in central Germany in the 18th century, notably Borna disease virus (BoDV1).

**Structure:** Bornavirions are enveloped and approximately 100-130 nm in diameter, with a presumed helical nucleocapsid structure (Figure 92).

**Genome organization:** Bornaviruses possess single, negative-strand RNA genomes approximately 9 kilobases in size. They encode six proteins (N, P, X, M, G, and L), following a gene order reminiscent of other Mononegavirales members. However, their unique transcription strategy involves splicing to generate certain mRNAs, facilitated by the virus's nuclear transcription location. Similar to other Mononegavirales, it is likely that a single promoter near the 3' end of the genome initiates transcription. The genome also contains four transcription termination sites and three reinitiation sites that have been identified.

G

P

ssRNA

Nucleocapsid protein

L

M

**Figure 92: Structure of bornavirus**

**Replication:**

* **Attachment-** The bornavirus glycoprotein (GP) is implicated in receptor attachment and subsequent fusion, particularly through its hydrophobic domain, GP-C. While specific host receptors remain unidentified, they are known to be expressed on neurons.
* **Transcription-** Transcription of bornavirus involves the nucleocapsid (N, P, L proteins, and RNA) trafficking to the nucleus. The process requires N, P, and L proteins, with L likely responsible for capping and polyadenylating viral mRNAs. Cellular splicing machinery also processes some transcripts, with the most abundant encoding the N protein.
* **Protein synthesis-** Bornavirus proteins (N, P, M, G, L, and X) are synthesized, including two forms of N (p40 and p38) and the catalytic subunit L. These proteins localize to the nucleus, reflecting their roles in RNA synthesis and viral replication.
* **Genome replication-** Genome replication, a nuclear event, depends on N, P, and L proteins. Truncations at genome ends in persistently infected cells suggest a regulatory role in downregulating viral replication and transcription. RNA cleavage facilitates viral RNA persistence without activating innate immune responses.
* **Viral assembly and release**- Bornavirus nucleocapsids containing N, RNA, and associated proteins (P, L, M) form in the nucleus. They are exported for virion assembly and maturation, likely involving transmission via both extracellular virions and nucleocapsids between cells, especially evident in cultured cells.

**Clinical presentation and epidemiology:**

**a. Borna disease in mammals-** Borna disease is a rare neurological disorder primarily affecting horses and sheep, with outbreaks most common in central Germany. The natural hosts for Borna disease virus 1 (BoDV-1) are mammals, and transmission occurs via contact with infected urine or feces, potentially through the olfactory route. The virus spreads within the host by axonal transmission, infecting neurons in a noncytopathic but persistent manner, leading to immunopathologic damage. BoDV-1 has been linked to neurological diseases in various mammals, including cats, where it causes a fatal condition known as staggering disease.

**b. Bornaviruses in birds-** In the early 1970s, veterinarians observed a mysterious disease in captive parrots, characterized by severe digestive and neurological problems, including proventriculus enlargement. The disease, known as proventricular dilatation disease (PDD), identified to be caused by bornavirus. Avian Bornaviruses are reported worldwide, with higher incidences in areas with a high density of pet bird populations.

**c. Bornaviruses in human-** Human infection with Bornaviruses is rare, but there have been isolated cases suggesting a potential zoonotic (animal to human) transmission. Infections have been linked to severe cases of encephalitis (inflammation of the brain). Most human cases have been reported in Europe, where animal Bornavirus infections are more prevalent. However, the overall distribution is not well-mapped due to the rarity of the cases.

**Laboratory diagnosis:** Diagnosing Borna disease virus (BDV) infections involves-

1. **Serological tests-**

* **ELISA-** Detects antibodies against BDV in serum samples.
* **Immunofluorescence Assay (IFA)-** Uses fluorescence-labeled antibodies to detect BDV antigens in infected cells.

1. **Molecular methods-** Detects BDV RNA in blood, cerebrospinal fluid, or tissue samples. Real-time PCR (qPCR) is often used for its sensitivity and quantification capabilities.
2. **Virus isolation-** Involves culturing the virus from clinical samples in susceptible cell lines. The presence of BDV can be confirmed by observing cytopathic effects or using immunostaining techniques.
3. **Histological examination-** Observes characteristic histopathological changes in tissues, such as encephalitis in brain tissues.
4. **Whole genome sequencing-** Provides detailed information on the viral genome, helping in the identification and study of different strains and mutations.
5. **Western blotting-** Detects specific BDV proteins in samples, confirming the presence of the virus or the host's immune response to it.

**Treatment and prophylaxis:** Currently, there is no approved vaccine for BDV in humans. Infected animals should be isolated to prevent the spread of the virus. Amantadine and riavirin can be effective. Corticosteroids may be used to reduce CNS inflammation.

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https://doi.org/10.1016/B978-0-12-803109-4.00022-2

**Rhabdoviridae**

Rhabdoviruses (Table 56) are bullet-shaped, enveloped viruses with a single-stranded RNA genome. The Rhabdoviridae family, named from the Greek word "rhabdos" meaning rod, includes viruses that infect a wide range of hosts including mammals, reptiles, birds, fish, insects, and plants. Some rhabdoviruses can replicate in both vertebrates and arthropods.

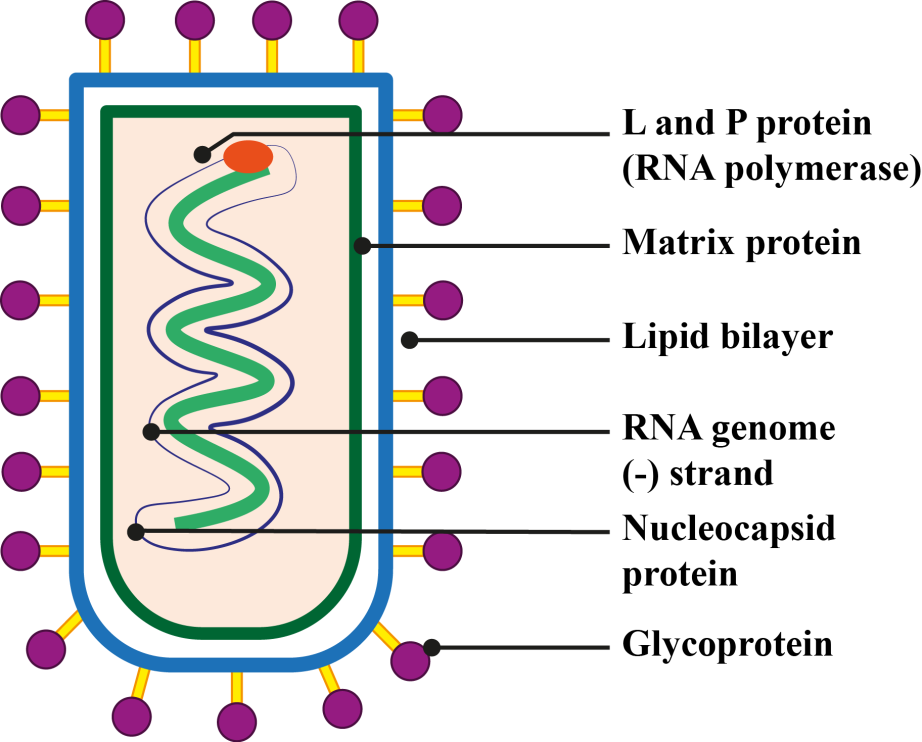
**Table 56: Classification of rhabdoviruses causing human infections**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Genus | Notable viruses` | Host | Clinical illness | Geographical distribution |
| Lyssavirus | Rabies virus (RABV) | Mammals (including humans), bats | Rabies: fatal encephalitis, hydrophobia | Worldwide, especially in low- and middle-income countries |
| EBLV-1 | European serotine bat (Eptesicus serotinus) | Rabies-like illness | Europe |
| EBLV-2 | *Myotis dasycneme*, *Myotis daubentonii* | Rabies-like illness | Europe |
| Duvenhage virus (DUVV) | Bats, humans | Encephalitis | Southern and Eastern Africa |
| Mokola virus (MOKV) | Cats, dogs, shrews, rodents | Rabies-like illness | Southern Africa |
| Australian bat lyssavirus (ABLV) | Fruit bats (*Pteropus spp*.), insectivorous bats | Rabies-like illness | Australia |
| Irkut virus (IRKV) | Murina leucogaster bats | Rabies-like illness | China, Russia |
| Ledantevirus | Le Dantec virus (LEDV) | Unknown, possibly bats | Febrile illness, rash, delirium, parkinsonism | West Africa, possible wider African distribution |
| Kumasi virus (KURV) | Fruit bat (*Eidolon helvum*) | Mild febrile illness | Ghana |
| Nkolbisson virus | Mosquitoes, humans | Febrile illness | Cameroon |
| Tibrovirus | Bas-Congo virus (BASV) | Unknown | Hemorrhagic fever | Democratic Republic of the Congo (DRC) |
| Ekpoma viruses (EKV-1, EKV-2) | Unknown | Mild febrile illness | Nigeria, detected in a Chinese worker from Angola |
| Vesiculovirus | Vesicular stomatitis virus (VSV) | Cattle, horses, pigs, humans | Vesicular stomatitis: flu-like symptoms, vesicles | Americas (primarily in the USA, Central, and South America) |
| Chandipura virus (CHPV) | Humans, sandflies | Encephalitis-like illness, febrile illness | India, Africa |

**Rabies virus**

The term "rabies" comes from the Latin word for "madness," which itself is derived from the Sanskrit word "rabhas," meaning "violence." The Greek term for rabies, "lyssa," also means madness and gives the genus its name, Lyssavirus. Pasteur developed a rabies vaccine by inoculating animals with CNS material and successfully immunized a boy named Joseph Meister in 1885. In 1903, Adelchi Negri identified pathognomonic inclusions in neurons of rabies-infected animals, aiding in diagnosis.

