**DNA viruses**

**Hepadnavirus**

Viral liver infections have had a profound impact on human health, causing significant illness and death. Advances in science and technology have enabled the control and treatment of these infections, primarily through vaccination. Viral hepatitis refers to inflammation of the liver caused by viral infections. Hepatitis viruses are classified into different types, labeled as hepatitis A, B, C, D, and E. Viral hepatitis, liver inflammation caused by viral infections, poses a global public health challenge, affecting millions worldwide and leading to acute and chronic health problems. Understanding the epidemiology, transmission routes, clinical manifestations, diagnosis, and management of viral hepatitis is crucial for healthcare providers to effectively diagnose, treat, and prevent these infections, ultimately reducing their impact on public health.

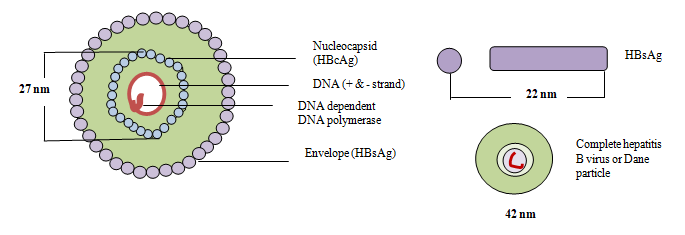
We will discuss Hepatitis A and E under RNA virus chapter.

**Hepatitis B**

Originally identified as the cause of "serum hepatitis," Hepatitis B virus is a leading cause of parenterally transmitted viral hepatitis, resulting in both acute and chronic liver infections. The incubation period for Hepatitis B varies from 1 to 6 months. Acute infection typically presents with clinical presentations similar to other types of viral hepatitis. While acute Hepatitis B often manifests as anicteric and asymptomatic, severe illness with jaundice or acute liver failure can occur in some cases.

**Structure:**

* The hepatitis B virus particle is about 42 nm in size and consists of a dense core, approximately 27 nm in diameter, surrounded by an outer envelope containing the surface protein (HBsAg) embedded in lipid derived from host cells.
* Infected liver cells produce excess surface antigen, which is secreted in 22-nm particles and tubular structures. These particles contain major surface proteins, including non-glycosylated and glycosylated forms, as well as minor components known as middle proteins containing the pre-S2 domain.
* The virion's surface also contains large surface proteins, including pre-S1 and pre-S2 regions, which are absent in 22 nm particles but may be found in tubular forms in highly viremic individuals. Detection of these large surface proteins in serum indicates the presence of the virus. The pre-S1 region likely binds to the specific HBV receptor on hepatocytes.
* The nucleocapsid of the virus consists of the viral genome surrounded by core antigen (HBcAg).
* The genome is approximately 3.2 kilobases long and has a unique structure, comprising two linear DNA strands held in a circular configuration by base-pairing at the 5' ends. One of the strands is incomplete, with the 3' end associated with a DNA polymerase molecule capable of completing the strand when supplied with deoxynucleoside triphosphates.

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**Figure 41: Structure of Hepatitis B**

**Clinical presentation:**

The incubation period for hepatitis B is typically 1-6 months, with an insidious onset and less prominent fever compared to hepatitis A. Extrahepatic complications such as arthralgia, urticaria, and occasionally polyarteritis or glomerulonephritis may occur due to circulating immune complexes containing viral surface antigen. Most adults recover from acute hepatitis B within 1-2 months, with a mortality rate of about 0.5-2.0%, higher in post-transfusion cases. Approximately 1% of patients, especially those co-infected with delta virus, develop fatal fulminant hepatitis. 1-10% of cases become chronically infected, either remaining asymptomatic carriers or progressing to recurrent or chronic liver disease, cirrhosis, and occasionally hepatocellular carcinoma over decades. There are 2 stages of hepatitis B:

Acute Hepatitis- Lasts less than six months, during which the immune system can clear the virus, though some cases progress to chronic hepatitis if the immune response is inadequate.

Chronic Hepatitis- Persists for more than six months, with the virus evading immune clearance. Patients may remain asymptomatic for years, but complications can arise without proper medical management.

**Pathogenesis:** Hepatitis B virus infects hepatocytes in the liver by binding to specific receptors on their surface. Once inside, viral DNA replicates via reverse transcription, generating new viral particles. The immune system recognizes infected hepatocytes, triggering both innate and adaptive responses. Immune dependent natural killer cells and cytotoxic T cells detect and eliminate infected cells, while inflammation helps clear the virus. However, HBV can evade clearance, leading to chronic infection and potential complications like liver cancer. Immune evasion mechanisms include viral protein interference and DNA integration. Inadequate immune responses may result in asymptomatic carrier states, particularly in infants and immunocompromised individuals.

**HBV antigen {antigenic diversity) and antibody:**

**a. HBSAg-** HBsAg is a protein located on the outer envelope of the HBV virion. It is primarily composed of surface proteins that form filamentous and spherical particles It facilitates the entry of the virus into hepatocytes, initiating the infection process. HBsAg exhibits antigenic diversity due to genetic variations within the S gene of HBV. These variations give rise to different antigenic determinants, including:

* **"a" determinant:** This determinant is shared among all HBV strains and is essential for diagnostic tests. It is the main target of neutralizing antibodies generated by vaccination.
* **Type-specific determinants (dy and w-r):** These determinants contribute to the classification of HBV strains into different subtypes, such as adw, adr, ayw, and ayr. Geographic variations exist in the prevalence of these subtypes. The ayw subtype predominates in regions spanning from West Asia through the Middle East to Western and Northern India, while adw is prevalent in Europe, Australia, and the Americas. adr is commonly found in South and East India and the Far East, whereas ayr is exceptionally rare. Other surface antigenic reactivities, such as a, x, f, t, j, n, and g, have been identified, but further comprehensive studies are needed to fully understand their significance.

Detection of HBsAg in serum or plasma is the primary marker used for diagnosing HBV infection. It indicates the presence of active viral replication and ongoing HBV infection. HBsAg screening is essential for identifying individuals with HBV infection, allowing for early intervention and prevention of transmission. HBsAg may persist in the bloodstream for varying durations depending on the stage of infection. In acute infection, HBsAg levels typically decline following the resolution of infection. However, in chronic HBV infection, HBsAg may persist for prolonged periods, indicating ongoing viral replication and chronic liver disease.

**Hepatitis B surface antibody (anti-HBs)-** The presence of anti-HBs in the bloodstream indicates a robust immune response against HBV, either from past infection or vaccination. It is a hallmark of immunity to HBV and is considered protective against future HBV infection. Adequate levels of anti-HBs (typically ≥10 mIU/mL) confirm successful vaccination and immunity to HBV. In acute infections, the immune system generates sufficient surface antibodies to eliminate the virus within a few weeks of symptom onset.

**b. HBcAg-** Hepatitis B core antigen (HBcAg) is an internal antigen found within the nucleocapsid core of HBV. It is not present on the surface of the virus but is encapsulated within the viral particle along with the viral genome and forms the structural core of the viral nucleocapsid, providing stability to the viral genome. During viral replication, HBcAg plays a role in packaging and protecting the viral DNA. It also involves in the assembly of new viral particles within infected hepatocytes.

Detection of HBcAg in liver tissue samples, typically using immunohistochemistry or immunofluorescence techniques, is indicative of active viral replication and ongoing HBV infection. The antigen is not detectable in serum or plasma. HbcAg is highly immunogenic and elicits both humoral and cellular immune responses in infected individuals. The cellular immune response, particularly cytotoxic T lymphocytes (CTLs), targets cells expressing HbcAg, leading to the elimination of infected hepatocytes. This immune response plays a crucial role in controlling HBV infection and reducing viral load.

**Anti-HBc antibodies-** Hepatitis B core antibodies, the first detectable HBV antibodies, usually emerge within weeks after infection, appearing in both acute and chronic cases. They serve as markers of prior HBV exposure and persist long after HBV infection. It exists as IgM or IgG, with IgM appearing early and IgG persisting longer. Detection of anti-HBc antibodies, particularly IgM, aids in diagnosing acute HBV infection, while IgG anti-HBc suggests prior infection or immunity.

**HbeAg-** Hepatitis B e antigen (HbeAg) is a soluble protein that originates from the core antigen (HbcAg) gene of HBV. It circulates in the bloodstream and serves as an indicator of active viral replication and high infectivity.The presence of HbeAg in the blood is associated with increased viral replication and indicates that the virus is actively reproducing in the liver. Therefore, its detection is crucial for assessing the progression of HBV infection and determining the need for antiviral treatment. HbeAg is commonly detected during the early stages of HBV infection, particularly in cases of acute infection or during the replicative phase of chronic infection. Its persistence in the bloodstream is often linked to higher viral loads and increased risk of transmission to others.

**Anti-HBe antibodies-** hepatitis B "e" antibodies typically remain detectable for one or more years. The presence of these antibodies usually corresponds to a decrease in hepatitis B virus activity, leading to reduced liver damage. Anti-HBe antibodies develop in response to the presence of HBeAg, a marker of active viral replication. These antibodies emerge after the acute phase of infection or during the transition from the replicative to the non-replicative phase of chronic hepatitis B. Detecting anti-HBe antibodies in the blood suggests a decline in viral replication, potentially indicating a lower risk of infectivity.

**HbxAg-** Hepatitis B x antigen (HBxAg) is a protein encoded by the X gene of the HBV. It is a small protein with transactivating effects on both viral and cellular genes. HBxAg plays a significant role in the replication and pathogenesis of HBV. HBxAg has been implicated in the development of hepatocellular carcinoma (HCC), the most common type of liver cancer. It promotes oncogenesis by disrupting cellular signaling pathways, promoting cell proliferation, and inhibiting apoptosis. Additionally, HBxAg can interact with cellular proteins involved in DNA repair and chromosomal stability, potentially leading to genomic instability and tumor formation. Detecting HBxAg in patients with chronic HBV infection may aid in identifying individuals at increased risk of developing HCC, allowing for early surveillance and intervention to prevent or manage liver cancer.

**Anti-HBx antibodies-** Anti-HBx antibodies are immune proteins generated in response to HBxAg, a protein produced by the HBV X gene. Detection of these antibodies suggests exposure to or past infection with HBV. Specific binding between HBx antibody and antigen confirms the specificity of the recombinant HBx protein.

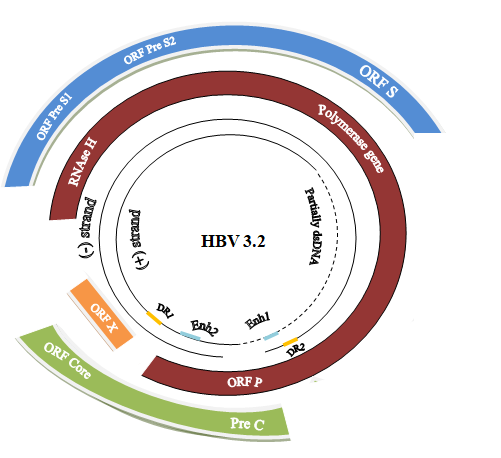
**Viral genome:** The hepatitis B viral genome (Figure 42) is a unique structure comprising two linear strands of DNA arranged in a circular configuration. It is approximately 3.2 kilobases in length. One of the DNA strands, known as the plus strand, is incomplete and associates with a DNA polymerase molecule. This polymerase can repair the gap in the plus strand when supplied with deoxynucleoside triphosphates. The genome contains four overlapping genes:

**S gene-** The genetic sequence responsible for the surface antigen comprises the S region along with two Pre-S regions, Pre-S2 and Pre-S1. This sequence generates various proteins, including the major surface protein (S protein), as well as the middle (M) and large (L) proteins. Translation beginning from the Pre-S2 region yields the M protein, while translation from the Pre-S1 region results in the L protein. The L protein is exclusive to the virion, whereas both M and S proteins are present in circulating HBsAg particles.

**C gene-** The C gene consists of two segments: C and Pre-C. Translation of the C region yields the core antigen (HBcAg), which forms the nucleocapsid core particles. HBcAg remains intracellular and can be detected in hepatocytes via immunofluorescence. Antibodies to HBc, both IgM and IgG, are present in the blood, with IgG persisting long after other serological markers have disappeared, indicating prior HBV infection. Translation from the Pre-C region results in the production of HBeAg, a soluble protein that can be secreted. HBeAg presence in the blood serves as a marker of HBV replication and high infectivity.

**P gene-** Largest gene encodes the DNA polymerase enzyme essential for viral replication.

**X gene**- The X gene encodes for the hepatitis B x antigen (HBxAg), a small protein with transactivating properties on viral and cellular genes. HBxAg enhances HBV replication and may contribute to the development of hepatocellular carcinoma. Additionally, it can affect the replication of other viruses like the human immunodeficiency virus. HBxAg and its antibody are found in patients with severe chronic hepatitis and hepatocellular carcinoma.

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**Figure 42: HIV genome**

**Viral mutations:** Mutations in HBV have been observed, leading to different clinical presentations and potential challenges in prophylaxis.

**Pre-core mutants-** These are prevalent in Mediterranean regions, causing severe chronic hepatitis by inhibiting the production of hepatitis B e antigen (HBeAg). Patients with pre-core mutants often test positive for anti-HBe and anti-HBc antibodies.

**Escape mutants-** These variants are observed in infants born to HBeAg-positive mothers and in liver transplant recipients who have received combined immunization. Escape mutants have mutations in the common "a" determinant of HBsAg, rendering them resistant to neutralization by anti-HBs antibodies. If these mutants become more common, they could complicate hepatitis B prophylaxis efforts.

HBV replicates primarily within hepatocytes, with its DNA existing in the cell nucleus either freely or integrated into the chromosome. Though HBV DNA and proteins have been detected in extrahepatic sites like the bone marrow and lymph nodes, the significance of this presence remains unclear. While HBV cannot be grown in conventional culture systems, limited production of the virus and its proteins is achievable through cell line transfection. Additionally, HBV proteins have been successfully cloned in bacteria and yeast. Experimental infection in chimpanzees serves as a valuable laboratory model for studying HBV.

**Resistance:** Hepatitis B virus exhibits a notable resistance to environmental factors. It can endure at room temperature for extended durations and remains relatively stable to heat, withstanding temperatures of 60°C for up to 10 hours, albeit with a substantial reduction in infectivity by 100- to 1000-fold. Despite its resilience, HBV is susceptible to chemical agents. Exposure to hypochlorite with 10,000 ppm available chlorine or 2% glutaraldehyde effectively deactivates HBV, although the hepatitis B surface antigen (HBsAg) may remain intact following such treatment.

**Epidemiology:** Hepatitis B is a global concern, affecting only humans with no animal reservoir. Carriers sustain the virus long-term, with sporadic outbreaks occurring, particularly in institutional settings. Carrier prevalence varies globally, with India's intermediate rates affected by regional disparities. Carriers, detected by HBsAg for over six months, constitute a significant population, with males more commonly affected. Worldwide, over 350 million carriers exist, with India ranking second only to China. The prevalence of chronic HBV infection was estimated at 257 million in 2015, with the highest burden in the Western Pacific and African regions. HBV contributes significantly to cirrhosis and liver cancer deaths globally. The virus exhibits ten genotypes and 35 sub-genotypes, with varying distributions and associated risk factors, such as vertical transmission, impacting chronic disease and hepatocellular carcinoma rates.

* **Super carriers** are highly infectious individuals with elevated levels of HBsAg, HBeAg, DNA polymerase, and HBV in their bloodstream, often accompanied by increased transaminase levels. Some exhibit exceptionally high antigenemia and viremia, with quantities reaching up to 10^13 HBsAg particles, equivalent to 500 µg of protein, and 10^8 HBV per ml of blood. In India, approximately a quarter of carriers are HBeAg positive.
* **Simple carriers**, on the other hand, have low infectivity and reduced levels of HBsAg in their blood. They typically test negative for HBeAg, HBV, and DNA polymerase. Over time, many super carriers transition to becoming simple carriers.

**Transmission:** Transmission of Hepatitis B virus (HBV) occurs through parenteral, sexual, and perinatal modes.

**1. Parenteral transmission-** Parenteral transfusion, once a common source of infection, has diminished with strict donor screening. While blood is the primary carrier, other bodily fluids like semen and saliva can transmit the virus, albeit less efficiently. Therapeutic and diagnostic procedures, along with non-medical practices like tattooing and acupuncture, now pose significant risks. Infection can even occur through contact with minute traces of infected material. Direct contact with open skin lesions and household transmission are common, especially in developing countries. Although HBV can survive in mosquitoes and bed bugs, they don't transmit the virus. Infants born to infected mothers are at risk during childbirth, with exposure to infected fluids being the main transmission route.

**2. Perinatal transmission-** This is commonly observed from carrier mothers to their infants. The risk to babies is significantly higher if the mother tests positive for HBeAg (ranging from 60% to 90%) compared to when the mother is HBeAg negative (5% to 15%). True congenital infection, occurring in utero or via the placenta, is rare. Infection typically occurs during birth through contact of maternal blood with the fetus's skin and mucosa, or shortly after delivery. While ingestion as a mode of transmission has been reported, its efficiency is very low. However, avoiding breastfeeding from carrier mothers can be safer if adequate alternative nutrition for the babies is ensured. Infected neonates usually do not exhibit clinical symptoms but remain carriers for life, with a risk of developing hepatocellular carcinoma decades later.