**Structure:** Rabies virus is a single-strand RNA virus with a non-segmented, negative-sense (antisense) genome consisting of 11,932 nucleotides. The virus encodes five proteins: nucleocapsid protein (N), matrix protein (M), phosphoprotein (P), glycoprotein (G), and RNA-dependent RNA polymerase or large polymerase protein (L). Virus particles are bullet-shaped with an encapsidated RNA forming a ribonucleoprotein (RNP) core, which consists of helical genomic RNA associated with the N, P, and L proteins. The RNP serves as a functional template for transcription and replication. The G and M proteins are associated with the lipid bilayer envelope that surrounds the RNP core. The M protein lines the viral envelope and forms an inner leaflet between the envelope and RNP core, playing a critical role in viral assembly, budding, and modulation of viral genome replication and transcription. The G protein produces spike-like projections on the surface of the viral envelope, playing an important role in viral entry and membrane fusion, and in viral release (Figure 93).



**Figure 93: Structure of rabies**

**Replication:** The virus is sensitive to various chemicals and can be inactivated by heat, light, and specific substances. It dies at room temperature but can survive for weeks if stabilized with 50% glycerol or stored at 4°C. For long-term preservation, it can be stored at -70°C or lyophilized. When using dry ice, the virus must be sealed in vials to prevent inactivation by CO2.

**Antigenic properties:** The genus Lyssavirus includes rabies virus and several antigenically and genetically related viruses such as Lagos bat, Mokola, Duvenhage viruses, and two suggested subtypes of European bat lyssaviruses. Cross-protection studies indicate that animals immunized with traditional rabies vaccines may not be fully protected against these other lyssaviruses.

Rabies viruses can be categorized as either:

* **Fixed viruses**- Adapted by passage in animals or cell culture.
* **Street viruses**- Wild type.

The use of monoclonal antibodies and genetic sequencing helps differentiate street rabies viruses, identifying viral variants originating in major host reservoirs worldwide and suggesting likely sources of human exposure when a definitive animal bite history is absent.

**The M protein-** It ines the viral envelope, forming an inner leaflet between the envelope and RNP core. It plays a crucial role in viral assembly, budding, and modulation of viral genome replication and transcription.

**The G protein-**

* It forms spike-like projections on the viral envelope and is vital for viral entry and membrane fusion and release.
* Binds to acetylcholine receptors in neural tissues.
* Induces hemagglutination inhibiting (HI) antibodies, and exhibits hemagglutinating activity.
* Stimulates neutralizing antibodies and cytotoxic T cell immunity,
* Heat-inactivated, acts as a serotype-specific antigen, and purified glycoprotein can serve as a safe and effective subunit vaccine.

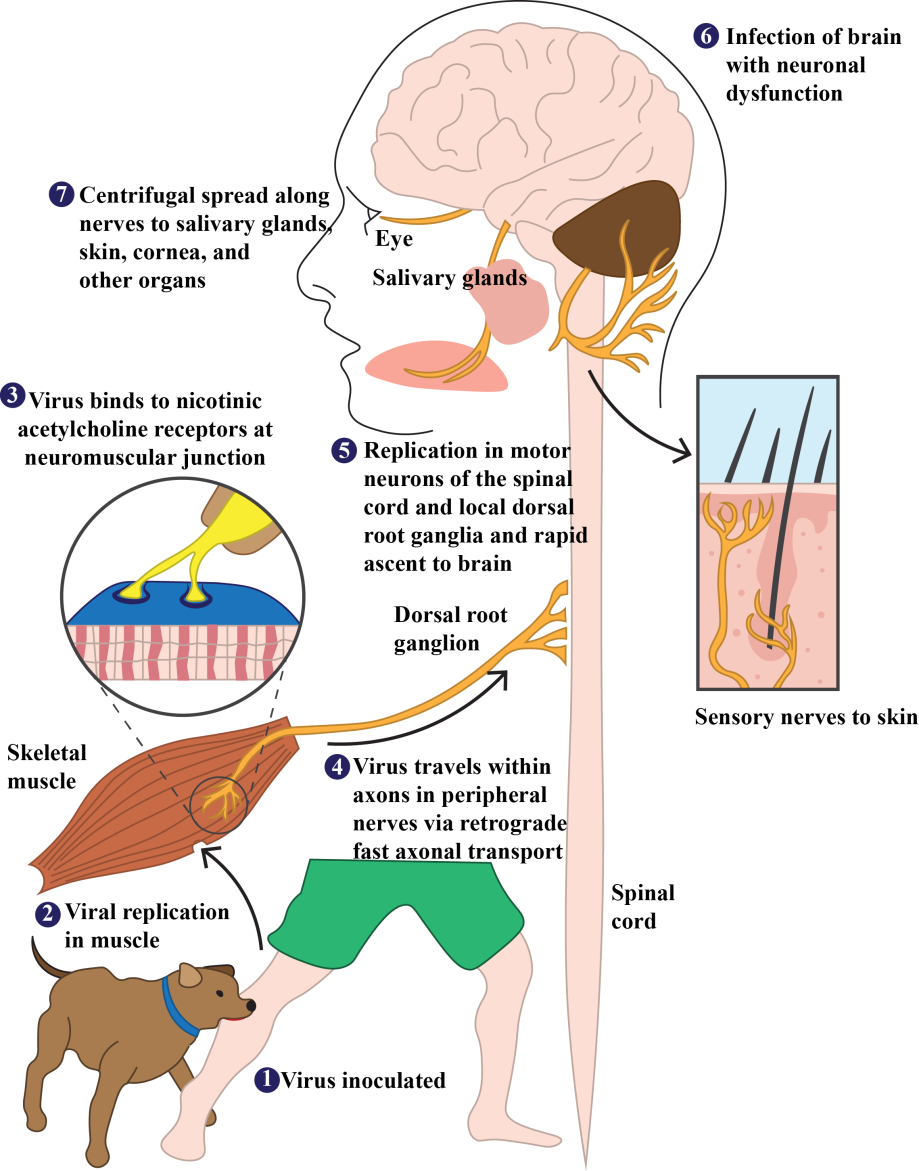
**Nucleoprotein-**

* The nucleocapsid protein induces complement-fixing antibodies but not protective.
* It is a group-specific antigen with cross-reactions with some rabies-related viruses.
* Antiserum prepared against the nucleocapsid antigen is used in diagnostic immunofluorescence tests.

**Other identified antigen-** Other identified antigens include-two membrane proteins, glycolipid, and RNA-dependent RNA polymerase.

**Replication:** The replication process of rabies virus resembles that of other negative-stranded RNA viruses. Initially, the virus attaches to host cell membranes through the G protein, enters the cytoplasm either through fusion with the membrane or pinocytosis, and sheds its coat to release the ribonucleoprotein (RNP) core. The core uses the virion-associated RNA-dependent RNA polymerase to start transcribing five separate messenger RNAs, each coding for a specific viral protein. Once these proteins are synthesized, the genomic RNA undergoes replication, producing full-length positive-stranded RNA, which serves as a template for generating new negative-stranded RNA progeny.

**Pathogenesis:** The rabies virus is typically transmitted through bites from infected mammals, though mucosal exposure or contaminated skin lesions can also rarely lead to transmission. Aerosol transmission in laboratories or caves with bats, and iatrogenic transmission via tissue or organ transplants, have been documented but are uncommon. Once transmitted, the virus remains near the entry site during an incubation period lasting weeks to months. It binds to nicotinic acetylcholine receptors at neuromuscular junctions (Figure 94), facilitating its concentration and subsequent uptake into peripheral motor neurons. From there, it spreads through axons of peripheral nerves via retrograde fast axonal transport, initially infecting local dorsal root ganglia and causing neurologic symptoms such as paresthesias and pain. The virus further disseminates throughout the central nervous system and beyond via centrifugal spread along neuroanatomic connections, reaching organs like the salivary glands and even the heart and gastrointestinal tract.



**Figure 94: Pathogenesis of rabies**

**Immune response:** Different factors such as the species of the host animal, the specific viral variant, the concentration of the inoculum, the site and severity of exposure, and the immune status of the host are linked to varying susceptibility and incubation periods in rabies infection. Protective immunity is primarily mediated by virus-neutralizing antibodies like IgG. Cytokines such as interferon, produced in response to rabies virus infection or vaccination, have been shown to potentially prevent disease onset if administered early. However, clinical trials using high doses of alpha interferon did not prevent mortality in all cases.

**Host range:**

**a. Animals-** All mammals are susceptible to rabies infection, though susceptibility varies between species. Cattle, cats, and foxes are highly susceptible, while skunks, opossums, and fowl are relatively resistant. Humans and dogs have an intermediate susceptibility, with pups being more vulnerable than adult dogs. Experimental infection can be induced in any laboratory animal, but mice are preferred due to their ability to be infected by any route and their development of encephalitis and death within 5-30 days after intracerebral inoculation.

**b. Street virus-** The rabies virus isolated from natural human or animal infections is termed the street virus. It can cause fatal encephalitis in laboratory animals following inoculation by any route, with a variable incubation period of about 1-12 weeks. Intracytoplasmic inclusion bodies, known as Negri bodies, can be found in the brains of animals that die from street virus infection. These bodies, composed of a finely fibrillar matrix and rabies virus particles, are most abundant in the cerebellum and hippocampus.

**c. Fixed virus-** After several serial intracerebral passages in rabbits, the rabies virus becomes known as the fixed virus. This variant is more neurotropic but much less infective by other routes. It causes fatal encephalitis after a short and fixed incubation period of 6-7 days following intracerebral inoculation. Negri bodies are usually not present in the brains of animals that die from fixed virus infection. The fixed virus is used for vaccine production.

**d. Chick embryos-** Rabies virus can be grown in chick embryos, typically through yolk sac inoculation. Serial propagation in chick embryos has led to the development of attenuated vaccine strains such as Flury and Kelev. Strains adapted to duck eggs, which yield high amounts of the virus, have been used to prepare inactivated vaccines.

**e. Tissue culture-** Rabies virus can grow in several primary and continuous cell cultures, such as chick embryo fibroblast, and porcine or hamster kidney cells. However, cytopathic effects are not appasrent, and the yield of the virus is low. Fixed virus strains adapted for growth in human diploid cell, chick embryo, and Vero cell cultures are used for vaccine production.

**Epidemiology:** Human rabies transmission through direct person-to-person contact has not been recorded, though the virus is present in the saliva of patients. Examining or nursing rabies patients poses no danger with suitable precautions. Rabies is present in terrestrial animals worldwide, except in Australasia, Antarctica, and certain islands like Britain. There are two epidemiological types: urban rabies, transmitted by domestic animals such as dogs and cats, and sylvatic rabies, involving wild animals like jackals, wolves, foxes, mongooses, skunks, and bats. Most human rabies cases follow dog bites, but in endemic areas, almost any animal can transmit rabies. In India, antirabies treatment is considered following bites from any animal except rats. In the USA, where domestic rabies is controlled, most infections are from wild animal bites.

**Reservoir-** The primary natural reservoir of rabies is in mustelids and viverrids, achieving latency within these populations. Foxes, wolves, and jackals acquire the infection from these reservoirs, spreading it to dogs and other domestic animals. Rabies also circulates in bats with distinct cycles involving vampire bats that transmit a fatal paralytic disease to cattle and humans, and insectivorous and frugivorous bats with different infection patterns. Bat rabies is largely confined to the Americas, with a few strains isolated from bats in Europe of unknown significance.

**Rabies in other countries-** Worldwide, at least 55,000 human rabies cases occur annually, primarily due to endemic dog rabies in developing countries, especially in Asia and Africa. Economic and cultural factors hinder effective dog rabies control. In contrast, in North America, most human rabies cases are caused by bat rabies variants. Many cases lack a history of bat bite or contact, likely because small bat bites often go unnoticed, preventing timely prophylactic treatment. In the U.S. and Canada, the rabies virus variant in silver-haired and tricolored bats is responsible for most human cases. Brazilian free-tail bats and vampire bats also transmit rabies in the Americas.

**Rabies in India-** Rabies is endemic in India, causing over 30,000 human deaths annually, with more than 700,000 receiving antirabies vaccine. Control measures include registration, licensing, and vaccination of pets, and the destruction of stray animals. With an estimated dog population of over 16 million, the problem is immense. Effective control also requires managing wild vectors like jackals and foxes and reservoir species like mustelids and viverrids. Control measures include vaccinating 70% of the dog population. However, India's large stray dog population, estimated at 25 million, poses a significant challenge, requiring community and political commitment.

Non-governmental organizations and global health companies play crucial roles in India's efforts to reduce rabies deaths and enhance public health through mass dog vaccination (implemented by National Rabies Control Program –NRCP and Animal Husbandry Departments) and education campaigns. India is actively involved in global initiatives, such as those led by WHO and OIE, aiming to eliminate dog-mediated rabies by 2030. Humane Society International (HSI) contributes significantly through advocacy, community engagement, and veterinary training. The National Centre for Disease Control (NCDC) coordinates national efforts to prevent and manage communicable diseases, including rabies.

**Clinical presentation:** Clinical rabies typically manifests after an incubation period ranging from 20 to 90 days but can be as short as a few days or extend over a year in rare cases. During this period, the rabies virus likely remains near the site of entry.

## A. Human rabies progression-

### I. Prodrome- The initial stage is characterized by non-specific symptoms such as fever, headache, malaise, fatigue, and loss of appetite. Early neurological symptoms like pain or tingling at the site of virus entry may occur. Patients also experience emotional disturbances including anxiety, agitation, irritability, and insomnia. Rarely, symptoms like increased libido or priapism may manifest.

### II. Acute encephalitic phase- Encephalitic rabies involves episodes of hyperexcitability, autonomic dysfunction (e.g., hypersalivation, cardiac arrhythmias), and characteristic hydrophobia, where attempts to drink water trigger painful spasms. This symptom is due to selective infection of brainstem neurons. Encephalitic rabies progresses to coma and widespread organ failure.

**III. Paralytic rabies-** Paralytic rabies begins with weakness in the bitten area and spreads to quadriparesis and facial weakness, with less sensory involvement and sphincter dysfunction. Unlike encephalitic rabies, hydrophobia is absent, and the disease course may last longer.

### IV. Coma and death- Some patients progress into a comatose state during the later stages of the disease. Death typically occurs within 1-6 days after symptom onset, often due to respiratory failure during convulsions.

## B. Rabies in dogs-

### I. Prodrome in dogs- Rabies in dogs starts with behavioral changes such as restlessness, snapping at imaginary objects, and licking or gnawing at the site of the bite. This stage lasts 2-3 days.

### II. Furious rabies- The more common form is characterized by aggressive behavior, with dogs becoming indiscriminately violent, drooling saliva, and experiencing paralysis and convulsions before death.

### III. Dumb rabies- In this paralytic form, dogs appear huddled and unable to feed. Though they may not exhibit aggression, attempts to handle or feed them can be dangerous. This form is equally infectious as the furious type, with about 60% of rabid dogs shedding the virus in saliva. Death typically occurs within 3-5 days after symptoms appear.

**Laboratory diagnosis:** Diagnosing rabies is generally straightforward in non-immunized patients exhibiting hydrophobia after a bite from a known rabid animal. However, in regions with high vaccination rates among domestic animals, the diagnosis becomes more complex. During the incubation period, diagnostic tests are ineffective, and any potential exposure to rabies should prompt immediate prophylactic treatment.

**A. In human-** Specimens for rabies testing include corneal smears, skin biopsies from the face or neck, saliva collected antemortem, and brain tissue postmortem.

**I. Direct microscopy-**

* **Antemortem:** Rabies virus antigens are detected using immunofluorescence with monoclonal antibodies tagged with fluorescein isothiocyanate. Immunoperoxidase staining can also identify antigens in tissues. A skin biopsy from the nape of the neck, above the hairline, is recommended, as the rabies virus tends to localize in hair follicles. This test reveals the presence of rabies virus in about 50% of samples during the first week of symptoms, with increasing detection rates thereafter.
* **Postmortem:** Diagnosis is made by identifying Negri bodies in the brain, although they may be absent in about 20% of cases.

**II. Virus isolation-**

* **Animal inoculation:** Virus isolation from brain tissue, CSF, saliva, and urine is done by intracerebral inoculation in mice. This method is most effective early in the disease. The inoculated mice are monitored for illness, and their brains are examined for Negri bodies or by immunofluorescence.
* **Tissue culture:** A more rapid and sensitive method involves isolating the virus in tissue culture cell lines, with identification by immunofluorescence as early as 2-4 days post-inoculation. The virus identity can be confirmed by a neutralization test with specific anti-rabies antibodies.

**III. Molecular methods-** RT-PCR is another diagnostic procedure used to detect rabies virus RNA in CSF, saliva, or tissue samples. This method is highly specific and can also determine the geographic and host species origin of the rabies virus. RT-PCR is particularly useful for analyzing decomposed brain material, where older techniques often fail.

### IV. Serological testing- The rapid fluorescent focus inhibition test (RFFIT) measures neutralizing anti-rabies antibodies. Detectable antibodies appear by day 6 of clinical illness in some untreated patients, with 50% showing antibodies by day 8 and nearly all by day 15. High antibody levels in CSF are diagnostically valuable, even in patients who have received post-exposure treatment (PET). Specific oligoclonal bands in CSF, not found in serum, can confirm CNS infection.

### V. Imaging-

* **Computed tomography (CT)**: CT scans are usually normal early in the disease course unless hypoxia occurs. Prolonged critical care support may reveal cerebral swelling.
* **Magnetic resonance imaging (MRI)**: MRIs may show increased T2 signal areas in the hippocampi, hypothalamus, brainstem, and occasionally other regions. Late-stage gadolinium enhancement indicates blood-brain barrier breakdown. In paralytic rabies, MRI of the spinal cord and nerve roots can be useful. No imaging finding is pathognomonic for rabies.

**B. In animals-** Diagnosing rabies in animals, particularly those that have bitten humans, is crucial for assessing the risk of infection and guiding post-exposure treatment. The entire carcass, head, or brain tissue, especially the hippocampus and cerebellum, should be sent to the laboratory for testing.

**I. Demonstration of negri bodies-** Brain impression smears are stained using Seller's technique and examined for intracytoplasmic inclusion bodies. This is common in laboratories without immunofluorescence or biological test facilities.

**II. Immunofluorescence-** This method is more sensitive than visualizing Negri bodies, provides immediate results, and can be used to examine salivary glands to determine if the animal was shedding the virus.