**3. Sexual (horizontal transmission) contacts-** HBV can be sexually transmitted globally, with heightened significance in developed nations, especially among sexually active homosexual individuals. The risk of transmission, whether through heterosexual or homosexual contact, escalates with an increase in the number of partners and the duration of such relationships. Notably, HBV infection cases have been reported even after artificial insemination procedures.

**4. Unsafe medical practices-** Occupational hazards pose a significant risk of HBV infection among various groups and professions. These include healthcare workers such as medical and paramedical personnel involving the use of contaminated needles or medical equipment, staff in blood banks, dialysis units, and medical laboratories, as well as individuals working in mental health institutions, barber shops, and within the sex industry. Dentists and doctors have occasionally been linked to minor outbreaks. In non-endemic countries like Britain, individuals carrying HBV are restricted from engaging in invasive medical procedures, and they are also prohibited from pursuing medical studies.

**5. Injection drug use-** Sharing needles or syringes contaminated with HBV-infected blood is a common route of transmission among injection drug users.

**6. Unsafe tattooing or piercing-** Procedures involving the use of unsterilized equipment for tattooing, piercing, or acupuncture can lead to HBV transmission if the equipment is contaminated with infected blood.

**7. Household contacts-** Close household contacts of individuals with chronic HBV infection are at increased risk of transmission through exposure to blood or other bodily fluids.

**Laboratory diagnosis of Hepatitis B virus:**

**1. Serological assays for HBV diagnosis**-

Various serological assays (Figure 43) detect HBV-specific antigens and antibodies, aiding in determining infection susceptibility or immunity post-infection or vaccination. Tests include rapid diagnostic tests (RDTs), enzyme immunoassays (EIAs), chemiluminescence immunoassays (CLIAs), and electrochemiluminescence immunoassays (ECLs), analyzing serum, plasma, whole blood, or oral fluid specimens. Reports are given qualitatively or quantitatively in international units (IU) or signal per cutoff (S/Co) values.

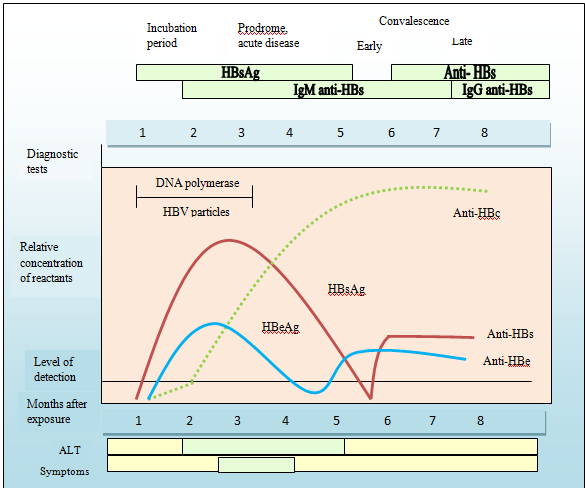
**a. HBsAg detection-** HBsAg, an envelope protein found on the surface of HBV particles known as Dane particles, serves as a marker for current HBV infection when detected in the serum. Confirming HBsAg positivity often involves a second surface antigen test, particularly in regions with HBsAg prevalence < 0.4, before further HBV DNA evaluation. Hepatitis typically manifests 90 days (with a range of 60-150 days) after HBV exposure, with HBsAg detectable in the blood approximately six weeks (ranging from 1 to 10 weeks) post-exposure.

During the immunological window period, HBsAg may vanish rapidly without HB surface antibodies appearing, with IgM antibodies serving as the sole evidence of infection during this time. Persistent HBsAg positivity beyond six months suggests the development of chronic infection. Quantitative immunochemiluminescence analysis aids in assessing HBsAg levels, particularly in chronic hepatitis B (CHB) patients, and proves beneficial as a marker for interferon alfa (IFN-α)-treated CHB patients who are HBeAg negative.

**b. Anti-HBs antibody detection-** Anti-HBs or HBsAb presence signifies recovery and immunization against HB infection, either through vaccination or past infection. Those with chronic carrier relatives or partners are advised vaccination if their triple serological screening tests are negative. A protective anti-HBs titer should be ≥10 mIU/ml.

**c. Anti-HBc detection-** Detection of anti-HBc confirms HBV exposure but does not indicate vaccine-induced immunity. IgM antibodies, along with HBsAg positivity that last for less than 6 months, suggest acute infection. Individuals positive for anti-HBc and negative for HB surface antibodies are chronically infected, with reduced risk of HBV reactivation. Vaccination is not clinically beneficial for those positive only for core antibodies or both anti-HBc and anti-HBs.

**d. HBeAg and anti-HBe detection-** HBeAg presence indicates active viral replication and contagiousness, while anti-HBe appearance suggests low viral replication and infection resolution, aiding in determining the chronic hepatitis B (CHB) infection phase.

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**Figure 43: Clinical and serological events in HBV**

**2. Biochemical parameters and fibrosis markers-** Liver fibrosis severity is assessed using biochemical parameters like AST, ALT, GGT, ALP, bilirubin, serum albumin, gamma globulin, full blood count, and PT. Non-invasive methods like APRI are recommended by WHO for predicting advanced liver fibrosis due to liver biopsy's invasiveness and limitations of other techniques. ALT levels should also be monitored according to WHO guidelines for disease severity assessment.

**3. Molecular diagnostic techniques for HBV-** Various molecular diagnostic techniques are employed for HBV DNA quantification, genotyping, detection of drug resistance mutations, and analysis of precore/core mutations.

**4. HBV DNA quantification-** HBV DNA quantification is crucial for assessing infectivity in individuals and evaluating the risk of mother-to-child transmission in HBsAg-positive pregnant women. It aids in treatment decision-making and monitoring treatment response in CHB patients. Quantification methods include UV spectrophotometry, real-time PCR, digital PCR, loop-mediated isothermal amplification (LAMP), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), and others. The recommended detection limit for HBV DNA is 10 IU/ml.

**HBV DNA genotyping, drug resistance, PreC/Core mutations:**

HBV exists in ten genotypes (A to J) and over 40 sub-genotypes with distinct geographical distributions and clinical implications. Genotypes and sub-genotypes influence disease progression, treatment response, and prognosis. HBV genotyping is not essential for initial diagnosis but is valuable for evaluating antiviral therapy responses and monitoring patients at risk of hepatocellular carcinoma (HCC). Genotyping methods include reverse hybridization, PCR-based techniques, oligonucleotide microarray chips, and sequencing followed by phylogenetic analysis. Drug resistance mutations are detected using sequence-based assays, with Sanger sequencing considered the gold standard. Real-time PCR is commonly used for its accuracy and speed in detecting drug resistance mutations.

**Treatment:** Hepatitis B virus treatment aims to accomplish several key objectives, including reducing liver cancer-related deaths, minimizing the need for liver transplants, slowing or reversing liver disease progression, and decreasing infectivity.

**Table 15: Treatment of HBV**

|  |  |
| --- | --- |
| Treatment | Usage |
| Interferon therapy | Interferon-alpha (IFN-α), available in standard and pegylated formulations, activates the body's antiviral defense mechanisms, leading to extended treatment responses in many individuals. However, it can have significant side effects. |
| Nucleos(t)ide analogues (NUCs) | NUCs like lamivudine (LAM), telbivudine, entecavir (ETV), adefovir (ADV), and tenofovir (TDF) inhibit HBV DNA replication. They vary in terms of resistance rates and side effects, with newer options showing improved safety profiles. |
| HBV/HIV coinfection | Treatment strategies for individuals coinfected with HBV and HIV are guided by specific recommendations, with newer NUCs favored over interferon therapy due to cost and side effect considerations. |
| Resistance and combination therapy | Resistance to NUCs can occur, prompting research into combination therapies involving NUCs and interferon to enhance treatment efficacy by targeting different stages of the HBV lifecycle. |
| Tenofovir Alafenamide (TAF) | TAF, a newer NUC, shows promise in HBV treatment with favorable pharmacokinetics and antiviral activity. Studies indicate it is well-tolerated with manageable side effects. |

**HBV vaccination:** Preventive measures involve avoiding high-risk behaviors such as unprotected sex, injectable drug use, and direct or indirect contact with bodily fluids from infected individuals. Although health education, disposable needle use, and blood, semen, and organ donor screening help mitigate risks, they may not entirely eliminate them, especially in developing nations. Universal immunization (Table 16) stands as the most effective method for prevention, offering both passive and active approaches.

**Table 16: Immunization of HBV**

|  |  |
| --- | --- |
| Immunization | Usage |
| Active immunization | Genetically engineered vaccines, produced by cloning the HBV S gene into baker's yeast, offer superior efficacy. Administered with alum adjuvant IM, typically in the deltoid or thigh, the vaccine consists solely of non-glycosylated HBsAg particles. A three-dose regimen at 0, 1, and 6 months yields robust seroconversion rates, with long-term clinical protection. Special formulations containing all HBsAg antigenic components may enhance seroconversion and are available.  Eg:- India- Engerix-B, Shanvac-B, Elovac-B  Abroad-Engerix-B, Recombivax HB,  Twinrix |
| Passive immunization | Hyperimmune hepatitis B immune globulin (HBIG), derived from individuals with high anti-HBs titers, is administered intramuscularly (IM) shortly after exposure to the virus. While it may not prevent infection, it does protect against illness and carrier status. Eg:- Hepagam B, Bayhep B, HyperHEP B |
| Combined immunization (for non-immune exposed individuals) | Combined immunization is advised for individuals who are not immune and have been exposed to HBV. For newborns born to mothers who are carriers of HBV, it's recommended to administer a single 0.5 ml injection of HBIG intramuscularly immediately after birth, followed by the complete vaccine series at a separate anatomical site. The initial vaccine dose should be administered within 12 hours of birth. In cases where HBIG is unavailable, reports suggest that administering the vaccine alone can still offer protection. |

**Hepatitis C**

Efforts to identify the group of 'non-A non-B' viruses through experimental infection in chimpanzees resulted in the discovery of the hepatitis C virus (HCV). Currently, it stands as the primary cause of post-transfusion hepatitis in developed countries. Hepatitis C can lead to both acute and chronic liver disease, ranging from mild illness to serious complications such as cirrhosis (scarring of the liver) and liver cancer.

**HCV structure:** HCV has not been cultivated in culture but has been cloned in Escherichia coli. It is a 50-60 nm virus with a linear, single-stranded RNA genome, enclosed within a core and surrounded by an envelope, carrying glycoprotein spikes. Structurally, HCV resembles flaviviruses and has been classified as a new genus, Hepacivirus, in the family Flaviviridae. The virus exhibits considerable genetic and antigenic diversity, with at least six different genotypes and numerous subtypes identified, indicating high mutability.

**Epidemiology and global impact:** HCV infection is confined to humans and is predominantly transmitted through a vast pool of carriers, estimated at approximately 200 million worldwide. Its epidemiology closely resembles that of hepatitis B, with transmission occurring primarily through blood transfusions and other forms of contact with infected blood or blood products. High-risk populations include injectable drug users, transplant recipients, and individuals with compromised immune systems. Though sexual transmission may be less prominent, vertical transmission from mother to baby is a possibility. Globally, HCV infection is widespread, with carrier rates ranging from 1-20 percent. In India, the burden of HCV infection is substantial, with an estimated 12.5 million cases, constituting a quarter of all chronic hepatitis instances in the nation.

**Clinical presentation:**

**Acute hepatitis C** infection often presents with mild or even without any symptoms, resembling flu-like ailments that may be overlooked. Nevertheless, some individuals may exhibit noticeable signs such as jaundice, fatigue, abdominal discomfort, and other related symptoms.

**Chronic hepatitis C** poses a more insidious threat, potentially resulting in enduring liver impairment and the eventual onset of cirrhosis or liver failure. While some individuals with chronic hepatitis C may not display symptoms for many years or even decades, others may suffer from fatigue, abdominal swelling, and manifestations of liver dysfunction..

**Diagnostic tools and screening:** Diagnosing hepatitis C typically involves blood tests aimed at identifying both HCV antibodies and viral RNA. Screening is particularly advised for individuals with high infection risks, such as those with a history of injection drug use, recipients of blood transfusions, healthcare workers with blood exposure, and newborns to HCV-infected mothers. Antibody detection via ELISA constitutes the key basis of diagnosis, utilizing a range of structural and non-structural proteins cloned in E. coli as antigens. Given the delayed and sporadic appearance of antibodies in HCV infection, confirmation through immunoblot assay is often recommended. Molecular techniques like PCR and branched DNA assay offer high sensitivity and specificity by detecting HCV RNA in blood, providing rapid and accurate results within days of HCV exposure.

**Treatment and management:** While certain individuals may spontaneously clear the infection with their immune system, not all new infections necessitate immediate treatment. However, chronic hepatitis C always requires treatment intervention. Antiviral drugs such as sofosbuvir and daclatasvir are utilized in the management of hepatitis C. Direct-acting antiviral (DAA) medications have emerged as highly effective therapeutic options. DAA therapy is often administered in combination with other antiviral medications to maximize efficacy and reduce the risk of viral resistance. Prevention Strategies

**Preventive strategies:**

1. Safe injection practice
2. Blood screening
3. Universal Precautions (Healthcare settings should adhere to universal precautions, which involve assuming that all blood and body fluids are potentially infectious).
4. Healthcare settings should adhere to universal precautions, which involve assuming that all blood and body fluids are potentially infectious
5. Appropriate medical care to prevent mother to child transmission.

**Vaccine:** Challenges in developing a vaccine for hepatitis C include the significant variability in genetic sequences between viral groups and the considerable diversity among isolates in the N-terminal region of envelope glycoprotein.

**Hepatitis D**

In 1977, Rizzetto and his team in Italy identified a novel viral antigen present in the liver cell nuclei of patients with hepatitis B virus (HBV) infection. This antigen was attributed to the hepatotropic virus delta or Hepatitis D Virus (HDV). HDV is a defective RNA virus that relies on HBV for its replication and expression, meaning it cannot survive or replicate independently.

**Morphology:** HDV appears as a spherical particle measuring 36 nanometers, with an outer coat comprised of the hepatitis B surface antigen enveloping a circular single-stranded RNA genome. While it shares some similarities with plant viruses like viroids or satellite viruses, it is proposed to belong to a distinct genus called Deltavirus due to its unique characteristics.

**Clinical presentation:** HDV is transmitted in the same manner as HBV. There are two recognized types of infections:

* **Co-infection-** Both delta and HBV are transmitted simultaneously, typically resulting in acute hepatitis B, ranging from mild to severe.
* **Superinfection-** Delta infection occurs in individuals already infected with HBV, often leading to more severe and chronic illness, worsening the underlying HBV infection. No association has been established between HDV and hepatocellular carcinoma.
* **Role of liver transplant-** Liver transplantation is indicated in cases of fulminant liver failure due to HDV infection. Despite appropriate medical therapy, post-transplantation recurrence of HDV infection can occur. Communication and coordination for transplant services are crucial for the successful management of these patients.

**Epidemiology:** In a recent comprehensive meta-analysis, it was estimated that globally, 62-72 million people are infected with hepatitis D virus (HDV). This infection affects approximately 10.58% of individuals who carry the hepatitis B surface antigen (HBsAg). High prevalence areas include western and middle Africa, the Amazon Basin, parts of Europe, the Middle East, and parts of Asia. Recent studies have shown a concerning rise in HDV prevalence among intravenous drug users.

**Pathogenesis:** HDV, discovered in 1977, is a single-stranded RNA virus reliant on hepatitis B virus (HBV) for replication. It is classified into eight genotypes, with HDV-1 being the most prevalent in North America, Europe, and the Middle East. Transmission primarily occurs through parenteral exposure to infected blood or body fluids.

**Screening and diagnosis:** Screening for HDV is recommended for all HBsAg-positive individuals, especially those with worsening liver disease. Initial testing involves detecting total anti-HDV antibodies, followed by confirmation through serum reverse transcriptase-PCR assays for HDV RNA. Quantification of serum HDV RNA helps assess the need for antiviral therapy.

**Treatment:** Current evidence supports treating chronic HDV infection in patients with detectable viral RNA and evidence of active liver disease. Interferon therapy remains the mainstay treatment, but its efficacy is limited, with sustained virologic response rates rarely exceeding 25%. Ongoing research focuses on novel antiviral agents targeting various steps of the HDV life cycle.

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**Pox virus**

Poxviruses represent a diverse family of large, complex DNA viruses that infect a wide range of hosts, including humans and other animals. These viruses have a unique structure, replication cycle, and pathogenesis, making them significant subjects of study in virology and immunology.

**Poxvirus classification:** Thebelow table shows the details of poxviridae classifications (Table 17)..

**Table 17: Classification of poxviridae**

|  |  |  |
| --- | --- | --- |
| Family | Genus | Species with features |
| Chordopoxviridae (infect vertebrates) | Orthopoxvirus | Variola (major)- causes smallpox; narrow host range (humans); eradicated globally |
| Variola (minor) – causes alastrim or moderate smallpox, narrow host range (humans); characterized by a rash of pus-filled lesions; seen in Europe, Asia, Africa. |
| Vaccinia virus – causes vaccinia; localized pustule and mild discomfort; complications such as eczema vaccinatum or generalized vaccinia may occur; broad host range (humans, cattle, buffalo, swine, rabbits); spread worldwide |
| Cowpox virus- causes cowpox; skin lesion ulcerates in a specific area; transmitted from cats, okapi, cattle or rodents (broad host range); observed in regions across Asia and Europe. |
| Monkeypox virus-causes monkeypox; characterized by pustules; 15% mortality rate; broad host range (Squirrels, monkeys, anteaters, great apes, humans); observed in  Western and central Africa |
| Parapoxvirus | Orf virus-Causes contagious pustular dermatitis; broad host range (sheep, goats, humans); spread worldwide |
| Pseudocowpox – causes Milker’s nodules (occupational skin disease; occurs in cattle herd); narrow host range (cattle, humans); spread worldwide |
| Bovine papular stomatitis virus- causes bovine papular stomatitis; papules and ulcers in mouth; narrow host range (cattle, humans); seen worldwide |
| Capripoxvirus | Sheeppox virus- causes shippox; characterized by fever, respiratory symptoms; nodules and scabs on the skin; narrow host range (sheep, goats); observed in Africa, Asia |
| Goatpox virus- causes goatpox; respiratory distress and skin lesions; narrow host range (goats, sheep); seen in Africa, Asia |
| Leporipoxvirus | Myxoma virus 9myxomatosis or skin tumor) and Rabbit fibroma virus (fibroma or benign tumor) - Narrow host range [rabbits (Oryctolagus and Sylvilagus spp.)]; found in Americas, Europe, and Australia |
| Avipoxvirus | Fowlpox virus, canarypox, crowpox, pigeonpox; narrow host range (chickens, turkeys, other bird species); spread worldwide |
| Suipoxvirus | Swinepox virus- causes swinepox; skin lesions and pustules; narrow host range (swine); seen  worldwide |
| Entomopoxvirinae (infect insects) | Molluscipoxvirus | Molluscum contagiosum virus- causes molluscum contagiosum; dome shaped bump; skin contact; broad host range (humans, nonhuman primates, birds, kangaroos, dogs); seen worldwide |
| Yatapoxvirus | Yabapox virus- causes localized skin tumors; narrow host range (monkeys, humans); observed in West Africa |
| Tanapox virus- causes localized skin lesions; transmission from arthropod bites; found in West Africa |
| Unclassified | Squirrel Poxvirus- affects red and grey squirrels; narrow host range; seen in Europe and North America | |
| Salmonid gill poxvirus- affects Atlantic salmon (Salmo salar); narrow host range; observed in Norway | |

**Variola virus-** Variola, a member of the Orthopoxvirus genus, triggered the onset of smallpox, a highly contagious and potentially fatal infectious disease. This enveloped virus carried a 186 kbp dsDNA with approximately 200 predicted genes. Transmission occured via inhalation of airborne particles. Variola virus had a narrow host range similar to ectromelia virus. Within the genus, variola exhibited two distinct variants: variola major and variola minor. Both had distinct clinical and epidemiological profiles, with variola major being more virulent, causing fatality rates of 30% compared to less than 1% for variola minor.

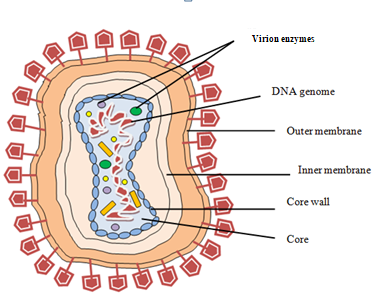
Symptoms of variola major include high fever, body aches, and a characteristic rash that progresses from red spots to fluid-filled vesicles and then to pustules, which eventually crust over and fall off. Variola major often leads to more severe complications such as pneumonia, encephalitis, and death. Variola minor, on the other hand, presents with similar symptoms but tends to be less severe. The rash in variola minor is typically less dense and progresses more slowly than in variola major.

Smallpox was once endemic worldwide, except in Australia and certain islands, due to its lack of an animal reservoir. The last naturally acquired case occurred in Somalia in 1977. Eradication achieved through ten years of concerted global efforts, including widespread vaccination since 1977 and antiviral treatment with cidofovir, led to the disease's elimination in 1980 (WHO declaration on May 8, 1980).

**Vaccinia virus-** In 1796, Edward Jenner pioneered small pox immunization by utilizing the cowpox virus. Over time, the vaccinia virus supplanted cowpox virus through sustained passage of the original vaccine virus via human-to-human transmission enduring modifications. Its genome consists of linear, dsDNA approximately 190 kilobase pairs (kbp) long, encompassing roughly 250 genes. Vaccinia possesses several advantageous characteristics crucial for the eradication of smallpox, including its remarkable thermal stability, robust induction of both humoral and cell-mediated immune responses, ease of propagation, incorporation of multiple genetic elements (genes encoding antigens for HBV, HIV, rabies, certain neuropeptides) and non-oncogenic nature. The absence of vaccinia genome integration into the host genome, coupled with its exclusive replication within the host cell cytoplasm, ensures its safety as a vaccine.

Vaccinia virus strains commonly employed during the eradication campaign included the New York City Board of Health (NYCBH), Lister, and EM-63. These live vaccines were often administered via scarification using a bifurcated needle, resulting in a cutaneous reaction caused by local virus replication. The formation of a scar at the inoculation site, known as the "take," historically served as the indicator of protection against smallpox. Occurrences of Vaccinia virus in domestic animals, notably buffalo in India, were linked to contact with vaccinated humans during eradication efforts. Continued outbreaks in buffalo even after vaccination termination suggest long-term virus maintenance in the population, with distinct genetic differences from standard vaccine strains. Despite its detailed study and potential as a vector for recombinant vaccines, its pathogenicity limits human use.

**Pox virus structural characteristics:** Poxviruses are characterized by oval or brick-shaped particles measuring 200-400 nm in length (Table 44), first observed by Buist (1887) under light microscopy. Paschen developed staining techniques in 1906, allowing the visualization of viral particles, particularly elementary bodies (Paschen bodies), in smears obtained from smallpox lesions. The virions exhibit ridged external surfaces, often arranged helically. Viral particles typically possess an external enveloped virion and intracellular mature virion (infectious and contains a distinct envelope). The internal structures of poxviruses appear as flattened capsules or barrels, with variations based on species, often resembling rounded bricks. These complex particles carry a single, linear, double-stranded DNA genome and consist of more than 100 different proteins.

****

**Figure 44: Structure of poxvirus**

Outer membrane

Core

Core wall

**Resistance:** Poxviruses are resistant to freeze-drying and heat, which contributes to their stability and ability to persist in the environment. Poxviruses exhibit resistance to 50% glycerol and 1% phenol, yet they are easily deactivated by formalin and oxidizing disinfectants.

**Antigenic properties:** Various surface antigens on the outer envelope of poxviruses are targets for the host immune response. Proteins forming the core structure of the virion are also antigenic. Poxviruses express agglutinogens and hemagglutinins, which are antigens capable of inducing agglutination of fowl erythrocytes. Poxviruses share a common nucleoprotein (NP) antigen, which serves as a target for host immune recognition. The LS antigen is a notable complex consisting of two components: the heat labile (L) antigen and the heat stable (S) antigen, contribute to the antigenic profile of poxviruses and are targets for host immune responses.

**Clinical presentation of smallpox:** Smallpox has been eradicated globally since 1977. The eradication effort employed freeze-dried vaccine and a simple, effective vaccination technique. Variola virus stocks have been destroyed, but smallpox vaccine stocks are maintained for potential bioterrorism threats.

* **Incubation period-** The incubation period for smallpox is typically around 7 to 17 days following exposure to the virus. During this phase, the virus replicates within the body without causing any noticeable symptoms.
* **Prodromal stage-** Lasts for 2 to 4 days and is characterized by nonspecific symptoms resembling a flu-like illness. These symptoms may include fever, malaise, headache, body aches, fatigue, and sometimes vomiting. Patients may experience an abrupt onset of high fever, often reaching above 101°F (38.3°C), accompanied by severe headache and backache.
* **Rash stage-** At this stage, the characteristic rash of smallpox begins to emerge. This stage marks the onset of illness and is highly characteristic of the disease.The rash typically starts on the face and spreads to the arms, legs, and trunk, progressing in a centrifugal (from center to periphery) fashion.Lesions appear initially as red macules (flat, red spots) that rapidly evolve into raised papules.Over the next few days, the papules transform into fluid-filled vesicles, which subsequently develop into pustules, deeply embedded in the skin and are surrounded by a red halo.
* **Crust and scab stage-** As the pustules mature, they become filled with a thick, opaque fluid and eventually form crusts. The crusts and scabs are deeply embedded in the skin and are firmly adherent. They gradually detach over a period of 2 to 3 weeks, leaving behind characteristic pitted scars.Patients may experience intense itching, which can lead to scratching and potential secondary bacterial infections.
* **Convalescent stage-** Once all scabs have detached, the patient enters the convalescent stage.Symptoms gradually subside, and the patient begins to recover. However, weakness and fatigue may persist for several weeks following the resolution of the rash.

**Complications:** Smallpox can lead to various complications, including secondary bacterial infections of the skin, pneumonia, encephalitis (inflammation of the brain), and ocular complications if the eyes are affected.

**Multiplication:**

**1.** **Attachment and entry-** Poxvirus replication (Figure 45) begins with attachment of the virion to specific receptors on the surface of the host cell. Attachment relies on specific polypeptides, including 32 KDa, 29 KDa, and the STE domain (54-58 KDa), facilitating cellular penetration. Upon attachment, the virus enters the host cell either through direct fusion with the plasma membrane or via endocytosis.

**2. Early gene expression-** Following entry, the poxvirus releases its DNA genome into the host cell cytoplasm. Early gene expression initiates, leading to the synthesis of nonstructural proteins necessary for viral replication and modification of host cell machinery. Replication occurs predominantly in the cytoplasm, utilizing DNA-dependent RNA polymerase. As DNA replication progresses late gene expression ensues, resulting in the production of structural proteins required for virion assembly.

**3. Virion assembly and release-** Virion assembly occurs in specialized cytoplasmic sites known as viral factories. Structural proteins, along with newly synthesized viral DNA, are packaged into assembling virions. Mature virions are released from the host cell either through cell lysis or budding through cellular membranes, completing the replication cycle.

**Pathogenesis:** Typically, infection arises from penetration through compromised skin and tends to remain confined to the affected area. Human monkeypox, however, can be contracted through direct contact or via airborne particles reaching the respiratory mucosa. In the initial stages, viremia disseminates the infection to internal organs during the incubation phase, followed by a subsequent viremia that propagates the virus to the skin.

**Immune response:** Host immunity against poxvirus involves intricate interactions among cells, yielding antiviral antibodies by B cells and virus-specific responses by T cells. Antibodies directly engage viral components, hindering attachment, aggregating viruses, or enhancing phagocytosis via Fc receptors. T cells, including CD8+ and CD4+ subsets, are vital in combating poxviruses. Antibody-dependent cytotoxicity (ADCC) entails leukocytes binding to virus-specific antibodies and lysing infected cells. Poxviruses employ diverse immunomodulatory proteins to evade immune responses, hindering apoptosis, interferon production, and T cell activity. They induce immunosuppression by infecting B and T cells and inducing suppressor factors, impairing immune function. Poxvirus infections impact host macromolecular synthesis and employ various mechanisms to counteract interferon responses, highlighting the importance of understanding this interplay for effective antiviral strategies and vaccines.

Cell membrane

Adsorption

Penetration

Virus

Loss of outer membrane

DNA replication

(2-5 hours)

Uncoating of core, releasing viral DNA

Core

Early transcription

Late transcription

Early mRNA

Late mRNA

Early enzymes

Late enzymes

+

Early virion proteins

Late virion proteins

Morphogenesis

(4-20 hours)

Spherical immune particles

Release by cell disruption or exocytosis

**Figure 45: Poxvirus replication**

**Laboratory diagnosis**: Crucial for confirming infection, guiding patient management, and implementing control measures-

**1. Clinical evaluation:**

* **Lesion examination-** Poxvirus infections typically present with characteristic lesions, including papules, vesicles, pustules, and scabs. The location, morphology, and distribution of lesions can provide important diagnostic clues.
* **Patient history-** Information about recent travel, contact with infected individuals or animals, and vaccination history can help narrow down the differential diagnosis.

**2. Microscopic examination-**

* **Direct microscopy-** Examination of clinical specimens (e.g., lesion swabs, tissue biopsies) under a microscope can reveal characteristic cytoplasmic inclusions known as Guarnieri bodies. These intracytoplasmic aggregates are composed of viral particles and are typical of poxvirus infections.
* **Electron microscopy-** High-resolution electron microscopy allows for the visualization of poxvirus particles, which have distinctive brick-shaped morphology. This method provides direct evidence of viral presence and morphology.

**3. Molecular techniques-**

* **Polymerase Chain Reaction (PCR)-** PCR amplifies specific regions of viral DNA, enabling rapid and sensitive detection of poxvirus nucleic acids in clinical samples. Targeting conserved genes such as those encoding viral DNA polymerase or major envelope proteins allows for reliable identification. Quantitative Real-Time PCR techniques provide information about viral load, which can be useful for monitoring disease progression and assessing treatment response.
* **Sequencing-** Genetic sequencing of PCR products facilitates strain identification and epidemiological investigations, particularly in outbreaks or cases with unusual presentations.

**4. Serological Assays-**

* **Enzyme-Linked Immunosorbent Assay (ELISA)-** ELISA detects poxvirus-specific antibodies (IgM and IgG) in patient serum. A rise in antibody titer between acute and convalescent-phase samples can confirm recent infection. ELISA can also be used for seroprevalence studies and surveillance.
* **Immunofluorescence Assay (IFA)-** IFA utilizes fluorescently labeled antibodies to detect poxvirus antigens in patient samples. It is particularly useful for rapid diagnosis and can be applied to various clinical specimens.

**5. Viral Culture-**

* **Cell culture-** Isolation of poxviruses in susceptible cell lines such as Vero cells allows for virus propagation and subsequent identification based on characteristic cytopathic effects. Cell culture is essential for strain characterization and isolation of live virus for further studies. Both variola and vaccinia viruses exhibit distinct growth characteristics and host range, allowing for their differentiation. In chick embryos aged 11-13 days, both viruses generate pocks on the chorioallantoic membrane (CAM) within 48-72 hours. Variola pocks (Figure 46) appear small, shiny, white, convex, and non-necrotic, while vaccinia pocks (Figure 46) are larger, irregular, flat, greyish, and often necrotic, with some showing hemorrhagic features. Additionally, their 'ceiling temperatures', above which pocks cease to form, differ: vaccinia's ceiling temperature is 41°C, variola major's is 38°C, and variola minor's is 37.5°C.

Pocks

Vaccinia

Variola

**Figure 46: Pocks formation**

* **Tissue culture-** In tissue cultures of monkey kidney, HeLa, and chick embryo cells, both variola and vaccinia viruses can grow. Vaccinia typically induces cytopathic effects within 24-48 hours, whereas variola's effects manifest more slowly. Guarnieri bodies, which are eosinophilic inclusion bodies comprising virus particles in a matrix, can be observed in stained preparations, primarily with vaccinia. Furthermore, vaccinia forms plaques in chick embryo tissue cultures, a trait not seen with variola.
* **Animal inoculation-** In vivo propagation of poxviruses in laboratory animals (e.g., mice, rabbits) can confirm the infectivity of clinical samples and aid in virus isolation, especially when cell culture attempts are unsuccessful. Experimentally, vaccinia virus can infect a broad array of animals, including monkeys, calves, sheep, and rabbits, resulting in vesicular lesions when introduced via scarification. In contrast, variola virus-induced lesions are primarily observed in monkeys. Scarification of rabbit corneas with variola virus leads to keratitis, with typical Guarnieri bodies observable in corneal sections. Intranasal administration of variola virus to monkeys results in a self-limiting infection

**6. Immunohistochemistry-**

* **Tissue staining-** Immunohistochemical staining of tissue sections with poxvirus-specific antibodies allows for the detection of viral antigens within lesions. This method provides histopathological evidence of viral infection and can aid in distinguishing poxviruses from other pathogens causing similar lesions.

**Other poxviruses:**

**Molluscum contagiosum**

Molluscum contagiosum is a globally occurring condition characterized by benign, self-limiting skin lesions or papular eruptions. It's considered a distinct human infection, with no known transmission between humans and other animals. However, similar lesions containing poxviruses have been observed in non-human species like horses and chimpanzees.

**Subtypes:** Upon digestion by restriction endonuclease, four subtypes have been identified. Despite variation, the disease presentation remains similar across all subtypes. The genome of MCV subtype I has been sequenced, revealing several unique gene products crucial for its pathogenesis and evasion of the immune system. These include an IL-18–binding protein and apoptosis inhibitors, among others.