**III. Virus isolation**: This involves inoculating mice with samples from the brain, CSF, saliva, or urine and monitoring for signs of rabies.

**Treatment:** Until recently, rabies was considered invariably fatal, and treatment efforts were limited to sedation. However, it has been shown that complete recovery from rabies is possible with intensive supportive care and management of complications, even though no specific antirabies agent is available. (Table 57)

**Table 57: Risk categories and post exposure prophylaxis of rabies**

|  |  |  |
| --- | --- | --- |
| Category of exposure | Types of exposure | Recommended prophylaxis |
| I | Licks on healthy unbroken skin;contact of skin with secretions of rabid animals or human cases | Exposed area should be washed with soap and water followed by application of antiseptics; no prophylaxis needed if prior history is available |
| II | Licks on fresh cuts; uncovered skin nibbling; minor wounds less than 5; scratches without oozing of blood | Wound management; rabies vaccination |
| III | Multiple transdermal bites; Oozing of blood on neck, head, face, palm, fingers; bites from wild animals | Wound management; rabies vaccination; administration of immunoglobulin |

**Prophylaxis:**

**A. Elimination of rabies in animal vectors-** Canine rabies **is** endemic in many countries, especially in Asia and Africa. Mass vaccination of dogs is crucial for its elimination. Oral vaccination of wildlife vectors (foxes, raccoons, coyotes) has effectively controlled wildlife rabies in various regions. No effective control method for rabies in insectivorous bats.

**B. Pre-exposure vaccination-** Cell culture vaccines mostly given to laboratory staff working with rabies virus, veterinary doctors etc., Booster doses are administered periodically based on serum antibody titers. The vaccination can be administered intramuscularly or intradermally for pre-exposure. Intradermal vaccination is cost-effective as it reduces vaccine volume and allows multiple individuals to be vaccinated from a single vial.

**C. Post-exposure prevention-** To prevent rabies after exposure, it is crucial to follow NRCP guidelines in India. Key factors include the type of exposure, the species involved, the animal's health status, and the ability to observe the animal for 10 days (for dogs, cats, and ferrets) or test its brain tissues. If a dog, cat, or ferret stays healthy for 10 days post-exposure, rabies transmission is unlikely. Prophylaxis involves wound cleansing, administering four doses of a modern cell culture rabies vaccine (Table 58), and injecting human rabies immune globulin into and around the wound, with any remaining amount given intramuscularly. Needs to be considered in the following situations:

* bites by all warm-blooded animals
* Exposure to wild animal bites (category III risk)
* Contact with secretions of rabid patients

**D. Wound management-** Immediate thorough flushing and washing (upto 15 mins) of all wounds with soap and water and application of povidone iodine or antiseptic having virucidal activity.

**Table 58: Details of rabies vaccines**

|  |  |  |
| --- | --- | --- |
| Vaccines | Type | Details |
| Neural | Pasteur's cord vaccine | Earliest vaccine using dried rabbit spinal cord. |
| Infected brain vaccines | Various preparations; generally poor immunogens, may contain infectious agents, and are encephalitogenic. |
| Semple vaccine | Developed in 1911 in India; 5% suspension of sheep brain with fixed virus inactivated with phenol. |
| Beta Propiolactone (BPL) | Modified Semple vaccine; uses BPL for inactivation, considered more antigenic with smaller doses needed. |
| Suckling mouse brain vaccine | Uses infant brain tissue; occasional neurological reactions, impractical in large quantities. |
| Non-neural | Duck Egg vaccine | Initially used but discontinued due to poor immunogenicity; a more potent version was later supplanted by tissue culture vaccines. |
|  | Tissue Culture vaccines | Human diploid cell (HDC) vaccine developed by Koprowsky, Wiktor, and Plotkin; Fixed RABV grown in a human diploid cell line, initially WI-38, later switched to MRC-5, resulting in the licensing of HDCV in the mid-1970s.; highly antigenic and safe but costly. Includes primary cell culture and Vero cell vaccines; In India, the HDC, purified chick embryo cell (PCEC), and purified Vero cell (PVC) vaccines are all available and effective for immunization. |
| **Subunit vaccines** | Glycoprotein subunit of the virus surface; acts as the protective antigen; cloned to produce recombinant vaccines; still under investigation. |

**E. Passive immunization:**

* **Equine Rabies Immune Globulin (ERIG):** Safer than crude serum, though not completely risk-free.
* **Human Rabies Immune Globulin (HRIG):** Preferred due to safety but limited availability and higher cost. Administered before or simultaneously with the first vaccine dose (Table 59), never afterward.

**Table 59: Rabies vaccine dosages**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Types of prophylaxis | Route of administration | Dose of vaccine | Dosage | Site of injection |
| Pre-exposure | Intra-dermal | 0.1 ml/dose | Day 0,7 and booster on either day 21 or 28 | Adults-Deltoid muscle  Infants & children- Anterolateral thigh |
| Intra-muscular | 1 entire vaccine vial | Day 0,7 and booster on either day 21 or 28 |
| Post-exposure | Intra-dermal | 0.1 ml/dose | Day 0,3,7 and 28 |
| Intra-muscular | 1 entire vaccine vial | Day 0,3,7,14 and 28 |
| Re-exposure | Intra-dermal | 0.1 ml/dose | Day 0 and 3 |
| Intra-muscular | 1 entire vaccine | Day 0 and 3 |

**Vesicular Stomatitis Virus (VSV)**

VSV, a member of the Rhabdoviridae family and genus Vesiculovirus, affects hoofed livestock in North and South America. While infections are generally mild, outbreaks can cause significant economic impacts due to blister-like lesions on the mouth, tongue, teats, and hooves of affected animals. VSV is one of several vesicular diseases indistinguishable from foot and mouth disease virus (FMDV) without specific testing. Consequently, animals are quarantined and veterinary health officials are alerted until FMDV can be ruled out, causing considerable anxiety among livestock producers. VSV outbreaks are sporadic and rare in the United States. The virus primarily affects horses, cattle, swine, deer, and humans, and is typically introduced into herds by biting insects, though it can also spread through contact among infected animals. Human infections generally result from exposure to infected animals and present as a general febrile illness.

**Chandipura Virus (CHPV)**

CHPV, endemic to India, has been isolated from various insects and animals, including humans. First identified in 1965 from the blood of two patients, CHPV is associated with neurological disease, particularly in children under 15 years of age. Although there is some debate about the exact disease burden in India, outbreaks continue to be reported with improved diagnostic methods. Case fatality rates range from 55% to 77%, and there is evidence suggesting increasing virulence of CHPV. Research on CHPV neuropathogenesis is ongoing using a suckling mouse model.

**Other Rhabdoviruses**

Other notable rhabdoviruses include bovine ephemeral fever virus (BEFV), which affects cattle and buffalo in tropical and subtropical regions and is transmitted by insects. Rhabdoviruses also cause several fish diseases, such as spring viremia of carp virus, infectious hematopoietic necrosis virus, and viral hemorrhagic septicemia virus, leading to significant mortality and morbidity in both wild and cultured fish populations.

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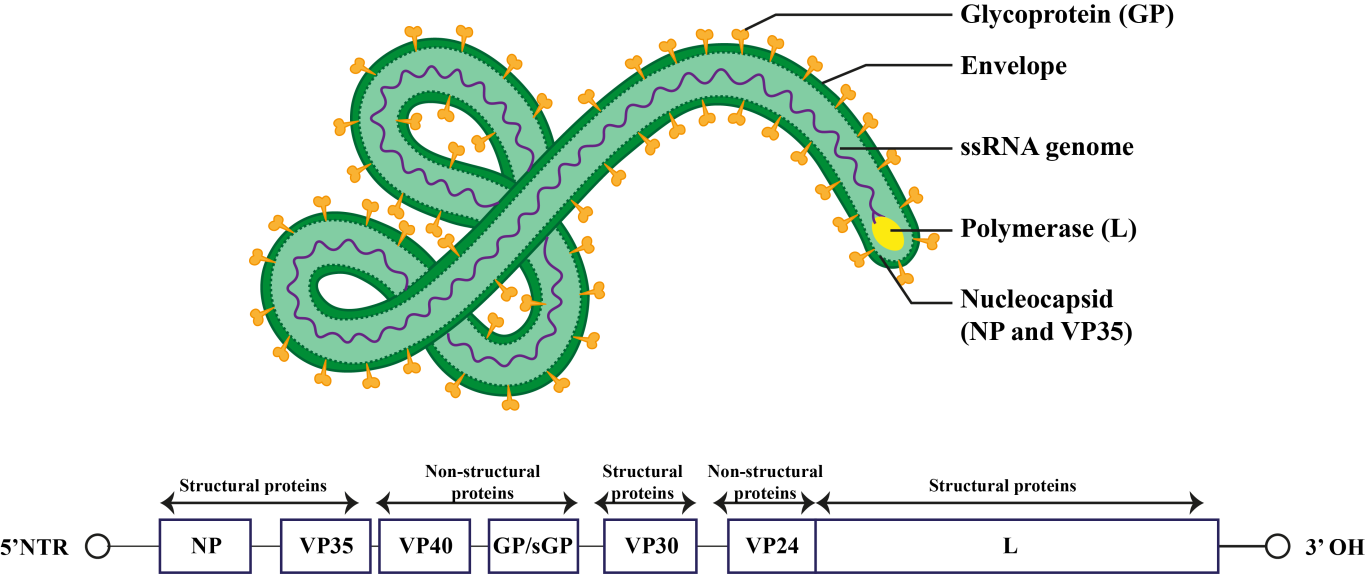
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**Filoviridae**

The family Filoviridae comprises two genera: Marburgvirus and Ebolavirus. Filoviruses cause some of the most severe hemorrhagic fevers, with high mortality rates. Within the Filoviridae family, Marburgvirus has a single species, Lake Victoria marburgvirus (MARV), the prototype being strain Musoke. The Ebolavirus genus includes four recognized species: Ivory Coast ebolavirus (ICEBOV), Reston ebolavirus (REBOV), Sudan ebolavirus (SEBOV), and Zaire ebolavirus (ZEBOV). The prototype for the genus is the ZEBOV strain Mayinga. Marburg virus was first identified in 1967 in Europe, linked to African green monkeys, with subsequent sporadic outbreaks in Africa. Ebola virus was first identified in 1976 in Zaire and Sudan, with multiple subtypes causing periodic outbreaks in Africa.