**Pathogenesis:** Molluscum contagiosum lesions have been recognized for their distinct pathology since the 19th century. Henderson and Paterson provided the first description of characteristic molluscum bodies in 1841. Infection begins with viral replication in the deeper layers of the epidermis before spreading upwards. The incubation period varies but can be prolonged, lasting from 2 to 7 weeks, or even up to 6 months. The epidermis thickens and extends into the underlying dermal layers. Henderson-Paterson bodies, or molluscum bodies, form in the stratum spinosum and grow larger as cells migrate to the surface.

Basal cell layer hyperplasia occurs, while the basement membrane structure remains intact. The enlarged epidermal cells, containing large acidophilic granules (molluscum bodies), protrude above the skin, resembling a tumor. Inflammatory infiltrate is minimal until late in the disease process, just before natural lesion resolution occurs. The host immune response plays a crucial role in controlling the virus infection. Initially, innate immune mechanisms such as natural cells, macrophages, and interferons help limit viral spread.

Despite the immune response, the virus can establish a persistent infection in the skin. This persistence is facilitated by several viral factors, including the ability to evade host immune surveillance through expression of immunomodulatory proteins such as IL-18–binding protein and apoptosis inhibitors.

**Clinical symptoms:** Following skin break, lesions initiate as small papules, maturing into discrete, 2-mm to 5-mm diameter, smooth, dome-shaped, pearly or flesh-colored nodules, often hollow, centrally depressed. Lesions may yield cheesy off-white or yellowish material upon expression. Typically, 1 to 20 lesions appear, occasionally numbering in the hundreds due to concurrent infections or mechanical spread, possibly merging along scratch marks and resulting in additional satellite lesions.

In children, lesions predominate on the trunk and proximal extremities, while in adults, they favour the trunk, pubic area, and thighs; however, transmission to other sites via autoinfection is possible. Molluscum contagiosum severe infections in HIV patients may manifest along the beard line in males, with occasional facial and ocular involvement, such as lesions on the bulbar conjunctiva. Individual lesions persist for approximately 2 months, with the disease often lasting 6 to 9 months.

**Laboratory diagnosis:** The distinctive appearance of lesions typically allows for clinical diagnosis. Examination of expressed cheesy material under transmission negative-stain electron microscopy often reveals abundant brick-shaped virions. The virus has not been successfully cultured in standard tissue culture methods. Diagnostic histopathology is characteristic, with polymerase chain reaction (PCR) methods available.

**Epidemiology and prevention:** The virus is prevalent worldwide, with a notable increase in cases coinciding with the AIDS epidemic. While transmission typically occurs through mild skin trauma or shared items like towels, recent evidence suggests a significant role for sexual transmission, especially involving genital lesions. Concerns arise in childcare and school settings due to paediatric infection. Preventive measures such as covering lesions and practicing hand hygiene can mitigate transmission risks in these environments.

**Treatment:** The virus causes benign infection and tend to resolve spontaneously within months to years except immunocompromised individuals (persist or recur). However, treatment may be sought for cosmetic reasons, particularly for facial or multiple lesions. Various treatment options exist, including cryotherapy, mechanical curettage, and chemical treatments such as podophyllin, cantharidin. Adverse effects, such as irritation, are common with chemical treatments. Antiviral creams like 3% cidofovir and immune-modulating therapies like cimetidine or topical imiquimod may also be beneficial.

**Monkeypox**

The monkeypox virus was initially discovered in 1959 when monkeys imported from Singapore to a research facility in Denmark became sick. The first confirmed human case occurred in 1970 when the virus was isolated from a child in the Democratic Republic of Congo who was suspected to have smallpox. Coincidental immunity to the monkeypox virus was attained through vaccinia vaccination; however, the eradication of smallpox and subsequent termination of vaccination campaigns facilitated the emergence of monkeypox as a clinically significant pathogen.

**Structure:** Monkeypox is a zoonotic infection, characterized by its brick-shaped structure, large virus (200 to 250 nm) with a genome composed of dsDNA. Transmission primarily occurs through contact with infected animals or humans, with evidence suggesting respiratory droplets, bodily fluids, and direct lesion contact as major modes of spread.

**Epidemiology:** Originally endemic to central and western Africa, monkeypox has seen sporadic cases outside Africa, including in the United States, Israel, Singapore, and the United Kingdom. Recent outbreaks, particularly among men who have sex with men (MSM), have raised concerns about its global spread. Various clades of the virus exist, each with different transmission dynamics and clinical outcomes.

**Pathogenesis and clinical presentation:** Following entry into the body, the virus replicates locally before spreading to regional lymph nodes and causing a secondary viremia. This leads to symptom onset, characterized by fever, lymphadenopathy, and eventually mucosal and skin lesions. The course of infection progresses through distinct stages, including macular, papular, vesicular, and pustular phases, before resolving within a few weeks. Complications, including bacterial superinfection and permanent scarring, can occur but are generally rare.

**Laboratory diagnosis:** Diagnosis of monkeypox relies on laboratory confirmation, which can be achieved through viral culture, PCR testing, or serological assays. Clinical and epidemiological criteria aid in identifying suspected cases, particularly in endemic areas. Prompt and accurate diagnosis is crucial for implementing appropriate infection control measures.

**Treatment and prevention:** Currently, no specific antiviral therapy exists, and treatment focuses on supportive care. Isolation of infected individuals, symptom management, and vaccination of close contacts are essential preventive measures. Experimental therapies such as brincidofovir and tecovirimat show promise but require further evaluation. Public education and awareness campaigns are vital for preventing outbreaks and limiting transmission.

**Parapoxviruses**

Parapoxviruses infect both humans and animals and are characterized by their ability to cause localized skin lesions. Examples include Orf virus, which primarily affects sheep and goats, and Bovine papular stomatitis virus, which infects cattle. While parapoxvirus infections are generally self-limiting, they can pose economic challenges in agricultural settings.

* **Orf (Contagious Pustular Dermatitis)-** Orf, primarily a disease of sheep and goats, can be transmitted to humans through contact. In humans, the disease manifests as a single papulovesicular lesion with a central ulcer, commonly found on the hand, forearm, or face. Morphologically, the virus causing orf resembles the paravaccinia virus and is unrelated to the variola-vaccinia group.
* **Milker's Node (Paravaccinia)-** Milker's node, also known as paravaccinia, is a minor occupational disease contracted by humans during the milking of infected cows. Lesions present as small ulcerating nodules and are unrelated to cowpox. Unlike cowpox, the virus causing milker's node does not grow in eggs but can be cultured in bovine kidney cultures. Morphologically, it resembles the orf virus.

**Avipoxviruses**

Avipoxviruses primarily infect birds and are responsible for diseases such as Fowlpox and Canarypox. These viruses can have significant implications for poultry farming, leading to reduced productivity and economic losses. Avipoxvirus infections can also impact wild bird populations, highlighting their ecological importance.

**Yatopoxvirus**

Includes tanapox and yabapox virus-

* **Tanapox-** The virus, discovered in 1957 in Kenya's Tana River basin, primarily impacts humans in Africa, notably in Kenya and the Democratic Republic of the Congo. Its distribution is predominantly confined to Africa. Although direct primate-to-human transmission is rare, speculation suggests possible involvement of insect or arthropod intermediaries. No instances of human-to-human transmission have been reported. Typically, tanapox infection begins with a brief febrile illness followed by the eruption of hyperpigmented macules, evolving into papules and firm nodules. The disease progresses benignly, resolving within about 6 weeks. Confirmation of Tanapox virus presence can be achieved through electron microscopy and nucleic acid testing.
* **Yaba Monkey Tumor Virus-** Yaba monkey tumor virus is a distinct species of Yatapoxvirus, primarily affecting African green monkeys. It was first isolated as the cause of cutaneous tumors in rhesus monkeys. In monkeys, it causes benign histiocytomas that typically resolve within 1 to 2 months. Human infections, primarily through accidental needle sticks in animal handlers, result in localized skin lesions at the site of inoculation. Human infections have not been recently reported, and the virus remains primarily associated with benign tumors in African green monkeys.

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**Herpesvirus**

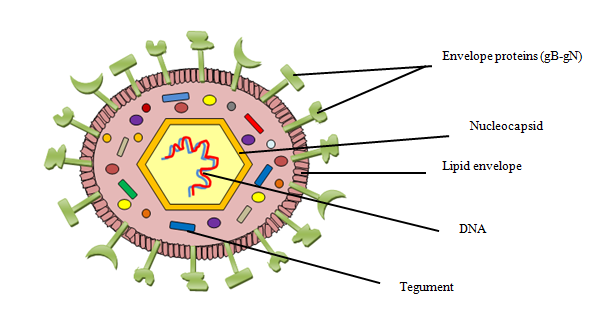
HSV can cause various illnesses, including skin, CNS, and internal organ infections, some potentially life-threatening. The term "herpes," from Greek, has been used for centuries. Cold sores were noted as early as AD 100, genital herpes in 1736. The herpesvirus family includes over a hundred species of DNA viruses affecting humans and animals, known for establishing latent infections with periodic reactivation.

**Classification of herpesvirus:** Below table shows the details of classifications of herpesviridae (Table 18).

**Table 18: Classification of herpesviridae**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sub family | Species | Common name with abbreviation | Site of infection | Disease caused |
| Alpha | Human herpesvirus type 1 | Herpes simplex virus type 1 (HSV-1) | Neuronal epithelial cells | Oral herpes, herpes keratitis, herpes gladiatorum, congenital defects, genital herpe |
| Human herpesvirus type 2 | Herpes simplex virus type 2 (HSV-2) | Neuronal epithelial cells | Genital herpes, congenital defects |
| Human herpesvirus type 3  (HHV-3) | Varicella zoster virus (VZV) | Neuronal epithelial cells | Chickenpox, shingles, herpes zoster ophthalmicus, postherpetic neuralgia |
| Beta | Human herpesvirus type 5  (HHV-5) | Cytomegalovirus (CMV) | Secretory glands, kidneys, Monocytes | Congenital defects, mononucleosis |
| Human herpesvirus type 6  (HHV-6) | Human B cell lymphotropic virus (HBLV) | Lymphocytes, monocytes, neural cells | Roseola infantum |
| Human herpesvirus type 7  (HHV-7) | R K virus | Lymphoid tissues | Roseola infantum |
| Gamma | Human herpesvirus type 4  (HHV-4) | Epstein-Barr virus (EBV) | Lymphoid tissues, epithelial cells, B cells | Mononucleosis, Burkitt lymphoma, Hodgkin lymphoma |
| Human herpesvirus type 8  (HHV-8) | Kaposi sarcoma–associated herpesvirus (KSHV) | B cells, endothelial cells | Kaposi sarcoma, lymphoma |

**Structure:** HSV virions are spherical particles (Table 47) enveloped in a lipid bilayer membrane, which is adorned with viral glycoproteins. Beneath this envelope lies an icosahedral capsid housing the viral DNA genome. Comprising 162 capsomeres arranged in a T = 16 symmetry, the capsid encapsulates the viral genetic material. Surrounding the capsid is a protein layer known as the tegument, which plays crucial roles in viral replication and assembly. This structure aids in the organization and function of the virus. The overall structure of the herpesvirus includes an enveloped virion measuring approximately 200 nm and a naked virion about 100 nm in diameter.



**Figure 47: Structure of HSV**

**Replication:** Herpesviruses replicate within the nucleus of host cells, generating Cowdry type A intranuclear (Lipschutz) inclusion bodies. HSV initially attaches to host cells via specific interactions between viral glycoproteins and cellular receptors on the cell surface. This attachment is crucial for subsequent entry into the host cell. Once inside the host cell, the viral capsid releases its genetic material, which consists of double-stranded DNA, into the cytoplasm. HSV replicates its DNA using host cell enzymes and machinery. Newly synthesized viral DNA, along with viral proteins produced during transcription and translation, assemble to form new viral particles within the nucleus. Mature virions are released from the host cell through budding or cell lysis.

**HSV glycoproteins:** The glycoproteins (Table 19) play key roles in the initial stages of infection, including attachment to host cells, penetration of the cell membrane, and fusion with cellular membranes during entry.

**Table 19: Glycoproteins of HSV**

|  |  |
| --- | --- |
| HSV glycoproteins | Used for |
| Glycoprotein D (gD) | Crucial for viral entry into host cells. It binds to specific cell surface receptors, facilitating viral attachment and fusion with the host cell membrane. |
| Glycoprotein B (gB) & glycoprotein H (gH) | essential for viral entry. They form a complex with gD and are involved in membrane fusion. |
| Glycoprotein G (gG) | Important for viral spread and evasion of the host immune response. It helps the virus to establish latency and also facilitates cell-to-cell spread. |

**HSV-1**

HSV-1, a member of the Alphaherpesviridae subfamily, features linear dsDNA and an icosahedral capsid enveloped with spikes, typically 100-110 nm in diameter. Its pathogenesis involves primary infection of epithelial cells, latency in neurons, and reactivation. HSV-1 primarily causes vesicular eruptions in orolabial and genital mucosa, with various presentations such as orolabial herpes, herpes gladiatorum, and ocular HSV infection. Antiviral therapy helps manage the infection.

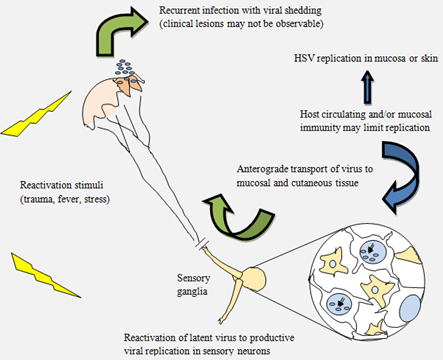
**Epidemiology:** HSV-1 is highly prevalent worldwide, affecting about two-thirds of the global population under 50. Humans are the sole natural reservoir, with transmission typically occurring during childhood or adolescence through non-sexual contact with infected saliva or lesions. Reactivation leads to recurrent infection, often with asymptomatic viral shedding.

In the US, neonatal HSV infection occurs in approximately 1 in 1000 newborns, usually due to exposure during vaginal delivery. While women with recurrent genital herpes have a low risk of transmitting HSV to their newborn, those acquiring genital HSV during pregnancy face a higher risk.Herpes encephalitis is the primary cause of fatal encephalitis, while ocular HSV infection can lead to blindness.

Understanding these epidemiological factors is vital for effective prevention and management. HSV-1 antibody prevalence rises with age and inversely correlates with socioeconomic status. Although HSV-1 infection is nearly ubiquitous in many parts of Asia and Africa, prevalence rates are declining in Western populations, particularly among higher socioeconomic groups.

**Pathogenesis:** HSV-1 enters the body via direct contact with infected bodily fluids or abraded skin, initiating replication in epidermal and dermal cells. It then travels along sensory nerve fibers to reach ganglia like the dorsal root (Figure 48) or trigeminal ganglia, establishing latency. Other ganglia may also be infected based on the initial site. Within the ganglia, HSV-1 enters a latent phase where viral replication is limited, and the virus remains dormant. During latency, the viral genome persists as an episome within the nuclei of neurons. Latency allows the virus to evade immune surveillance and remain hidden from the host's immune system.

Periodically, HSV-1 undergoes reactivation triggered by factors like stress, fever, hormonal changes, or ultraviolet radiation. This leads to the production of infectious virions and viral shedding, with migration along sensory nerves to the initial infection site, causing recurrent lesions. HSV-1 employs immune evasion strategies like down-regulating MHC class I molecules and interfering with interferon production. Infection triggers an inflammatory response marked by immune cell infiltration, contributing to clinical manifestations such as erythema, edema, and vesicular lesion formation.



**Figure 48: The recurrence pattern and asymptomatic shedding of HSV-1**

**Clinical presentation:** The presentation and progression of HSV-1 infection vary depending on several factors, including the site of infection (Table 20), the age and immune status of the individual, and the specific viral type involved.