**Structure:** Filoviruses are pleomorphic, long filamentous (14 μm) or in shorter U, 6, or circular shapes (Figure 95). All filovirus particles have a uniform diameter of 80 nm. MARV virions from culture fluids are consistently shorter (795–828 nm) than other filoviruses. The virions are surrounded by a lipid envelope derived from the host cell plasma membrane, featuring surface glycoprotein (GP) spikes (7 nm). This lipid envelope protects the helical nucleocapsid.

**Genome organization:** The filovirus genome (Figure 95) consists of a non-segmented, single-stranded, negative-sense linear RNA molecule, contributing to 1.1% of the total virion mass. The genome is approximately 19 kb in size. The genome contains seven genes arranged linearly (Table 60): nucleoprotein (NP), virion structural proteins (VP35, VP40, VP30,VP24), glycoprotein (GP), and the RNA-dependent RNA polymerase gene (L).



**Figure 95: Structure and genome organization of Filoviruses**

These genes are flanked by noncoding sequences at the 3′ and 5′ ends, which contain signals for replication and encapsidation. The genes possess highly conserved transcriptional start and stop signals, and some genes overlap, particularly in the genus Ebolavirus. The 3′ (leader) and 5′ (trailer) ends of the genome are conserved and highly complementary, playing crucial roles as promoters in RNA transcription and replication. While both Marburg and Ebola viruses produce the surface glycoprotein GP1,2, their production mechanisms differ. Marburgvirus produces its glycoprotein through direct transcription of viral RNA, whereas Ebola virus uses transcriptional editing by the RNA-dependent RNA polymerase.

**Table 60: Viral proteins of Filoviruses**

|  |  |  |
| --- | --- | --- |
| Category | Protein | Function |
| Ribonucleoprotein Complex (RNP) | NP (Nucleoprotein) | Encapsidation, found in the ribonucleocapsid complex. |
| VP30 | Filovirus-specific nucleocapsid protein; minor phosphoprotein involved in transcription and replication |
| VP35 | Phosphoprotein involved in transcription and replication, type 1 interferon (IFN) antagonist, blocks virus-induced transcription and IFN-beta promoter activation |
| L | RNA-dependent RNA polymerase, essential for transcription and replication |
| Envelope-Associated Proteins | GP1,2 (Glycoprotein) | Major surface spike protein, receptor binding, and membrane fusion, processed into GP1 and GP2 by proprotein convertase furin, forms trimeric heterodimers on viral surface |
| VP40 | Major matrix protein, induces filamentous particle formation, interacts with GP1,2 in morphogenesis |
| VP24 | Minor matrix protein, involved in virus assembly and budding, interacts with NP and VP35, speculated to play a role in species adaptation |
| Nonstructural Proteins | pre-sGP | Soluble precursor protein, cleaved into sGP and delta peptide, sGP detected in blood of EBOV-infected patients, may interact with neutrophils via CD16b. |

**Resistance:** Marburg and Ebola viruses remain stable at room temperature (approximately 20°C) but are largely inactivated within 30 minutes at 60°C. These viruses can also be destroyed by ultraviolet and gamma irradiation, lipid solvents, beta-propiolactone, and common disinfectants such as commercial hypochlorite and phenolic solutions.

**Replication:** Filovirus transcription and replication occur in the cytoplasm of the infected cell and are mediated by a single virus-encoded polymerase. The negative-sense RNA genome is transcribed into monocystronic, polyadenylated subgenomic RNA, which is then translated into seven structural proteins. In the case of Ebola viruses, a single glycosylated nonstructural protein is produced through RNA editing and/or frameshifting (-1) at a specific site in the glycoprotein open reading frame. Replication proceeds via a full-length, positive-sense antigenome that serves as a template for negative-sense progeny genomes. Viral particles mature at the plasma membrane.

**Pathogenesis:** Human infection typically occurs via contact with infected skin or secretions, entering through skin lesions or mucous membranes. Monocytes/macrophages and dendritic cells are primary sites of viral replication, especially in lymph nodes, liver, and spleen, leading to viremia. Infected macrophages release proinflammatory cytokines, contributing to tissue inflammation and potentially disrupting host responses. Endothelial cells, directly affected by virus and indirectly by inflammatory mediators, show altered barrier functions and increased adhesion molecule expression, causing vascular disturbances and hemorrhage, impacting disease severity.

**Immune response:** Recovery mechanisms from filovirus infections remain unclear in both humans and experimental animals. Robust cell-mediated immunity is hypothesized as the likely route to recovery, although definitive proof is lacking. In fatal cases, high viremia typically coincides with a lack of detectable immune response, the reasons for which are unknown. The heavily glycosylated filovirus glycoprotein likely influences its interactions with the immune system. Additionally, filovirus glycoproteins contain motifs similar to retroviral p15E, known for immunosuppressive effects in experimental models.

**Epidemiology:** The geographic distribution of filoviruses (Table 61) is primarily determined by actual virus isolates rather than serosurveys, which often use unreliable tests for Ebola antibodies. Ebola virus species such as Zaire, Sudan, and Cote d’Ivoire originate from African tropical forests or nearby savannas, typically emerging during rainy seasons. The Reston Ebola species, found only in a Philippines export facility, suggests an Asian origin, possibly derived from infected macaques captured in Philippine forests.

Understanding the natural hosts and maintenance strategies of filoviruses remains a significant challenge, with limited efforts directed towards identifying reservoirs in tropical forests. Experimental evidence suggests potential chronic infection in mammalian reservoirs like bats, though conclusive findings are lacking. Human infections typically occur in rural African areas through contact with infected primates, and transmission among humans primarily occurs via direct contact with bodily fluids during caregiving or burial practices.

Efforts to control filovirus infections in nonhuman primates through quarantine have been crucial, especially in settings where reuse of medical equipment has facilitated nosocomial spread. Although airborne transmission has been suggested, it remains unproven in natural settings. Large outbreaks are often contained through public health measures like quarantine and barrier nursing once the disease's transmissibility and severity are recognized.

**Table 61: Subtypes of filoviruses**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Virus | Subtypes | First isolated | Geographical distribution | Clinical presesntation |
| Ebola | Zaire (EBOV) | 1976 in Yambuku, Democratic republic of Congo (DRC) | Central Africa (DRC, Gabon) | Severe hemorrhagic fever, high fatality rate |
| Sudan (SUDV) | 1976 in Nzara, Sudan | East Africa (Sudan, Uganda) | Hemorrhagic fever, lower fatality rate than EBOV |
| Reston (RESTV) | 1989 in Reston, Virginia, USA (from monkeys) | Asia (Philippines) | Asymptomatic in humans, pathogenic in monkeys |
| Ivory Coast (TAFV) | 1994 in Tai Forest, Ivory Coast | West Africa (Ivory Coast) | Hemorrhagic fever, similar to EBOV |
| Bundibugyo (BDBV) | 2007 in Bundibugyo, Uganda | East Africa (Uganda, DRC) | Hemorrhagic fever, intermediate fatality rate |
| Marburg | Marburg (MARV) | 1967 in Marburg and Frankfurt, Germany (from monkeys imported from Uganda) | East Africa (Uganda, Kenya), Southern Africa (Angola) | Severe hemorrhagic fever, high fatality rate |
| Ravn (RAVV) | 1987 in Kenya | East Africa (Kenya) | Severe hemorrhagic fever, similar to MARV |

**Pathology and course of infection in humans:**

**a. Human infection and case fatality rates**- Filovirus infections in humans have the highest case-fatality rates among viral hemorrhagic fevers, ranging from 30% to 90%. These infections often involve significant hepatic damage and hemorrhagic manifestations.

**b. Pathological changes-** Marburg and Ebola virus infections share similar histopathologic changes with other hemorrhagic fevers. Focal necrosis mainly affects the liver, spleen, kidney, and gonads. Electron microscopy, immunohistochemistry, and in situ hybridization studies link this necrosis to the presence of numerous viral particles, antigens, and nucleic acids. Minimal inflammatory response in necrotic areas suggests that necrosis is due to the virus's cytopathic effect and ischemia. Early infection affects extrahepatic sites, as indicated by a high AST-ALT ratio and normal bilirubin levels. Increased endothelial permeability leads to extensive visceral effusions, pulmonary interstitial edema, and renal dysfunction, which are critical aspects of the shock syndrome in filovirus infections.

**c. Hematological changes**- Severe thrombocytopenia is common, with remaining platelets unable to aggregate normally. There is also an early, profound lymphopenia followed by dramatic neutrophilia with a left shift.