**Table 20: HSV-1 infections and infection sites**

|  |  |
| --- | --- |
| Infection site | HSV-1 infection |
| Orolabial Herpes (Cold Sores) | **Primary Infection:** Typically occurs 3 days to a week post-exposure, marked by fever, malaise, and tender lymphadenopathy followed by painful vesicles on the mouth and lips.  **Recurrent Infection:** Milder symptoms with a prodrome of tingling and burning, often affecting the vermilion border of the lip. |
| Cutaneous Infections | **Fever blisters (herpes febrilis)-** Commonly manifest on the face, particularly on the cheeks, chin, around the mouth, or on the forehead, leading to lesions.  Infants- In infants, lesions may appear on the buttocks resembling napkin rash.  **Eczema herpeticum (Kaposi Varicelliform Eruption)-** widespread vesicles and ulceration, atopic dermatitis, leading to punched-out erosions with hemorrhagic crusts and secondary impetigo; occurs in children with eczema (Figure 49a).  **Herpetic Sycosis-** Affects the beard area, especially in males who shave closely, presenting as follicular papules progressing to erosions. |
| Digit and Periungual Infection | **Herpetic whitlow (occupational herpes)-** Deep blisters (Figure 49b) on digits, potentially leading to lymphadenopathy and mimicking acute paronychia or blistering dactylitis; observed in healthcare workers (doctors, dentists, nurses) |
| Mucosal Infections | The buccal mucosa is affected, leading to gingivostomatitis, pharyngitis, or recurrent herpes labialis; ulcerated vesicles. |
| Ocular Infections | **Primary Ocular HSV-1-** Presents as keratoconjunctivitis with unilateral or bilateral symptoms such as tearing, photophobia, and corneal ulcers.  Recurrence: Often unilateral and may lead to blindness, especially when manifested as keratitis or dendritic corneal ulcers. |
| Nervous System Infections | HSV-1 encephalitis is the most common sporadic viral encephalitis worldwide, characterized by fever and focal neurological symptoms.  HSV-1 meningitis is usually self-limiting, with lymphocytic pleocytosis in cerebrospinal fluid. |
| Visceral Infections | HSV=1 esophagitis, tracheobronchitis, and pneumonitis may occur, leading to dysphagia, substernal pain, or respiratory symptoms.  Hepatitis, erythema multiforme, and disseminated HSV-1 infection can occur in immunocompromised individuals. |
| Neonatal infection | Transplacental HSV-1 infection can lead to congenital malformations but is rare.  Neonatal herpes can occur during birth, leading to disseminated disease involving multiple organs; high mortality rate; potential neurological impairment in survivors. |

**(a) (b)**

**Figure 49: (a) Eczema herpeticum (Damour et al., 2020); (b) Herpetic whitlow (Hoff and Gerber, 2012)**

**Treatment:** Antiviral agents like idoxuridine, acyclovir, vidarabine, valaciclovir, famciclovir, and foscarnet are used for management. Early treatment with intravenous acyclovir improves encephalitis outcomes, while oral and topical medications are effective for less severe cases. Foscarnet is considered when resistance to other drugs develops.

**HSV-2**

HSV-2, affecting approximately 22% of adults in the United States, is the primary cause of genital lesions, presenting with nonspecific symptoms such as itching and irritation, leading to potential delays in diagnosis and treatment.

**Pathogenesis:** HSV-2 spreads through direct contact with shedding sections of a seropositive individual, targeting skin and mucous membranes. The virus initially invades epithelial cells, replicating intracellularly. After symptoms resolve, the virus becomes dormant in sensory nerve sheaths for about 10 to 14 days on average. Reactivation later in life can occur, with the virus traveling through sensory nerves to mucocutaneous sites, leading to vesicular clusters at dermatological sites innervated by the affected sensory neuron.

**Epidemiology:** HSV-2 infection primarily occurs through direct exposure to fluids from a seropositive individual, typically during sexual intercourse, with transmission peaking during puberty. Both primary and recurrent infections in pregnant women can lead to intrauterine transmission and congenital infection. Herpes genitalis, mainly caused by HSV-2, is a prevalent sexually transmitted infection (STI) transmitted through direct contact with open lesions. In the United States, HSV-2 is a significant cause of genital ulcers, with over 23 million new cases reported annually worldwide. Seropositivity for HSV-2 rises with sexual activity, particularly among women and non-Hispanic African Americans, yet the majority of infections remain undiagnosed. Globally, HSV-2 is one of the most common viral STIs, with approximately 11% of the population aged 15-49 infected. Prevalence is notably higher in low- and middle-income countries, especially in sub-Saharan Africa. Public health initiatives emphasize awareness, education, and access to testing and counseling services for those at risk of HSV-2 infection.

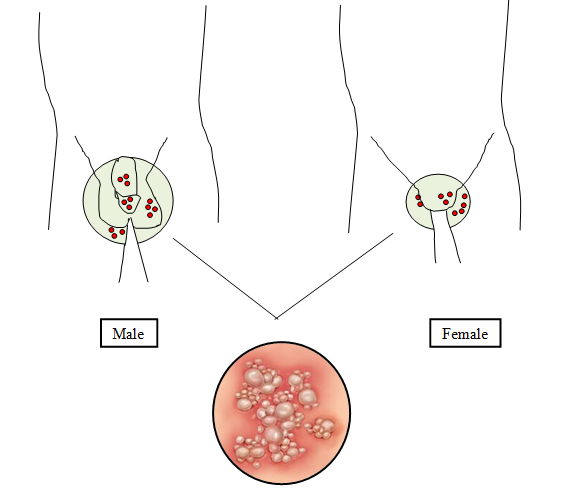
.**Clinical presentation:** HSV-2, or herpes simplex virus type 2, typically causes genital herpes, characterized by painful, recurrent genital lesions. Clinical presentations of HSV-2 infection include:

**Genital lesions:** The hallmark of HSV-2 infection (Figure 50) is the development of painful, fluid-filled blisters or ulcers on the genital area often entail prolonged symptoms, lasting 10 to 12 days. These lesions can appear on the penis, scrotum, vulva, vagina, cervix, or anus. The lesions may be accompanied by itching, burning, or tingling sensations.

* **Primary infection-** Initial infection with HSV-2 often presents with multiple painful lesions, fever, headache, muscle aches, and swollen lymph nodes in the groin area. The primary infection may be severe and can last for several weeks.
* **Recurrent episodes-** After the primary infection resolves, the virus remains dormant in nerve cells and may periodically reactivate, leading to recurrent outbreaks of genital lesions. Recurrences are often milder and shorter in duration compared to the primary infection but can still cause discomfort and distress.

**Asymptomatic shedding:** HSV-2 infected individuals may shed the virus asymptomatically, meaning they can transmit the virus to sexual partners even when no visible lesions are present. Asymptomatic shedding contributes significantly to the spread of HSV-2.

**Complications:** In addition to the physical discomfort caused by genital lesions, HSV-2 infection can have emotional and psychological effects due to stigma and concerns about transmission to sexual partners. In some cases, HSV-2 infection can lead to complications such as meningitis, urinary retention, and neonatal herpes if transmitted to a newborn during childbirth.

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**Figure 50: Genital herpes by HSV-2 (InformedHealth.org[Internet], 2022)**

**Treatment:** Genital herpes management focuses on preventing transmission and suppressing viral shedding through antiviral therapy and counseling regarding sexual transmission risks. Primary infections typically resolve within 19 days regardless of treatment. Antiviral agents, such as nucleoside analog-polymerase inhibitors like acyclovir, are the mainstay of therapy, reducing symptoms and viral shedding. Valacyclovir, inhibit viral replication and are recommended to prevent prolonged symptoms. Currently, no HSV-2 vaccine is available.

**Laboratory diagnosis of HSV-2:** The laboratory diagnosis of herpes simplex virus HSV infection involves various techniques aimed at detecting the virus.

**1. Viral culture-**

* **Method:** Clinical specimens, such as vesicle fluid, swabs from lesions, or cerebrospinal fluid (CSF), are inoculated onto cell culture monolayers.
* **Detection:** Cytopathic effects (typically observed within 24-48 hours) indicative of viral replication, such as cell rounding, syncytial formation, or intranuclear inclusions, are observed microscopically.
* **Advantages:** Highly specific and can provide virus isolation for further characterization.

**2. Microscopy-**

* **Method:** The microscopy technique for HSV involves preparing a Tzanck smear from lesions, preferably from the base of vesicles. The smear is then stained with a 1% aqueous solution of toluidine blue 'O' for 15 seconds.
* **Detection:** Multinucleated giant cells with characteristic features, known as Tzanck cells, are observed under the microscope. Additionally, intranuclear inclusion bodies may be visible in Giemsa-stained smears.
* **Advantage:** The Tzanck smear is a rapid, relatively sensitive, and inexpensive diagnostic method for HSV infection. It allows for quick visualization of characteristic viral features, aiding in the prompt diagnosis of herpes virus infections.

**3. Antigen detection-**

* **Method:** Enzyme immunoassays (EIAs) or direct fluorescent antibody (DFA) tests detect HSV antigens in clinical specimens.
* **Detection:** Target viral proteins, such as glycoprotein D, are identified using specific antibodies conjugated to enzymes or fluorescent dyes.
* **Advantages:** Rapid results, suitable for detecting viral antigens in lesions with low viral titers.

**4. Nucleic Acid Amplification Tests (NAATs)-**

* **Method:** Polymerase chain reaction (PCR) or nucleic acid sequence-based amplification (NASBA) techniques amplify and detect HSV DNA in clinical specimens.
* **Detection:** Targeted viral DNA sequences, typically from genes like glycoprotein B or DNA polymerase, are amplified and detected using fluorescent probes or gel electrophoresis.
* **Advantages:** High sensitivity and specificity, suitable for detecting low viral loads, and can differentiate between HSV-1 and HSV-2.

**5. Serological tests-**

* **Method:** Enzyme-linked immunosorbent assays (ELISAs) or immunofluorescence assays (IFAs) detect HSV-specific antibodies (IgM and IgG) in serum samples.
* **Detection:** Antibodies against HSV antigens, such as glycoprotein G, are detected.
* **Advantages:** Useful for detecting past HSV infections and distinguishing between primary and recurrent infections, but not suitable for diagnosing acute infections.

**6. Direct Immunofluorescence Assay (DFA)-**

* **Method:** Clinical specimens are stained with fluorescent-labeled antibodies specific to HSV antigens.
* **Detection:** Visualization of fluorescent-labeled viral antigens under a fluorescence microscope.
* **Advantages:** Rapid results and suitable for detecting viral antigens in lesions with high sensitivity.

**Varicella Zoster Virus (VZV)**

The Varicella-zoster virus (VZV), a member of the herpesvirus family, causes varicella (chickenpox) and herpes zoster (shingles). While varicella incidence has dropped due to vaccination, herpes zoster remains common, particularly in older adults. Vaccines exist for both diseases, aiming to prevent primary infection and alleviate symptoms.

Shingles, recognized since ancient times for its distinctive rash, contrasts with chickenpox, often mistaken for smallpox. In 1875, Steiner demonstrated VZV transmission by inoculating chickenpox fluid into volunteers. Subsequent research by von Bokay confirmed VZV's infectious nature, noting chickenpox in those exposed to herpes zoster patients.

**Structure:** VZV is structurally characterized by its symmetrical icosapentahedral shape, containing centrally located double-stranded DNA enveloped by a lipid-containing envelope with glycoprotein spikes. Its size ranges from 150 to 200 nm, with a naked capsid diameter of approximately 90 to 95 nm. The viral genome consists of about 125,000 base pairs encoding around 75 proteins, organized into unique long and short regions with terminal repeat sequences. Glycoproteins, such as gpI to gpV, are essential for VZV's infectivity and immune response, serving as targets for neutralizing antibodies.

**Resistance:** VZV is sensitive to environmental factors like detergent and air drying and spreads from cell to cell through direct contact. It can be isolated in various cell culture systems.

**Epidemiology:** Chickenpox, primarily seen in children, spreads through close contact with an incubation period of 10 to 20 days. While more prevalent in children, around 10% of adults are susceptible. The disease is usually endemic but can become epidemic during late winter and early spring. In contrast, herpes zoster, affecting about 20% of the population, typically strikes the elderly. It arises from VZV reactivation in those who previously had chickenpox, with the highest incidence in individuals over 60. Immunocompromised individuals are at higher risk for both diseases. Moreover, herpes zoster may manifest in children born to mothers who had chickenpox during pregnancy.

**Pathogenesis**: Varicella-zoster virus (VZV) triggers immune responses upon infection, including immunoglobulin production and cell-mediated immunity. After primary infection, VZV becomes latent in sensory nerve ganglion cells, but reactivation can lead to herpes zoster and complications like postherpetic neuralgia and Ramsay Hunt syndrome type II. Individuals over 60 face an increased stroke risk due to herpes zoster, highlighting the importance of vaccination, especially for those previously exposed to chickenpox.

Chickenpox arises after exposure to the virus, with histopathological findings similar to herpes zoster. Vesicles in the dermis result from viral replication, causing epithelial cell changes, occasionally leading to dermal necrosis and hemorrhage. As vesicles progress, the fluid becomes cloudy due to immune cell infiltration. Transmission occurs via respiratory droplets, followed by local replication and viremia. Chickenpox lesions' diffuse nature suggests viremia, backed by virus recovery from blood. The mechanism behind VZV reactivation remains unknown.

**Immune response:** Primary VZV infection prompts the production of IgG, IgM, and IgA antibodies against various viral proteins, facilitating virus neutralization and cell lysis. High-titer VZV immune globulin (VZIG) and maternally acquired IgG antibodies mitigate varicella severity. While humoral immunity develops over time, cellular responses, involving T lymphocytes, are crucial in controlling viral replication. T-lymphocyte recognition of VZV antigens leads to mild varicella in healthy children but severe infections in immunocompromised individuals. Cytotoxic T lymphocytes play a vital role in eliminating virus-infected cells. Factors influencing VZV reactivation include viral virulence and T-lymphocyte-mediated immunity, with immunosenescence and immunosuppressive therapy increasing herpes zoster risk. However, individuals recovering from herpes zoster typically regain robust T-lymphocyte responses, aiding in prolonged immunity against recurrent episodes.

**Clinical presentation:**

**Varicella (Chickenpox)-** Chickenpox (Figure 51a), largely benign in immunocompetent children, is declining in incidence due to widespread vaccination. The disease typically manifests with a rash, low-grade fever, and malaise, often preceded by a prodrome. Lesions progress through stages, from maculopapules to vesicles and scabs, appearing mainly on the trunk and face. Immunocompromised children may experience more severe symptoms, with longer healing times and increased risk of complications, including hemorrhagic lesions and secondary bacterial infections.

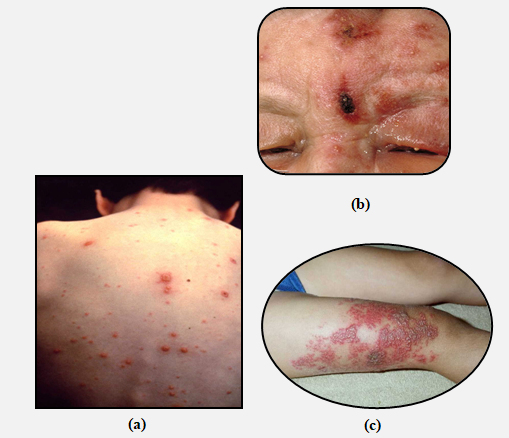
**Complications-**

* **Noncutaneous infections-** The central nervous system (CNS) is a common noncutaneous site of involvement, with cerebellar ataxia and encephalitis being notable complications. While cerebellar ataxia is usually benign, encephalitis can be life-threatening, leading to seizures and neurologic deterioration. Late cerebral angiitis and other manifestations, like meningitis and transverse myelitis, can also occur.
* **Varicella pneumonitis-** Varicella pneumonitis poses a significant risk, especially in adults and immunocompromised individuals, with potentially fatal outcomes. Other complications include myocarditis, nephritis, and hepatitis.
* **Perinatal infections-** Perinatal varicella, particularly when occurring near delivery, can be fatal due to the absence of protective antibodies in newborns. Congenital varicella can result in severe developmental abnormalities.
* **Reye's syndrome-** Reye's syndrome (swelling in the liver and brain), associated with aspirin use during chickenpox, underscores the importance of avoiding aspirin administration. Secondary skin infections, often by Staphylococcus aureus, are also concerning.
* **Chickenpox in Immunocompromised Individuals-** Chickenpox in immunocompromised individuals presents significant morbidity and mortality risks, with prolonged healing times and increased visceral involvement. Those undergoing hematopoietic cell transplantation face a particularly high risk, with a substantial incidence of infection and mortality, especially in the presence of graft-versus-host disease.

**Herpes zoster-** Herpes zoster (Figure 51c), commonly known as shingles, manifests as a unilateral vesicular eruption within specific dermatomes, often involving thoracic and lumbar segments. It shares similarities with varicella lesions but appears in a limited distribution. The rash typically resolves within two weeks, but residual pain and paresthesia may persist for an extended period.

**Complications –**

* **Opthalmic infections-** Herpes zoster ophthalmicus (Figure 51b), affecting the first or second branch of the trigeminal nerve, can lead to serious complications such as keratitis and secondary glaucoma. The onset is usually marked by pain preceding the characteristic rash by 48 to 72 hours.
* **Ramsay Hunt syndrome-** Ramsay Hunt syndrome, a rare form of herpes zoster, involves the facial nerve, presenting with facial palsy, pain, and vesicles in the ear canal.
* **Neuronal infections-** Acute neuritis and postherpetic neuralgia (PHN) are significant clinical manifestations, with PHN occurring in up to 25% to 50% of patients over 50 years old.Extracutaneous involvement may include the central nervous system (CNS), leading to conditions like meningoencephalitis, encephalitis, and granulomatous cerebral angiitis.Motor paralysis, Guillain-Barré syndrome, transverse myelitis, and myositis are neuromuscular disorders associated with herpes zoster.
* **Immunocompromised-** Immunocompromised patients, including those with HIV infection, are at increased risk of severe and chronic herpes zoster, often with cutaneous dissemination and visceral involvement.

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**Figure 51: (a) Varicella (chickenpox) Zoster (Ayoade and Kumar, 2024); (b) Herpes Zoster Ophthalmicus (Nair and Patel, 2024); (c) Herpes Zoster infection (Nair and Patel, 2024)**

**Laboratory diagnosis:**

**1. Direct detection methods-**

* **PCR-** PCR amplifies specific VZV DNA sequences, allowing for the detection of viral DNA in various samples like blood, CSF, skin lesions, or respiratory secretions.
* **Direct Fluorescent Antibody Staining (DFA)-** This method involves using fluorescent-labeled antibodies to detect VZV antigens in skin lesions or mucosal scrapings.