**d. Characteristic histopathological features-**

* **Liver-** Widespread hepatocellular necrosis, Councilman bodies, microvesicular fatty change, and Kupffer cell hyperplasia are observed. Filovirus inclusions within hepatocytes are numerous, especially in Ebola infections, and consist of viral nucleocapsid aggregates.
* **Spleen and Lymph Nodes-** Extensive follicular necrosis and necrotic debris are present.
* **Lungs-** Typically exhibit interstitial edema, hemorrhage, and features of diffuse alveolar damage.
* **Heart-** Myocardial edema and focal necrosis are present but do not involve significant inflammatory infiltrates.

**Clinical presentation:** Filovirus infections in both humans and nonhuman primates, such as Marburg and Ebola viruses, cause severe hemorrhagic disease, ranking among the most serious viral hemorrhagic fevers (VHFs). Following an incubation period of 4–10 days, symptoms begin abruptly with fever, severe headache, malaise, myalgia, bradycardia, and conjunctivitis. Within 2–3 days, symptoms escalate to include pharyngitis, severe nausea, vomiting, hematemesis, melena, prostration, and obtundation.

Bleeding manifestations appear as petechiae, ecchymoses, uncontrolled bleeding from venipuncture sites, and postmortem evidence of visceral hemorrhagic effusions. Around day 5, a maculopapular rash often emerges, aiding in diagnosis. Death from shock typically occurs 6–9 days after symptom onset. In pregnant women, abortion is common, and infants born to mothers who succumb to the infection are usually fatally infected. Survivors typically develop a humoral antibody response around days 7 to 11, though some experience prolonged recovery with potential sequelae such as arthralgia and psychosocial disturbances.

**Laboratory diagnosis:** Acute sera, postmortem tissue specimens, and materials collected during ecological investigations may contain filoviruses and should be handled with maximum safety measures, including respirators, gloves, gowns, and BSL-4 laboratory safeguards.

**I. Risk factors-** Occupational and travel history are crucial for narrowing the diagnosis. Filovirus exposure risks include rural travel, jungle or cave exposure, contact with sick humans, and contact with sick or dead primates.

**II. Differential diagnosis-** Common causes of febrile illness in travelers that need to be ruled out include rickettsioses, malaria, typhoid fever, leptospirosis, borreliosis, septicemic plague, and dysentery.

**III. Early diagnosis-**

* Seek an etiologic diagnosis at the earliest stages of illness.
* Test for virus, viral antigen, and viral RNA in serum or blood during the acute phase of illness.

**IV. Direct virus detection-** Virus antigen detection can be done through electron microscopy of tissue culture supernatants, blood, or serum, along with scanning for cytopathic effects and immunofluorescent staining of infected cells.

**V. Antibody detection**-

* **Immunofluorescence assays**- These use acetone-fixed virus-infected cells inactivated by γ-radiation to detect antibodies, but they can produce false positives.
* **ELISA-** This method, which uses a mild detergent extract of infected Vero cells adsorbed to plastic plates, is more reliable for detecting filovirus antibodies. IgM-capture ELISA is positive in early convalescence, and rising IgG levels can further confirm the diagnosis.
* **Western Blot**- This technique has been standardized and evaluated for the diagnosis of filovirus infections.

**VI. Virus isolation-**

* **Cell lines**: Filoviruses can be isolated and propagated using the Vero cell line, particularly the E6 clone, as well as MA-104 and SW13 cell lines. Primary cell cultures, especially monocytes, macrophages, and endothelial cells, are also susceptible to filovirus infection. **Animal Models**: In some cases, primary isolation in guinea pigs (for Marburg virus) or suckling mice (for Ebola virus) may be necessary.

**VII. Molecular methods-** If antigen levels are low, reverse transcriptase polymerase chain reaction (RT-PCR) is used to detect viral RNA.

**Treatment:** There is no specific antiviral treatment for filovirus hemorrhagic fever (HF); care is mainly supportive, focusing on fluid and electrolyte balance, especially potassium levels. Antibiotics do not alter the disease's progression. Hyperimmune horse serum can protect baboons from Ebola virus if given within 4 hours of infection, but it has limited use in humans due to immunogenicity concerns. Heparin is recommended only for clear cases of DIC. Human interferon and convalescent plasma have shown limited effectiveness in past (Kikwit Ebola and Franfurt Marburg) outbreaks. Patient isolation and healthcare worker protection are essential, using strict barrier nursing techniques and HEPA-filtered respirators when possible. To prevent the introduction of filoviruses, quarantining imported wild-caught monkeys and professional handling of these animals is crucial measures.

**Prevention:** Efforts to develop an Ebola virus vaccine began after the first outbreak in 1976 with formalin-fixed or heat-inactivated virus (subtypes Sudan and Zaire), but these early attempts provided inconsistent protection and insufficient immunity in animal models. The most successful development so far is the adenovirus-vectored GP gene vaccine, which has protected guinea pigs and macaques against lethal challenges and has reached phase I human trials. Despite progress, developing a successful human Ebola vaccine remains challenging, particularly in identifying a target population for efficacy testing. Potential targets include nonhuman primates in endemic areas, medical personnel in Africa, and global research workers. The need for a vaccine is emphasized by increased travel, bioterrorism threats, social unrest in endemic regions, and rising natural epidemics.

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**Slow viruses**

The term "slow virus" was introduced around 25 years ago by Sigurdsson (1954) to describe a disease in Icelandic sheep. Although the term is not entirely satisfactory, it is widely used to refer to viruses that persist long-term in the host and cause chronic disease after a lengthy incubation period. Human diseases associated with slow viruses often involve degenerative changes in the central nervous system, but the full range of conditions caused by these viruses is not yet known. There is potential significance for certain cancers and possibly multiple sclerosis, as they may be caused by slow viruses.

**Classification:** Slow virus diseases can be categorized into three main groups:

1. **Group A**- This group includes slowly progressive infections observed in sheep, caused by nononcogenic retroviruses known as lentiviruses. The human immunodeficiency virus (HIV), which causes AIDS, is also classified within this group due to its similar characteristics as a slow virus disease.
2. **Group B**- Prion diseases of the central nervous system (CNS) comprise this group. These diseases include scrapie, mink encephalopathy, Kuru, and Creutzfeldt-Jakob disease, collectively known as subacute spongiform viral encephalopathies.
3. **Group C**- This group encompasses two distinct CNS diseases in humans: subacute sclerosing panencephalitis (SSPE) and progressive multifocal leukoencephalopathy (PML).

**I. Group A slow viruses**

**A. Visna**

Visnais a demyelinating disease affecting sheep, characterized by a gradual onset of weakness that progresses to paralysis over approximately two years, ultimately leading to death. Transmission occurs via intracerebral inoculation with an incubation period ranging from months to years. The virus replicates in sheep choroid plexus or testes cells, causing syncytia formation and cell culture destruction. Visna virus is RNA-containing with a lipid envelope, resembling myxoviruses. It exists in two particle forms and can be inactivated by various methods including chloroform and heat.

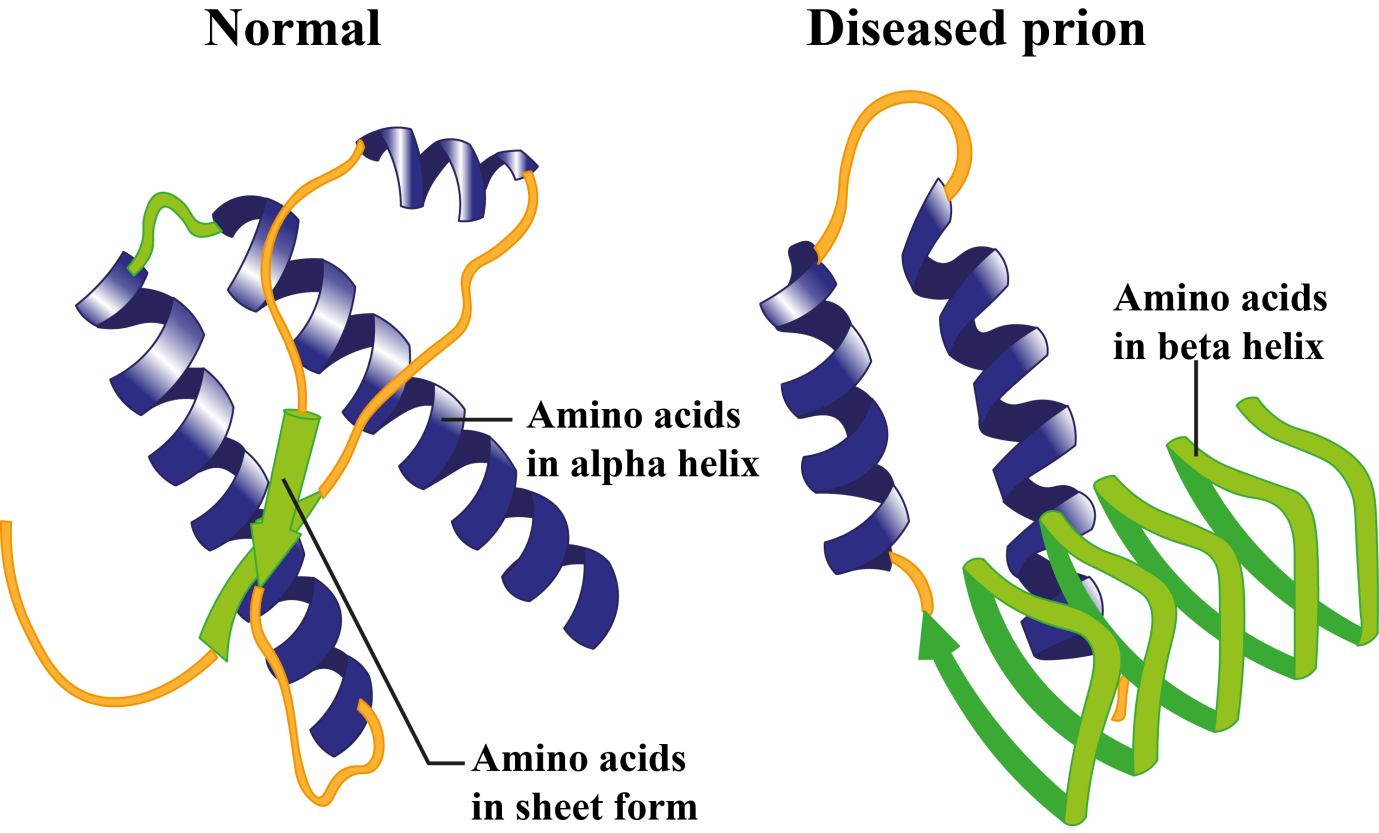
**B. Maedi**

It isalso known as progressive pneumonia, is a slowly advancing and fatal respiratory disease in sheep. It has an incubation period of 2-3 years and belongs to the lentivirus group.