**2. Serological tests-**. ELISA identifies IgM and IgG antibodies, indicating recent or past infection respectively, while IFA detects VZV-specific antibodies in serum or CSF using fluorescent-labeled antibodies and also facilitates antigen detection. Western Blot can be used particularly in cases where ELISA results are inconclusive.

**3. Viral culture-** Virus isolation can be attempted from buccal or cutaneous lesions in the early stages by inoculating human amnion, human fibroblast, HeLa, or Vero cells.

**4. Microscopy-** Multinucleated giant cells and type A intranuclear inclusion bodies can be observed in Tzanck smears, which are prepared by scraping the base of early vesicles and stained with toluidine blue, Giemsa, or Papanicolaou stain. Electron microscopy of vesicle fluid can also reveal the virus with typical herpes morphology.

**5. Histopathological examination-** Skin biopsy with histopathological examination may reveal characteristic features of VZV infection, such as intranuclear inclusions (Cowdry type A) and multinucleated giant cells.

**Treatment and prophylaxis:** The medical management of chickenpox and shingles focuses on reducing complications. Practices like hygiene, topical treatments, and antipyretics help manage chickenpox symptoms. Acyclovir is approved for both chickenpox and shingles, while prodrugs like valaciclovir and famciclovir show enhanced efficacy. However, the use of corticosteroids alongside antivirals remains controversial.

In immunocompetent individuals, chickenpox prophylaxis relies on vaccination. However, in hospitals, there's a risk of VZV transmission to immunosuppressed patients. Varicella-zoster immune globulin (VZIG) is recommended for high-risk individuals

**Zostavax**, a vaccine for herpes zoster prevention in older adults, reduces the incidence of herpes zoster and PHN, despite an increased risk. The FDA approved it for individuals aged 60 and above. An inactivated Oka vaccine is being studied for herpes zoster prevention after stem cell transplantation.

**Cytomegalovirus (CMV)**

Cytomegalovirus (CMV), the largest herpesvirus, causes various clinical conditions from congenital neonatal infection to infectious mononucleosis in young adults. CMV infection is widespread, reaching up to60-70% in U.S. cities and nearly 100% in some parts of Africa. It manifests differently, CMV establishes latency in various host cells and can reactivate due to immunosuppression or other factors.

**Structure:** CMV salivary gland viruses) is ubiquitous in nature and can be found in both humans and animals. They are identified by the enlargement of infected cells and distinctive intranuclear inclusions. The viruses posses icosahedral shape measuring 150 to 200 nm in diameter. The four key structural components are: an outer lipid envelope, tegument, nucleocapsid, and an internal nucleoprotein core covering its genome. The viral envelope contains lipoproteins and over 33 structural proteins, crucial for cell entry. Notably, the tegument includes structural proteins like the pp65 antigen, pivotal in diagnosis. A 64 nm linear dsDNA genome harbours non-overlapping open-reading frames for more than 230 proteins. Among these proteins is DNA polymerase, essential for viral replication and the primary target for approved antiviral medications.

**Epidemiology:** In the United States, CMV infects approximately 30% of children by the age of 5 and over 50% of adults by the age of 40. CMV seroprevalence tends to be higher among women, older individuals, those with lower socioeconomic status, and in developing countries. Among women of reproductive age globally, CMV seroprevalence ranges from 45 to 100%.

Transmission of CMV occurs through contact with infectious bodily fluids such as blood, saliva, urine, tears, seminal fluid, cervical secretions, and breast milk. Solid organ and stem cell transplantation can also lead to infection, with CMV being the most common opportunistic infection among solid organ transplant recipients.

**Pathogenesis:** The virus gains entry into host cells through interactions between viral glycoproteins and cellular receptors. Once inside the cell, the viral genome is released and undergoes replication, leading to the production of viral proteins and new viral particles. CMV enters a latent state in various cell types, including endothelial cells, epithelial cells, smooth muscle cells, and fibroblasts. During latency, the virus can multiply, and it may be disseminated by peripheral monocytes and circulating endothelial cells to distant sites within the body. Primary infection triggers the production of CMV-specific IgM antibodies, followed by the development of IgG antibodies, which persist for life. When reactivation occurs, virions are released into the bloodstream and other bodily fluids, often resulting in symptoms, especially in immunocompromised individuals.

**Clinical presentation:** Cytomegalovirus (CMV) infection is widespread, with the majority of cases being asymptomatic and leading to prolonged latency, occasionally reactivating. Clinical disease can result from either intrauterine or postnatal infections.

**1. CMV in newborns and infants-** Congenital CMV infection can result from maternal infection during pregnancy and may lead to various clinical manifestations, ranging from mild to severe. Clinical disease occurs in a minority of cases and may include symptoms such as petechial rash, neurologic abnormalities, and sensorineural hearing loss. Transmission risk is highest with primary maternal infection, but it can also occur with superinfection in CMV-immune women.

**2. Congenital infections-** Intrauterine CMV infection can have devastating consequences, including fetal death or cytomegalic inclusion disease in newborns, which can be fatal. This condition is characterized by widespread symptoms like hepatosplenomegaly, jaundice, thrombocytopenic purpura, and hemolytic anemia. Notably, cytomegalic inclusion disease is a major cause of microcephaly, with additional manifestations such as chorioretinitis and cerebral calcifications resembling congenital toxoplasmosis. Thrombocytopenia and hemolytic anemia are common in both congenital CMV disease and CMV mononucleosis in healthy adults. Moreover, skin eruptions, including maculopapular and rubelliform rashes, may occur, potentially triggered by immunologic reactions to antigens exposed during acute CMV infection.

**3. Infection in infants and children-** Cytomegalic inclusion disease mainly impacts infants born to mothers experiencing primary CMV infection during pregnancy. Conversely, infants born to mothers with CMV reactivation usually develop chronic subclinical infections. Perinatal transmission can happen through genital secretions or breast milk. In older children and adults, primary CMV infections are often asymptomatic. However, there's a possibility of developing a heterophile, antibody-negative, infectious mononucleosis, especially after receiving transfusions of CMV-infected blood.

**4. CMV mononucleosis**- Primary CMV infection in young adults can result in an infectious mononucleosis syndrome, presenting with fever, lymphadenopathy, and relative lymphocytosis. While the majority of infectious mononucleosis cases are attributed to Epstein-Barr virus (EBV), around 21% are caused by acute CMV infection. CMV mononucleosis is characterized by systemic symptoms, predominant fever, and less pronounced lymph node enlargement compared to EBV mononucleosis.

**5. Hematologic manifestations-** In CMV mononucleosis, a characteristic feature is relative lymphocytosis, with atypical lymphocytes accounting for over 10% of lymphocytes in the peripheral blood smear. A landmark study demonstrated CMV infection in patients who were previously seronegative, with a median age of 29 years. Fever is common, and all patients typically recover with the presence of atypical lymphocytes in the blood.

**6. Neurological complications-** CMV-induced infectious mononucleosis may rarely lead to meningoencephalitis, especially in immunocompetent individuals, presenting with symptoms such as severe headache, photophobia, lethargy, and pyramidal tract findings. Diagnosis can be aided by detecting CMV DNA using PCR in cerebrospinal fluid.

**7. Cardiac involvement-** Complications of CMV mononucleosis include myocardial involvement or myocarditis, evidenced by abnormalities such as T wave inversion on electrocardiogram. Myocarditis associated with CMV infection can be severe and potentially fatal.

**8. CMV in immunocompromised individuals-**

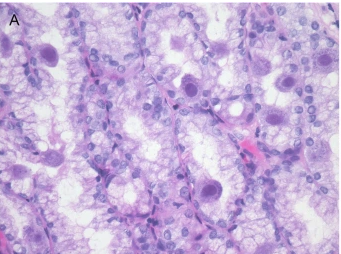
* **In AIDS patients-** CMV disease in AIDS patients primarily manifests as sight-threatening retinitis, especially when CD4+ T-cell counts drop below 50 cells/mm3. This condition results in retinal cell infection and progressive retinal destruction, potentially leading to irreversible blindness.
* **Others-** CMV disease can also occur in other immunocompromised patients, including those with leukemia, lymphoma, or inflammatory bowel disease. Manifestations are similar to those observed in transplant patients, with CMV colitis being increasingly recognized among patients with inflammatory bowel disease.

**9. CMV in transplant recipients-**

* **Solid-organ transplant recipients-** Primary infection occurs in CMV-seronegative recipients who receive organs from CMV-seropositive donors, constituting the highest-risk scenario for CMV disease. Secondary CMV disease can result from reactivation or superinfection and may manifest in various organs. CMV infection after organ transplantation can be asymptomatic or symptomatic, with tissue-invasive disease affecting organs such as the gastrointestinal tract and allograft.
* **Hematopoietic stem-cell transplant recipients-** They are at increased risk of CMV involving multiple organ dysfunction due to allostimulation and immunosuppressive drugs. CMV pneumonia is particularly common, and aggressive surveillance and preemptive strategies have decreased its incidence.

**Laboratory diagnosis:** CMV can be isolated from various bodily fluids such as urine, saliva, semen, and cervical secretions.

**1. Virus isolation-** Traditional method involving isolation and identification of CMV through cell cultures (Figure 52). Despite being specific, it's slow (takes 2-4 weeks) and less sensitive. Diagnosis can be confirmed by isolating the virus from bodily fluids like urine or saliva through inoculation into human fibroblast cultures. Early detection of viral growth can be achieved using shell vial cultures (16-48 hours) and staining the cells with fluorescently tagged antibodies against CMV early antigens. A simpler method involves observing cytomegalic cells, characterized by "owl's eye" inclusion bodies, in centrifuged deposits from urine or saliva, although this technique is less reliable.



CMV infected cell

**Figure 52: CMV owl’s eye inclusion in gastrointestinal tract of HIV patients (Sun et al., 2022)**

**2. Serology-**Serological techniques like complement fixation (CF), indirect hemagglutination (IHA), immunofluorescence (IF), and enzyme-linked immunosorbent assay (ELISA) are used. IgM antibody detection is particularly helpful in diagnosing congenital infections and may be necessary for screening blood or organ donors. Detects CMV-specific IgG and IgM antibodies in serum or plasma. Tests are Useful for diagnosing acute or previous CMV infections.

**3. Histopathology-** Involves examining tissue biopsies for characteristic CMV inclusion bodies using stains or immunohistochemistry.

**4. Molecular methods-**

* **Nucleic Acid Testing (NAT)-** Mainly PCR-based, it's highly sensitive for detecting CMV DNA. NAT can diagnose infection early, even before antibody production.

**5. CMV-Specific T-cell assays-** Measures cell-mediated immune response to CMV, aiding in predicting risk of CMV infection or disease after transplantation.

**6. Antigenemia-** Detects CMV antigens in neutrophils, helpful in diagnosing CMV in immunocompromised individuals. Can indicate disease severity and monitor treatment response.

**Prevention and treatment:** Preventive measures for CMV infection are primarily targeted at high-risk individuals like organ transplant recipients, those with weakened immune systems, and premature infants. Strategies include screening blood and organ donors, administering CMV immunoglobulins, and using prophylactic drugs such as acyclovir. However, acyclovir is not effective for treatment. Ganciclovir and foscarnet are preferred for treating CMV disease in AIDS patients, while vaccines remain experimental and not yet proven effective in protecting immunocompromised individuals.

**Epstein-Barr virus**

The Epstein–Barr virus (EBV) was the first human oncogenic virus discovered, capable of silently persisting in the body for a lifetime. In 1964, Epstein, Barr, and Achong identified the Epstein-Barr (EB) virus, within cultured lymphoma cells. This virus specifically targets B lymphocyte lineage cells, with receptors (CD 21 molecules) found only on human and certain subhuman primate B cells. Infection with EBV transforms these B cells, enabling their continuous growth in laboratory conditions.

**Structure:** EBV possesses a double-stranded DNA genome packaged within an icosahedral protein nucleocapsid, enveloped by a lipid membrane containing viral glycoproteins. Additionally, herpesviruses feature a protein layer called the tegument, situated between the capsid and envelope. The B95-8 laboratory strain of EBV, which was the first herpesvirus to have its genome sequenced, was found to have a 12-kilobase deletion. Subsequent studies have revealed that the wild-type EBV genome spans approximately 184 kilobases and codes for nearly 100 proteins.

**Epidemiology:** Serum antibody studies reveal widespread presence of Epstein-Barr virus (EBV) antibodies, with no gender bias. In developing countries, antibodies are acquired earlier, while in industrialized nations, 90-95% have them by adulthood. In the US and UK, around half seroconvert before 5 years old, with a second wave in the teens. EBV strains, type 1 and 2, can co-infect and transform B cells. Infectious mononucleosis is more common where primary EBV exposure is delayed, notably in adolescents from affluent backgrounds. Transmission primarily happens through saliva, especially during intimate oral contact like kissing (“Kissing Disease”), blood or marrow transfusion is rare. EB virus infection can lead to various clinical conditions, including infectious mononucleosis and EBV-associated malignancies like Burkitt's lymphoma and nasopharyngeal carcinoma.

**Replication:** EBV's host range is limited, primarily infecting B lymphocytes and nasopharyngeal epithelial cells. It binds to CD21 receptors via its major envelope glycoprotein, gp350, facilitating entry into host cells. The subsequent latent infection and growth transformation of B lymphocytes involve the expression of various viral proteins, including latent membrane proteins (LMP1 and LMP2), EBV nuclear antigens (EBNAs), and EBV-encoded RNAs (EBERs). These proteins promote B-cell growth and survival, mimicking signals necessary for normal B-cell function.

**Pathogenesis:** The Epstein-Barr (EB) virus first infects pharyngeal epithelial cells through CR2 receptors, then spreads to B lymphocytes, resulting in latent or lytic infections. Mononucleosis, characterized by B cell transformation, often presents with atypical lymphocytes. Reactivation of the virus leads to B cell proliferation, typically regulated by T cells but potentially causing lymphomas in immunodeficient individuals. Malaria in Africa might contribute to immune dysfunction in Burkitt's lymphoma. Although EBV triggers a complex immune response involving T cells and antibodies, it has evolved mechanisms to evade immune detection, such as mimicking cytokines like IL-10 and preventing host cell apoptosis. Histopathological findings in mononucleosis usually involve lymph node enlargement and splenic hyperplasia, with minimal hepatic changes, while nervous system involvement, though rare, can include neuronal degeneration and perivascular changes.

**Clinical presentation:** Clinical presentations of EBV are (Table 21)-

**Table 21: Characteristics of EBV infection**

|  |  |
| --- | --- |
| EBV infections | Characteristics |
| Infectious mononucleosis | primarily seen in non-immune young adults following primary infection; acute and self-limited illness; incubation period 4 to 8 weeks; symptoms include fever, sore throat, lymphadenopathy, and abnormal lymphocytes in peripheral blood smears; transient rash, particularly when treated with ampicillin, leading to a maculopapular rash due to an immune complex reaction  Complications: complications include hepatitis, hematological issues, neurological conditions, cardiac problems, pulmonary complications, and splenic rupture. Complications may prolong the recovery period, with some patients experiencing mental and physical fatigue during convalescence |
| X-Linked Lymphoproliferative Disease | A rare syndrome linked to mutations in the SAP gene; complications include hepatitis and hemophagocytic syndrome |
| Chronic Active Epstein-Barr Virus Infection | Extremely rare but severe form of infection; persistent illness lasting more than 6 months,; associated with elevated virus titers and major organ involvement.; poor prognosis; treatment limited to supportive care and therapies |
| Epstein-Barr Virus–Associated Hemophagocytic Lymphohistiocytosis | Excessive activation of lymphocytes and macrophages, leading to infiltration of various organs with prominent phagocytosis; children< 3 years are affected; symptoms include high fevers, pancytopenia, liver dysfunction, and coagulopathy |
| Oral Hairy Leukoplakia | An exception to asymptomatic replication, presents as a white "hairy" lesion, often on the tongue's lateral surface; occurs in immunosuppressed individuals; differs from oral candidiasis; diagnosis based on clinical appearance |
| Epstein-Barr Virus–Associated Malignant Diseases | **Lymphoproliferative Disease-**Induce uncontrolled proliferation of infected B lymphocytes among transplant recipients; lymphomatous infiltration of various organs. |
| **Burkitt’s Lymphoma-** Endemic in equatorial Africa; characteristic chromosomal translocations involving the c-myc oncogene; non-Hodgkin lymphoma characterized by rapidly growing tumors of the lymphatic system; risk increased in malaria endemic areas; virus's ability to immortalize B cells contributes to cancer |
| **Hodgkin’s Lymphoma-** Mixed cellularity and lymphocyte-depleted subtypes (particularly nodular sclerosis) ; role of virus has not been understood probably role in the transformation of B cells cause cancer; observed in younger patients. |
| Nasopharyngeal Carcinoma | 100% association with EBV; endemic regions are Southeast Asia, Africa and Mediterranean basin; infects epithelial cells in the nasopharynx and promotes their transformation into cancerous cells. |
| Multiple Sclerosis (MS) and Other Autoimmune Diseases | EBV infection triggers such conditions; presence of EBV antibodies in the blood is associated with an increased risk of developing MS and other conditions |

**Laboratory diagnosis:**

**1. Hematologic manifestation-**

* **Initial phase-** leucopenia due to decreased polymorphs.
* **Later phase-** prominent leucocytosis, appearance of abnormal mononuclear cells (lymphoblasts). These cells have deeply basophilic vacuolated cytoplasm and kidney-
* Mild and self-limiting Neutropenia and thrombocytopenia

**2. Serology-**

* **Paul-Bunnell test-** Conventional diagnostic approach for infectious mononucleosis; relies on heterophile antibodies (found in 90% of cases) causing agglutination of sheep erythrocytes. The delayed appearance of these antibodies might be linked to prolonged recovery periods. Commercial spot kits are available for detection. The infectious mononucleosis syndrome can result from infections by other agents such as cytomegalovirus and toxoplasmosis or occur as a reaction to non-infectious stimuli. However, the heterophile Paul-Bunnell test is positive only in EBV infections.
* **Differential agglutination test-** Confirmation requires absorbing agglutinins differently with guinea pig kidney and ox red cells. Treatment with guinea pig kidney and ox red cells removes Forssman antibody. Ox red cells remove the infectious mononucleosis antibody.; largely replaced by slide agglutination test using sensitized horse erythrocytes.