**II. Group B slow viruses (Prion diseases)**

Prion diseases are caused by the misfolding of a host-encoded prion protein (PrP), where PrP exists in two forms: a normal cellular form (PrPC) and a pathogenic misfolded form (PrPSc). PrPC undergoes modification in the endoplasmic reticulum and gets anchored to cell membranes via a  glycosylphosphatidylinositol (GPI) anchor. PrPSc, derived from the same genetic sequence, differs in its secondary and tertiary structure, primarily rich in beta sheets compared to alpha helices in PrPC. This structural difference makes PrPSc highly resistant to degradation and contributes to its pathogenicity. The PRNP (Prion Protein) gene encodes the prion protein (PrP), which is involved in neuroprotection, cell signaling, adhesion, copper binding, development, and immune response. Mutations in the PRNP gene can lead to prion diseases, also known as transmissible spongiform encephalopathies (TSEs).

**Structure:** Normal PrPC is a single polypeptide chain with approximately 253 amino acids in humans. It includes a signal peptide at the N-terminus, which is cleaved off in the mature protein. In prion diseases, PrPC undergoes a conformational change to become PrPSc. This involves a significant increase in beta-sheet content at the expense of alpha-helices (Figue 96).



**Figure 96: Normal and disease prion protein structures**

**1. Prion diseases in humans-**

**A. Kuru**

Kuru, a transmissible spongiform encephalopathy akin to scrapie in sheep, affected the Fore linguistic group in New Guinea, causing high mortality. Carlton Gajdusek received the Nobel Prize in Medicine in 1976 for his research on Kuru.

Early studies noted distinct age and gender patterns: childhood cases were evenly split, while adult cases predominantly affected females. Investigation revealed kuru spread through ritual endocannibalism, where women and children consumed deceased relatives' tissues, sparing adult males. Prohibition of cannibalism led to a significant decline in kuru mortality over decades, confirming transmission and revealing a 7-year average incubation period.

Clinical stages include ambulant, sedentary, and terminal phases with symptoms like ataxia, tremors, and cognitive decline. Neuropathological features include spongiosis and kuru plaques. Genetic studies highlight PRNP codons 127 and 129's role, with heterozygosity offering resistance. Geographic independence suggests environmental factors influence prion diseases.

**B. Sporadic Creutzfeldt-Jakob Disease (sCJD/ sCJDMM2)**

Creutzfeldt-Jakob disease (CJD) is a rare transmissible spongiform encephalopathy affecting humans, primarily as sporadic cases with no clear links between affected individuals. Sporadic CJD (sCJD) comprises 85% of cases worldwide, with an annual incidence of 1-2 cases per million. It affects both sexes equally, typically appearing between ages 55 and 75, though cases occur outside this range.

Clinical symptoms include rapidly progressive dementia, cerebellar dysfunction causing muscle coordination issues, and neurological deficits like visual disturbances, speech impairments, and abnormal gait. Myoclonus, agitation, and depression are common, often leading to akinetic mutism where patients become unresponsive. Pathological features include spongiform changes and PrPSc deposits in the CNS, with amyloid plaques in 5-10% of cases.

Diagnosis relies on rapidly progressive dementia with specific neurological signs or characteristic EEG findings. Differential diagnosis from other neurodegenerative disorders is crucial. Imaging techniques like MRI and EEG detect brain abnormalities—MRI shows atrophy, and EEG reveals pseudo-periodic sharp wave complexes. Genetic insights highlight PRNP gene variations, especially M129V homozygosity affecting disease susceptibility. Other polymorphisms like E219K influence CJD risk. Different M129V genotypes and prion strain types correlate with varied clinical manifestations in CJD cases.

**C. Variant CJD**

Variant Creutzfeldt-Jakob disease (vCJD), identified in Britain from 1996, raised concerns about BSE transmission through contaminated beef. Initially affecting younger individuals (aged 16-39 years), vCJD's association with BSE prions led to significant public health fears and cattle culling. Psychiatric symptoms like agitation and depression precede neurological signs such as ataxia and cognitive decline. Rapid disease progression averages 18 months from onset to death, often due to secondary infections. Diagnostic markers include EEG abnormalities, elevated 14-3-3 protein in CSF, and MRI findings like 'pulvinar' signs. Pathologically, kuru-like amyloid plaques and spongiform lesions are prominent in the cerebellum and cerebrum. All vCJD cases are homozygous for the M129 PRNP gene polymorphism, influencing disease susceptibility.

### D. Iatrogenic Creutzfeldt-Jakob Disease (iCJD)

Iatrogenic Creutzfeldt-Jakob disease (iCJD), first identified in 1974, is associated with medical procedures contaminated by prions. Cases stem from cadaveric corneal transplants, neurosurgical instruments, cadaveric dura mater grafts, and pre-1987 pituitary-derived human growth hormone (hGH). Most hGH-related cases occurred in France, while dura mater graft cases were prevalent in Japan.

Between the late 1950s and 1985, around 30,000 children globally received pituitary-derived hGH, with an estimated iCJD incidence of about 1 case per 100 treated individuals due to PrPSc contamination. Recombinant hGH since 1987 and improved dura mater graft processing have minimized iCJD risks. hGH-related iCJD has long incubation periods (4.5 to over 25 years), affecting PRNP M129 homozygotes akin to kuru. Dura mater graft-related iCJD resembles sporadic CJD with shorter illness durations (average 18 months) and incubation periods (1.5 to 18 years.

**E.** **Familial or Genetic CJD (f/gCJD)**

Familial CJD (fCJD) is linked to dominantly inherited mutations in PRNP, including point mutations and repeat insertions or deletions within the protein. The term "genetic CJD (gCJD)" is increasingly used due to cases without a clear family history. gCJD comprises 5-15% of all CJD cases, with mutations like E200K, I210V, D178N, and V180I showing geographic clustering.

The clinical and pathological features of gCJD are influenced by codon 129 genotypes and PrPSc type, with mutations acting as susceptibility factors rather than direct causative agents. Various mutations lead to phenotypic variability, affecting aggregation propensity and PrP retention in secretory pathways.

**F.** **Gerstmann-Sträussler-Scheinker Syndrome (GSS)**

GSS, caused by autosomal dominant PRNP mutations, is a rare TSE with an annual incidence of about 1 in 100 million. Onset typically occurs between 30 and 60 years, with a disease duration of 3.5-9.5 years. Symptoms vary widely among affected families and include cerebellar ataxia, dementia, gait abnormalities, dysarthria, myoclonus, spastic paraparesis, and parkinsonian signs.

Recent observations include leg hyperreflexia in a patient with the P102L mutation. Neuropathologically, GSS is characterized by amyloid plaques, variable spongiform changes, neuronal loss, astrocytic microgliosis, and occasionally neurofibrillary tangles, notably in cases like Y218N. The M129V polymorphism influences disease presentation, particularly with mutations like P102L, which may exhibit psychiatric symptoms such as apathy and depression.

**G.** **Fatal Familial Insomnia (FFI)**

FFI, an autosomal dominantly inherited prion disease linked to PRNP mutation D178N and polymorphism M129V, has seen nearly 100 cases worldwide across multiple countries. It affects both genders equally, with onset typically between 20 to 72 years and an average age of 49 years. Disease duration ranges widely, from 8 to 72 months, with an average of 18.4 months.

Clinical symptoms include severe insomnia, myoclonus, ataxia, dysarthria, dysphagia, pyramidal signs, and autonomic hyperactivation, varying by genotype. Polysomnography is critical for diagnosis, revealing sleep disruptions, while PET scans show thalamic and cingulate cortex hypometabolism, more pronounced in 129 MV genotype cases. Neuropathological findings include neuronal loss, astrogliosis in thalamic nuclei, and distinct PrP deposits in the cerebellum and subiculum entorhinal region.

**H.** **Sporadic Fatal Insomnia (sFI)**

sFI, a sporadic form resembling FFI, emerged in 1999 without familial history or PRNP mutations. About 24 cases worldwide show 129 MM homozygosity and PrPSc type 2. Known as "sCJDMM2 thalamic," sFI differs clinically and pathologically from sCJDMM2, affecting the thalamus over the cortex, potentially affecting circadian rhythms. Both involve MM2 PrPSc but vary in fragment characteristics, indicating other factors influence prion strain traits.