**3. Specific EBV antibodies-**

* The IgM antibody to virus capsid antigen (VCA) appears soon after primary infection and disappears in 1-2 weeks.
* IgG anti-VCA antibody persists throughout life, indicating past or recent infection.
* The appearance of antibody to EBV nuclear antigen (EBNA) is a marker for primary infection.
* Antibodies to early antigens (EA) are present in high titers in EBV-associated lymphomas
* Tests include immunofluorescence and ELISA.

**4. Virus detection-**

* EBV can be cultured from oropharyngeal washings or circulating lymphocytes, but routine cultivation is not widely available.
* Rapid diagnostic techniques based on DNA hybridization or monoclonal antibody techniques are limited in clinical use.
* EBV DNA can be detected in plasma, with viral load initially high but declining rapidly during the course of infection.

**5. Other laboratory abnormalities-** Liver function tests often show abnormalities, with elevation of hepatocellular enzymes and mild bilirubin elevation being common. Cryoproteins, primarily mixed cryoglobulins of IgG and IgM classes, are present in the majority of patients.

**Treatment & prevention:** Treatment for infectious mononucleosis relies mainly on supportive care, including activity management and pain relief, as most patients recover without specific therapy. Antiviral drugs like acyclovir have limited effectiveness, while corticosteroids are reserved for complicated cases. In lymphoproliferative diseases, reducing immune suppression and adoptive immunotherapy, such as rituximab, are pivotal. Oral hairy leukoplakia may respond to antiviral agents or topical therapy, especially in HIV-related cases. Prevention strategies involve public health measures and vaccine development. Developing an EBV vaccine faces challenges due to its mild presentation and association with malignancy. Current efforts explore targeting viral glycoprotein gp350 or known EBV MHC class I restricted cytotoxic T lymphocyte epitopes.

**Human herpesvirus 6 (HHV-6)**

HHV-6 was discovered in 1986 from patients with lymphoproliferative disorders and HIV. It exists in two variants, HHV-6A and HHV-6B, with HHV-6B being the main cause of exanthem subitum (ES). HHV-7, isolated in 1990, also contributes to ES.

**Structure:** HHV-6 belongs to β-herpesvirus family, exhibiting similarities with cytomegalovirus in terms of shared viral proteins and genomic structures. HHV-6A and HHV-6B, with a 90% nucleotide sequence similarity, possess distinct sequences and cell tropism, suggesting they could be classified as separate herpesvirus species. CD46 serves as the receptor for HHV-6, interacting with a complex of viral glycoproteins including gH, gL, gQ1, and gQ2. The HHV-6 genome comprises approximately 165 kilobase pairs of DNA.

**Epidemiology:** Over 95% of adults are seropositive for HHV-6. The virus is transmitted horizontally via saliva, with outbreaks reported in daycare centers. It infects various cells and is detected in pregnant women, suggesting potential fetal transmission.

**Pathogenesis:** HHV-6 infects various immune and non-immune cells, establishing latent and persistent infections. It impacts immune responses, including increasing susceptibility to HIV infection and inhibiting viral replication in co-infected cells.

**Clinical presentation**: HHV-6 causes infantile fever, febrile seizures, and exanthem subitum. It's associated with about one-third of febrile seizures in children up to 2 years old. Exanthem subitum typically presents with high fever and a rash.

**Exanthem subitum**, also known as Roseola Infantum or Sixth Disease, can be caused by either HHV-6B or HHV-7. In the United States, approximately 25% of HHV-6 infections lead to exanthem subitum, while in Japan, this figure is about 75%. The disease typically starts with a high fever lasting 3 to 4 days, followed by the development of a rash. This rash, which is macular or maculopapular in nature, initially appears on the neck or trunk before spreading to the extremities. Other symptoms may include cough, lymphadenopathy, erythema of the tympanic membranes, conjunctivitis, eyelid swelling, bulging fontanelles, diarrhea, or Nagayama spots. The median duration of symptoms is 9 days, and rare complications may include febrile seizures, meningitis, and encephalitis. Additionally, patients may exhibit changes in their blood cell counts, such as leukocytosis followed by leukopenia, with a relative increase in lymphocytes and, in some cases, thrombocytopenia.

**Diagnosis:** Serologic tests, PCR, and culture help diagnose HHV-6 infection. Detection of viral DNA in plasma may differentiate acute infection from latent states.

**Treatment:** HHV-6 is sensitive to ganciclovir, foscarnet, and cidofovir in vitro, but controlled studies on their efficacy in immunocompromised patients are lacking. Ganciclovir-resistant strains have been reported.

**Human herpesvirus 7 (HHV-7)**

HHV-7 was discovered by Frenkel and colleagues in 1990 in a healthy individual and subsequently linked to exanthem subitum. HHV-6 and HHV-7, collectively known as Roseolovirus, are ubiquitous viruses with more than 90% of adults having antibodies to both. HHV-7 genome size is about 145 kilobase pairs of DNA.

**Epidemiology:** HHV-7 infections typically occur later in childhood compared to HHV-6 infections. The virus is commonly transmitted through saliva, with a significant percentage of pregnant women testing positive for HHV-7 DNA. Reactivation rates are notable among transplant recipients.

**Pathogenesis:** HHV-7 exhibits a narrower tissue tropism compared to HHV-6, primarily infecting CD4+ T cells, epithelial cells in salivary glands, and cells in the lungs and skin. It induces degradation of MHC class I molecules.

**Clinical presentation:** Primary HHV-7 infection may be asymptomatic or present with fever, febrile seizures, or nonspecific symptoms like upper respiratory tract disease. HHV-7 is less frequently associated with CNS diseases compared to HHV-6.

**Laboratory diagnosis:** Seroconversion is the common diagnostic method for HHV-7, while detection of viral DNA in blood is more indicative of acute infection. HHV-7 has been cultured from PBMCs in research settings.

**Treatment:** HHV-7 is sensitive to antiviral drugs like foscarnet and cidofovir in vitro, but clinical efficacy in vivo is not well-established.

**Human herpesvirus 8 (HHV-8)**

Kaposi’s sarcoma-associated herpesvirus (KSHV), also known as HHV-8, was identified in 1994, linking it to Kaposi’s sarcoma (KS) and other diseases like primary effusion lymphoma (PEL) and multicentric Castleman’s disease. The initial description of KS dates back to 1872 by Moritz Kaposi, which depicted an aggressive and fatal disorder. However, the disease later evolved into a relatively milder form, with a resurgence in recognition during the AIDS epidemic in the 1980s.

**Virus characteristics:** HHV-8 belongs to the gamma-2 herpesvirus family and shares similarities with other rhadinoviruses. It exhibits both latent and lytic infection cycles, with the majority of infected cells being latently infected. Latent infection promotes cell survival, contributing to tumorigenesis. Lytic infection, although less common, can still influence tumorigenesis by stimulating nearby cell growth.

**Pathogenesis and epidemiology:** HHV-8 plays a significant role in Kaposi’s sarcoma, primary effusion lymphoma, and multicentric Castleman’s disease. Its prevalence varies worldwide, with higher rates in sub-Saharan Africa. Transmission occurs through sexual contact, saliva, vertical transmission, and blood transfusion. The virus is associated with specific diseases but usually remains dormant, causing disease only when immune suppression occurs.

**Clinical presentation:**

**Primary infection-** The primary infection syndrome for KSHV is not well-defined, often going unnoticed or asymptomatic. In children and HIV-negative men, primary infection presents with mild symptoms like rash, upper respiratory issues, and fever. In HIV-infected individuals, symptoms include fever, joint pain, and lymphadenopathy, resolving within weeks. Transplant recipients from KSHV-positive donors may experience severe complications like disseminated KS or marrow failure. These cases indicate that primary KSHV infection is typically self-limiting in immunocompetent hosts but can have severe consequences in immunosuppressed individuals.

**Kaposi’s Sarcoma (KS)-** Kaposi's Sarcoma (KS) primarily affects the skin, progressing from patches to nodules (Figure 53). Initially, lesions are violaceous and later turn brown due to hemosiderin deposition. KS lesions consist of vascular spaces, erythrocytes, malignant spindle cells, and mononuclear cells. Four variants of KS exist, with different epidemiological patterns.

* **Classical KS-** occurs in elderly Mediterranean men, while
* **Endemic KS**- prevalent in sub-Saharan Africa
* **Epidemic KS-** affect HIV-infected individuals, often more aggressively, involving various organs and mucosal sites.
* **Iatrogenic KS-** develops in immunosuppressed individuals, particularly transplant recipients, and may regress with immune modulation.

The introduction of highly active antiretroviral therapy (HAART) has reduced KS incidence in developed countries, but it remains the highest among cancers in HIV infection. Diagnosis is confirmed by biopsy, although early stages can be challenging to recognize. Differential diagnosis includes bacillary angiomatosis. Measurement of KSHV viral loads aids in diagnosis and predicting disease progression, though its clinical utility is limited.

**(c)**

**(b)**

**(a)**

**Figure 53: (a) Macular lesions in back and nodules at the arm; (b) KS plaques seen on the legs; (c) KS gingival nodules (Cesarman et al., 2019)**

**Other syndromes-** Various syndromes have been linked to KSHV infection, including pemphigus, bullous pemphigoid, sarcoid, multiple myeloma and primary pulmonary hypertension.

**Treatment & prevention:** Treatment options include steroids and chemotherapy. Several drugs show potential in inhibiting the lytic replication of KSHV, including ganciclovir, foscarnet, cidofovir, and adefovir, though acyclovir is not effective in this regard. Developing drugs that target latent infection could significantly advance KSHV-associated disease treatment. Lytic KSHV infection plays a role in the virus's biology and transmission. Studies have shown that ganciclovir reduces the incidence of KS in AIDS patients with CMV retinitis and that oral valganciclovir reduces oropharyngeal KSHV shedding, suggesting a potential for reducing KSHV transmission. However, the adverse effects of these drugs may limit their widespread use for prevention.

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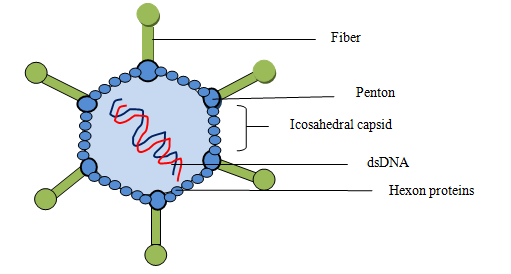
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**Adenovirus**

In 1953, Rowe and colleagues made a significant discovery by identifying a new cytopathic agent in adenoid samples, later recognized as adenoviruses, which were found in cases of acute respiratory disease (ARD).. Over the subsequent twenty years, more than 30 adenovirus serotypes were distinguished, leading to respiratory infections, keratoconjunctivitis, and infantile gastroenteritis. Adenovirus infections were prevalent, contributing to 5-10% of febrile illnesses in infants and young children.

**Structure:** Adenoviruses (Figure 54) possess a distinct structure characterized by a icosahedrons capsid, comprising 252 capsomeres. Among these, 240 hexons form the faces, while 12 pentons are located at the vertices. Each penton carries a slender fibre, giving the virion a space vehicle shape. Adenoviruses are nonenveloped containing dsDNA, associated with two major core proteins, features a 55-kDa protein covalently attached to its 5′ end. The virus exhibit a precise host range, infecting humans, animals, and birds.



**Figure 54: Structure of adenovirus**

**Resistance:** Adenoviruses exhibit stability for around a week at temperatures of 3 to 7°C and are rendered inactive at 50°C. They resist ether and bile salts. They can survive various environmental conditions, including temperature changes, pH fluctuations, and humidity levels, remaining viable on surfaces and in water for extended periods, facilitating their transmission through direct contact or aerosols. Chlorine-based compounds, quaternary ammonium compounds, alcohols, and hydrogen peroxide show varying effectiveness against adenoviruses. UV irradiation can reduce infectivity, but complete inactivation may require prolonged exposure or high-intensity light.

**Classification:** The Adenoviridae family (Table 22) comprises two genera: Mastadenovirus, which infects mammals, and Aviadenovirus, which infects birds. There are more than 50 adenovirus serotypes isolated from humans, categorized into groups A-F. Serotypes mostly found in HIV infected individuals. Mammalian adenoviruses share a common antigen detectable through complement fixation. Type-specific antigens are located on pentons and fibers.

**Table 22: Classification of adenovirus**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Groups (Subgenus) | Serotypes | Hemagglutination | | Tumorogenicity  (in vivo) | Cell transformation |
| **Groups** | **Cells** |
| A | 12,18,31 | IV | Little or no agglutination | High | + |
| B | 3,7,11,14,16,21,34,35 | I | Monkey (Complete agglutination) | Moderate | + |
| C | 1,2,5,6 | III | Rat (Partial agglutination) | Low or none | + |
| D | 8-10,13,15,17,19,20, 22-30, 32, 33,36-39,42-47 | II | Rat (Complete agglutination) | Low or none | + |
| E | 4 | III | Rat (partial agglutination) | Low or none | + |
| F | 40,41 | III | Rat (partial agglutination) | Low or none | + |

**Pathogenecity:** Adenovirus pathogenesis involves several stages and mechanisms that contribute to the development of infection and disease. Upon initial exposure, adenoviruses can enter the body through various routes, such as respiratory droplets or fecal-oral transmission. The following are key aspects of adenovirus pathogenesis:

* **Entry and tissue tropism-** Adenoviruses typically target epithelial cells lining mucosal surfaces in the respiratory, gastrointestinal, and ocular tracts. The specific tissue tropism often depends on the viral serotype and the presence of cellular receptors on target cells.
* **Viral replication**- Once inside host cells, adenoviruses undergo replication, leading to the production of viral proteins and nucleic acids. This replication process can cause cellular damage and trigger immune responses.
* **Cytokine release and inflammation-** Adenovirus replication and immune activation lead to the release of pro-inflammatory cytokines and chemokines, which contribute to the recruitment of immune cells to the site of infection. This inflammatory response can cause tissue damage and contribute to the symptoms of adenovirus-associated diseases.
* **Dissemination and systemic infection-** In severe cases or immunocompromised individuals, adenovirus infection can spread beyond the initial site of entry to other organs and tissues, leading to systemic infection. This dissemination can result in more severe manifestations of disease and complications.
* **Chronic or latent infection-** Some adenovirus serotypes can establish persistent or latent infections, where viral replication is maintained at low levels over an extended period. Chronic or latent infections may contribute to long-term health effects and complications, particularly in immunocompromised individuals.

**Epidemiology:** Adenovirus infections are widespread, with most individuals experiencing them by age 10, often with multiple serotypes involved. Roughly half of adenovirus infections go unnoticed, leading to underreporting in epidemiological data. In infants and young children, adenovirus infections are responsible for 5-10% of febrile illnesses, mainly affecting the respiratory tract. Adenovirus epidemics occur in winter or early spring but can happen throughout the year without a distinct seasonality. Transmission can occur through exposure to infected individuals, inhalation of aerosolized droplets, conjunctival inoculation, fecal-oral spread, or contact with contaminated surfaces. The incubation period ranges from 2 to 14 days, and latent adenovirus can reside in various tissues for years, potentially leading to reactivation in severely immunosuppressed patients. Asymptomatic carriage of the virus can persist for weeks or months, facilitating rapid spread during epidemics, especially in closed populations like hospitals, neonatal nurseries, long-term care facilities, and schools.

**Clinical presentation:** Adenovirus infections, while mostly self-limiting, can be severe, especially in specific demographics and with certain serotypes.