**I. Varibly protease-sensitive prionopathy (VPSPr)**

VPSPr, identified in 2008, is an atypical dementia affecting M129V homozygotes. No additional PRNP mutations were found, but many patients had family history of cognitive issues. It has a longer clinical course than sCJD but is confirmed as a TSE by neuropathology. Initially called PSPr, its unique feature is PrPDis susceptibility to PK, producing multiple fragments. Research is ongoing on its mechanisms, PMCA amplification, and animal transmissibility. Renamed VPSPr due to varying PK sensitivity across M129V genotypes (MM, MV, VV), with 129 VV cases most affected.

**2. Prion diseases in animals-**

**A. Scrapie**

Scrapie, a historical form of transmissible spongiform encephalopathies (TSEs), has been known since 1732 and affects sheep, goats, and moufflons. Clinical symptoms vary widely, including behavioral changes, blindness, ataxia, and intense pruritus leading to characteristic rubbing and nibbling responses. The incubation period ranges from 2 to 5 years, with death occurring within 2 weeks to 6 months of clinical onset. Neuropathological signs include spongiform vacuolation, astrogliosis, and the deposition of PrPSc amyloid plaques in the central nervous system (CNS). PrPSc is detectable in various tissues and secretions, contributing to horizontal transmission among animals.

Atypical scrapie cases in sheep and goats show distinct clinical patterns, with ataxia and incoordination prominent and pruritus less common. Genetic variations in PRNP, such as A136V and Q171R, influence susceptibility to scrapie. Selective breeding programs based on PRNP polymorphisms have successfully reduced scrapie incidence without negative impacts on animal health or productivity. In goats, lower prevalence of typical scrapie outbreaks correlates with specific PRNP variations, while atypical scrapie cases also reflect genetic influences on disease susceptibility.

### B. Transmissible Mink Encephalopathy (TME)

Transmissible Mink Encephalopathy (TME) is a rare transmissible spongiform encephalopathy affecting farmed mink, primarily for fur production. First identified in Wisconsin and Minnesota in 1947, subsequent outbreaks occurred in the US during the 1960s and 1970s, with the most recent in 1985. Cases have also been reported in Canada, Finland, East Germany, and the former USSR. Contaminated feed, possibly with scrapie or L-type BSE agents, is suspected as the source.

TME is transmissible to various animals including raccoons, striped skunks, ferrets, and cattle via different routes. Infections in mink show distinct clinical phenotypes with variable incubation periods. Clinical signs include aggressiveness, hyperesthesia, depression, ataxia, tremors, and eventually, somnolence and convulsions. Neuropathological features include spongiform degeneration, particularly in the cerebral cortex and thalamus. PrP deposits are found in several tissues but not as amyloid plaques.

Transmission among mink likely occurs through cannibalism or biting, with high mortality rates (60-100%) during outbreaks. Vertical transmission and environmental exposure are not significant transmission routes. Incubation periods range from 6 to 12 months, with death typically occurring within 2 to 8 weeks after onset.

**C. Chronic Wasting Disease (CWD)**

CWD is a neurological disorder affecting deer family members since 1967, spreading across states, provinces, and South Korea. It transmits through contact or environmental exposure among cervids. Humans, livestock, and transgenic mice resist; red deer and reindeer/caribou can get infected. PrPCWD is found in tissues and persists in soil. Prevalence varies (0.1-100%) with clinical signs like weight loss, rough coats, ataxia, and tremors, leading to death within a year. Neuropathology shows spongiform lesions and PrPCWD in brain regions. Ecological challenges include reduced survival and predation risks. Management includes genetic resistance and research on disease mechanisms.

**D. Bovine Spongiform Encephalopathy (BSE)**

BSE, or "Mad Cow disease," is a fatal neurodegenerative disease in cattle, characterized by tremors, ataxia, and aggressive behavior. PrPSc accumulates in the brain and other tissues. BSE has classical and atypical forms (H-type and L-type), detected mainly through surveillance. It spread globally, with a peak in the UK in the mid-1980s. BSE can infect zoo species and humans, causing variant Creutzfeldt-Jakob disease (vCJD). Transmission likely occurred through contaminated meat and bone meal in cattle feed, leading to strict bans and decline in cases. Genetic variants in cattle, like PRNP indels and E211K, influence susceptibility to BSE.

**E. Exotic Ungulate Spongiform Encephalopathy (EUE)**

It affects exotic zoo ruminants (Bovidae) like greater kudu, elands, Arabian oryx, ankole cattle, gemsbok, nyala, scimitar-horned oryx, and bison in the UK. Linked to BSE, it's transmitted through contaminated meat and bone meal (MBM). Mice injected with EUE brain homogenates develop similar TSE with neuropathological signs. EUE's clinical course varies by species, distinct from BSE and scrapie, with all cases fatal.

**F. Feline Spongiform Encephalopathy (FSE)**

It is a transmissible spongiform encephalopathy affecting domestic cats and captive wild members of the Felidae family. Cases have been reported mainly in the UK, with some instances in other European countries and zoos worldwide. The disease is linked to exposure to feed contaminated with BSE prions. Mice injected with brain material from FSE-affected cats and BSE-infected cattle show similar neuropathological signs and incubation periods, indicating a shared prion strain. FSE manifests with severe behavioral changes, ataxia, hyperesthesia, and other neurological symptoms. Neuropathological examination reveals spongiform degeneration and PrP deposits in various tissues.

**G. Transmissible spongiform encephalopathy (TSE)**

Non-human primates (NHP) such as Mayotte brown lemurs and Rhesus macaques from French zoos were diagnosed with a transmissible spongiform encephalopathy (TSE) between 1996 and 1999. These cases were linked to contaminated primate diets possibly containing meat from the UK. Experimental inoculations of lemurs with brain material from BSE-infected cattle showed similar neuropathological lesions to naturally infected lemurs, suggesting a shared prion strain. Immunohistochemical studies confirmed PrPres distribution in various tissues including the brain, spinal cord, tonsils, spleen, and gut-associated lymphatic tissues.

**III. Group C slow viruses**

**A. Subacute Sclerosing Panencephalitis (SSPE)**

Subacute sclerosing panencephalitis (SSPE), a rare disease affecting children and young adults, occurs at a rate of approximately 1 per 1,000,000 in the United States, with a higher prevalence among males (4:1 ratio) and 85% of cases reported in rural areas. It typically follows primary measles infection before age 2, with an average onset interval of about 7 years post-measles. Males are affected twice as often as females, and incidence rates vary geographically, with higher occurrences in rural and southeastern U.S. regions.

Clinical presentation involves progressive mental and motor function decline over years, leading to death within 1-3 years from symptom onset. Diagnosis relies on serological and electron microscopic evidence of measles virus infection in brain cells, as standard cultures fail to isolate the virus. Measles virus antibody levels in serum and cerebrospinal fluid are markedly elevated and diagnostically significant.

The pathogenesis of SSPE remains poorly understood, but animal studies suggest measles virus latency in the brain, with age at infection influencing symptoms. The measles vaccine, initially questioned for SSPE risk, carries about a tenfold lower risk compared to natural infection. Prospective studies indicate the vaccine's strain offers significant protection, reducing neuroinvasion and providing around 90% effectiveness against SSPE.

**B. Progressive Multifocal Leukoencephalopathy (PML)**

Progressive multifocal leukoencephalopathy (PML), a rare central nervous system disorder first described in 1958, primarily affects individuals with severe immunodeficiency, often linked to lymphoproliferative disorders or immunosuppression. Initial evidence of a viral role came from electron microscopy showing papova virions in oligodendrocyte nuclei. JC virus (JCV) has been isolated from human fetal spongioblast cultures, suggesting it as a likely cause.

Serological studies indicate widespread exposure to JC virus in the general population, suggesting PML arises under specific conditions of immunocompromise rather than from a highly lethal or exotic virus. In PML brains, viral antigens concentrate around demyelinated areas, with virions observed within oligodendrocytes, suggesting a direct relationship between viral presence and demyelination.

Patients with PML typically experience progressive deterioration in motor function, vision, and speech, with rapid disease progression leading to death within 3-4 months from symptom onset. The presence of giant astrocytes in PML lesions resembles those seen in malignant astrocytes of glioblastomas, suggesting a potential oncogenic role of isolated papovaviruses.

**Other slow virus diseases**

1. **Multiple Sclerosis (MS)-** A chronic demyelinating disease with a suspected viral origin, notably linked to measles virus. Measles antibodies found in MS patient CSF, and virus-like particles observed in lesions.
2. **Guillain-Barre Syndrome (GBS)-** Associated with various infectious agents including mumps, influenza, Epstein-Barr virus (EBV), and Mycoplasma pneumoniae. Also linked to vaccinations such as tetanus and polio. Echo viruses isolated from CSF, elevated EBV antibody levels in GBS patients.
3. **Amyotrophic Lateral Sclerosis (ALS)-** Primarily sporadic, with familial cases (5-10%) linked to autosomal dominant inheritance.. Possible association with chronic poliovirus infection due to similarities in motor neuron pathology. Intraneuronal inclusion bodies similar to rabies noted, significance debated.
4. **Parkinson's Disease (PD)-** Common chronic neurological disorder of unknown origin. Postencephalitic variant following von Economo's encephalitis suggests an infectious basis, supported by influenza virus antigen in affected brains.
5. **Presenile dementias-** Group of disorders affecting middle-aged adults, onset insidious and origins unclear. Creutzfeldt-Jakob Disease (CJD) proven transmissible, whereas Alzheimer's disease and Pick's disease not successfully transmitted despite numerous attempts in subhuman primates awaiting suitable incubation periods.

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