**1. Respiratory tract disease-**

Adenoviruses contribute significantly to upper respiratory tract infections and pneumonias in children, with common presentations including pharyngitis, tracheitis, and otitis media. Below are the conditions caused by different serotypes of the virus-

* **Pharyngitis and tonsillitis-**Adenoviruses stand out as the primary cause of nonbacterial pharyngitis and tonsillitis, often resembling a febrile common cold. Types 1-7 of adenovirus are frequently implicated in these respiratory infections.
* **Pneumonia-**In adults, adenovirus types 3 and 7 are linked to pneumonia, mirroring primary atypical pneumonia. However, in infants and young children, particularly type 7, pneumonia can progress to severe and sometimes fatal conditions.
* **Acute Respiratory Diseases (ARD)-** Outbreaks of acute respiratory diseases, often observed among military recruits, are commonly attributed to adenovirus serotypes 4, 7, and 21. These outbreaks represent a significant health concern in such populations.

**2. Ocular disease-** Adenoviruses can cause both mild and severe ocular diseases.

* **Pharyngoconjunctival fever-** Pharyngoconjunctival fever, characterized by febrile pharyngitis and conjunctivitis, predominantly affects the civilian population. This syndrome is typically associated with adenovirus serotypes 3, 7, and 14.
* **Epidemic Keratoconjunctivitis (EKC)-** EKC represents a severe ocular condition often seen in epidemic form, often causing painful corneal infiltrates and blurred vision. Mainly attributed to adenovirus type 8, with less common occurrences associated with types 19 and 37. EKC is highly contagious and can lead to prolonged morbidity.
* **Acute follicular conjunctivitis-** This condition involves nonpurulent inflammation of the conjunctiva, characterized by the enlargement of submucous lymphoid follicles and pre-auricular lymph nodes. Adenovirus types 3, 4, and 11 are commonly associated with this form of conjunctivitis, which shares clinical similarities with chlamydial conjunctivitis.

**3. Gastrointestinal tract disease-** Adenoviruses are commonly detected in fecal samples, but their direct connection to intestinal diseases remains inconclusive. adenovirus serotypes 40 and 41 are strongly associated with infantile diarrhea. Additionally, adenovirus types 2, 3, 8, and 31 have been implicated in causing diarrheal illnesses Adenoviruses are also implicated in mesenteric adenitis, which can occasionally mimic appendicitis and lead to intussusception, especially in children.

**4. Genitourinary tract disease-** Acute hemorrhagic cystitis, characterized by hematuria, is a benign illness often linked to adenovirus infections, especially serotypes 11 and 21. Cases of hemorrhagic cystitis and tubulointerstitial nephritis have been observed in transplant recipients. Adenoviruses have also been associated with nongonococcal urethritis in adult males.

**5.Central nervous system disease-**Adenoviruses can cause meningitis and meningoencephalitis either independently or as complications of respiratory infections. While spinal fluid findings can vary, meningoencephalitis is frequently associated with severe pneumonia, primarily due to serotype 7, but also other serotypes like 1, 6, and 12.

**6.Other syndromes and complications-**Adenovirus infections (Table 23), particularly in immunocompromised (hematopoietic stem cell transplantation and solid organ transplantation)individuals, have been associated with myocarditis, myositis, arthritis, and pancreatitis. Disseminated adenoviral disease, notably observed in pediatric and immunocompromised patients, manifests with diverse symptoms and involves various serotypes, including 3, 7, 21, and 30.Despite being less prevalent due to highly active antiretroviral therapy (HAART), adenovirus infections in HIV/AIDS patients can still result in rare but serious complications such as hepatic necrosis, pneumonia, and systemic infections.

**Table 23: Adenovirus syndromes**

|  |  |
| --- | --- |
| Serotypes | Disease |
| 1,2,5,6 | Respiratory infections in children |
| 3,4,7,14,21 | Febrile illness, sore throat, pneumonia |
| 4,7,21 | acute respiratory distress (ARD) in military individuals |
| 3,7 | Follicular conjunctivitis (related to swimming pool) |
| 8,19,37 | Epidemic keratoconjunctivitis (shipyard eye) |
| 40,41 | Diarrhoea |

**Laboratory diagnosis:** In immunocompetent patients, mild and self-limited adenovirus infections often do not warrant diagnosis. However, for immunosuppressed individuals or in outbreak scenarios, diagnosis becomes crucial.

**1. Tissue culture-** Adenoviruses are isolated from various sources (throat, eye, urine, feces) through inoculation into tissue cultures. Preliminary identification involves-

* 1. Observing cytopathic effects,
  2. Complement fixation tests,
  3. Hemagglutination tests with rat and monkey erythrocytes
  4. Typing by neutralization tests.

Adenoviruses, except types 40 and 41, can be detected via routine tissue culture, with a typical cytopathic effect observed within days. Specialized tissue culture techniques employing trypsinised monkey kidney cells or transformed human embryonic kidney cells are essential for the growth of adenovirus 40 and 41, since they do not thrive in regular cell cultures. Stool ELISA is employed for their identification.

**2. Direct detection-** Electron Microscopy is utilized for detecting fecal viruses, providing direct visualization of viral particles.

**3. Antigen detection-** Antigen detection methods, such as immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA), provide rapid diagnosis when culture is unavailable. IFA is useful for detecting viral antigens in nasopharyngeal and ocular infections, aiding in rapid diagnosis.

**4. Serology-** Serological diagnosis requires demonstrating a rise in antibody titers in paired sera. Single-sample examination is inconclusive due to the presence of adenovirus antibodies in the population.

**5. Molecular diagnosis-** PCR assays are employed for detecting viral DNA, offering high sensitivity and specificity in diagnosing adenovirus infections. Real-time PCR facilitates viral load monitoring in immunocompromised patients.

**6. Histopathology-** Tissue examination reveals characteristic histopathological findings, including interstitial pneumonitis and bronchitis. Inclusions known as "smudge cells," typical of adenovirus infections, are observed.

**Treatment:** Currently, there are no approved antiviral medications specifically for adenovirus infections. Clearance of the virus in transplant patients typically relies on immune reconstitution**.** Pre-emptive cidofovir therapy has shown effectiveness in pediatric transplant recipients, yet larger studies are needed for confirmation. Immunotherapy, such as adoptive T-cell therapy, is showing promise in inducing T-cell responses in pediatric patients. Intravenous immunoglobulin has been inconsistently effective.

**Prevention:** Specific preventive measures are necessary primarily for controlling outbreaks within closed communities. practicing good personal hygiene, Avoiding close contact with sick individuals, maintaining clean environment, especially in crowded or communal settings like schools, daycares, and healthcare facilities, can help prevent the spread of the virus.

**Vaccination:** Vaccines targeting Adenovirus types 4 and 7, initially created for the U.S. military in 1971, were exhausted by 1999. After undergoing new clinical trials and being manufactured by a different company, the same live nonattenuated vaccine for AdV-4 and -7 was reintroduced for military use in the United States in October 2011. Notably, antibodies developed against AdV-4 and -7 may offer cross-protection against other serotypes.

**Gene therapy:** In recent decades, adenoviruses has been extensively studied as vectors for gene therapy and vaccination, since they exhibit a capacity to carry DNA inserts of up to 7 kb. Typically, these viruses are modified to be replication incompetent by removing the E1 gene, allowing the insertion of genes of interest. Numerous human clinical trials have utilized adenovirus vectors, benefiting from their advantages such as high titer production and broad cell infectivity. Commonly used serotypes include Ad5 and Ad2, which have been extensively studied and engineered for gene delivery purposes.

Adenovirus vectors are also being developed as vaccines for infectious diseases and cancer due to their ability to provoke robust cellular immune responses. These vaccines contain genes encoding pathogen-specific antigens, stimulating strong CD8+ T-cell responses. Adenovirus vectors are being studied for vaccine development against infectious diseases including emerging pathogens such as SARS-CoV-2 and malaria, tuberculosis, and HIV-1, although efficacy trials for some diseases have faced setbacks. Additionally, adenovirus vectors are being explored in cancer treatment to deliver genes controlling cell growth or inducing antitumor responses, with some advancing to phase II and III trials for head and neck cancers.

**Adeno-associated viruses (AAVs)**

Adeno-associated viruses (AAVs) are small, non-enveloped viruses that belong to the family Parvoviridae. Unlike adenoviruses, AAVs are not known to cause any human diseases and are considered relatively harmless. However, they have gained significant attention in biomedical research due to their ability to serve as efficient and safe gene delivery vectors..   
Electron microscopy has revealed the presence of small icosahedral viral particles measuring 20-25 nm in diameter in adenovirus preparations. These particles, termed defective viruses or adenosatellite viruses, are unable to replicate independently and rely on helper viruses (adenoviruses or herpesviruses) for efficient multiplication in host cells. AAVs have a single-stranded DNA genome of approximately 4.7 kilobases (kb). This genome contains two open reading frames (ORFs) encoding Rep and Cap proteins, which are essential for viral replication and capsid formation, respectively. Adeno-associated viruses (AAVs), also known as dependoviruses, are detectable through electron microscopy and specific serological tests like complement fixation or immunofluorescence using specific antisera. While types 1, 2, and 3 of AAVs are of human origin and naturally infect humans, type 4 originates from simians. AAV vectors offer several advantages for gene therapy applications, including their ability to transduce both dividing and non-dividing cells, low immunogenicity, and long-term gene expression.

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**Polyomaviruses**

Discovered in 1971, human polyomaviruses JC virus (JCV) and BK virus (BKV) are widespread globally but typically do not cause symptoms in individuals with a healthy immune system. However, in people with weakened immune systems, JCV can lead to progressive multifocal leukoencephalopathy (PML), while BKV can result in nephropathy, hemorrhagic cystitis, and ureteral stenosis. Additionally, three newer human polyomaviruses—WU, KI, and Merkel cell carcinoma virus (MCV)—have been identified. WU and KI viruses, found in respiratory tract secretions, are named after their respective discovery institutions. These polyomaviruses represent a growing understanding of viral infections and their potential health implications.

**Structure:** Polyomaviruses, characterized by their small size and circular double-stranded DNA structure, belong to the Papovaviridae family, along with papillomaviruses. These viruses are ubiquitous in nature and species-specific, affecting humans, monkeys (e.g., simian virus 40 [SV40]), and mice (e.g., mouse polyomavirus). The outer layer of the polyomavirus virion consists of a capsid composed of a single protein called capsid viral protein 1 (VP1), arranged in 72 pentameric capsomers on an icosahedral lattice. These capsomers have a distinctive knobby appearance due to their structure, with additional capsid proteins, VP2 and VP3, associated within each capsomer.

**Pathogenesis:** Polyomaviruses, like other non-enveloped viruses, are thought to enter host cells through endocytic pathways, often by engaging cell-surface glycans containing sialic acid residues. While murine polyomavirus, SV40, and BKV use gangliosides as entry receptors, MCV may also require interactions with heparan sulfate. JCV, on the other hand, may need both a serotonin receptor and a sialylated glycan for entry, possibly influencing its tropism for central nervous system cells.

Once inside the cell, polyomaviruses traffic through the endoplasmic reticulum before releasing their genome into the cytoplasm. In permissive cells, early genes drive cell-cycle progression and facilitate viral DNA replication. However, in many cell types, the late phase of the viral life-cycle is blocked, possibly leading to viral latency where the viral genome is stably maintained with minimal gene expression. This latency may help the virus evade immune clearance and persist long-term within the host.

JCV primary infection typically presents without symptoms, followed by clinical latency.In patients with PML, JCV infects brain oligodendrocytes and astrocytes, leading to lytic infection and demyelination. BK virus primary infection, usually asymptomatic, occurs in childhood, after which the virus remains latent in the kidney. BK viruria is often benign, but in immunocompromised patients, reactivation can cause nephropathy and hemorrhagic cystitis.

**Epidemiology:** The epidemiology of JCV and BKV underscores their widespread prevalence in human populations. The seroprevalence increases with age, indicating common childhood exposure. often as asymptomatic infections.

**JCV-** JCV transmission primarily occurs via respiratory routes or ingestion of contaminated food or water. Following primary infection, the virus remains latent in the kidneys and lymphoid tissues. Reactivation, common in immunocompromised individuals, can lead to progressive multifocal leukoencephalopathy (PML). Immunocompromised conditions, notably in individuals with HIV/AIDS or receiving immunosuppressive therapy, heighten the risk of JCV reactivation and subsequent PML. Although JC viruria is not influenced by immune status, viremia is typically observed in immunosuppressed individuals, particularly those with PML.

**BKV-** Following primary infection, BKV establishes latency in renal tubular epithelial cells. Reactivation of the virus can lead to BK virus-associated nephropathy in kidney transplant recipients or hemorrhagic cystitis in bone marrow transplant recipients. Routes of transmission include respiratory secretions, fecal-oral transmission, and potentially through blood transfusions or organ transplantation. Ureteral stenosis occurs in 0.5% to 6% of transplant recipients.

**Clinical presetation:** Clinical presentations of polyomaviruses include (Table 24)-

**Table 24: Clinical presentations of polyomaviruses**

|  |  |  |
| --- | --- | --- |
| Viruses |  | Clinical manifestations |
| JC virus | Classic Progressive Multifocal Leukoencephalopathy (PML) | Induces multifocal demyelination in the white matter of the CNS, leading to a variety of neurological deficits. Symptoms include coordination difficulties, gait imbalance, cognitive dysfunction, visual impairments, and limb weakness, seizures (in some cases) |
| Granule Cell Neuronopathy | infect cerebellar granule cell neurons, leading to a distinct syndrome known as JCV granule cell neuronopathy . This condition manifests as cerebellar atrophy, gait ataxia, and incoordination without significant demyelination. |
| Encephalopathy | Targets cortical pyramidal neurons, resulting in a syndrome characterized by gray matter lesions on MRI and presenting with global cognitive decline and aphasia |
| Meningitis | JCV testing is not standard in CSF)analysis for meningitis, some studies have identified JCV as the sole pathogen in patients with typical meningitis symptoms. The exact incidence of JCV meningitis remains uncertain due to the lack of routine JCV PCR testing in CSF |
| BK virus | Nephropathy | BK virus targets the genitourinary tract cells and can cause various urinary tract complications in immunocompromised individuals, including asymptomatic hematuria, hemorrhagic and non-hemorrhagic cystitis, and ureteral stenosis. Additionally, BKV can lead to interstitial nephritis in patients with HIV or those who have had renal transplants. Symptoms of BKV nephropathy mimic graft rejection, with a gradual rise in serum creatinine levels, hematuria, and fever. Risk factors for BKV detection include high donor BK antibody titer and the potential absence of the HLA-C7 class I allele in both donor and recipient. |
| Ureteral Stenosis | Patients with ureteral stenosis after renal transplantation often do not experience pain due to the lack of innervation in the transplanted kidney. However, they may present with urinary obstruction and elevated serum creatinine levels. |
| Hemorrhagic Cystitis | Diagnosis of BKV-induced hemorrhagic cystitis is considered in post-engraftment bone marrow transplant patients presenting with hematuria, dysuria, urgency, frequency, or suprapubic pain. Severe bleeding and clot formation may lead to complications such as urinary tract obstruction and renal failure. Affects 10% to 25% of cases, |

**Laboratory diagnosis:**

**I. JC virus-**

**a. Imaging Diagnosis-** Radiographic imaging, including CT scans and MRI, is crucial for diagnosing PML. Lesions typically appear as multiple white matter lesions in the brain, with MRI being more sensitive than CT scans. These lesions often spare the cortex and are characterized by hyperintense signals.

**b. Brain Biopsy-** Brain biopsy serves as the gold standard for diagnosing PML, revealing demyelinated areas with reactive gliosis and enlarged astrocytes.

**c. Serology-** Serological tests measure the levels of antibodies against JCV in the blood. However, serology is generally less useful for diagnosing active JCV infection and is more often used to determine previous exposure to the virus.

**d. PCR-** CSF examination via PCR is an alternative diagnostic method, particularly when brain biopsy is not feasible. PCR detection of JCV DNA in CSF is sensitive and specific, though its sensitivity has decreased in the post-HAART era.

**e. Immunohistochemistry-** Immunohistochemistry and in situ hybridization techniques can be used to localize JCV antigens or nucleic acids within brain tissue samples. These techniques provide valuable information about the distribution and localization of the virus within the brain.

**f. Flow Cytometry-** In cases where PML is suspected, flow cytometry can be used to analyze the immune status of the patient, particularly the CD4+ T cell count in HIV/AIDS patients. A lower CD4+ T cell count increases the risk of developing PML.

**II. BK virus-**

**a. Urine cytology-** Detection of BK viruria and renal insufficiency suggests BKV-induced nephropathy. Urine cytology may show decoy cells, but renal biopsy is often necessary for confirmation due to the nonspecific nature of these findings.

**b. Renal Biopsy-** Renal biopsy reveals viral replication in tubular epithelial cells, aiding in the diagnosis of BKV-induced nephropathy. However, biopsy carries a risk of false-negative results due to the focal nature of the disease.

**c. Urinalysis-** In cases of BKV-associated complications such as hemorrhagic cystitis, urinalysis may reveal blood in the urine, along with other abnormalities such as leukocytes and protein.

**d. PCR-** BKV DNA detection in plasma is more useful for ruling out nephropathy than diagnosing it. A high plasma BK viral load may predict nephropathy histologic manifestations, but BK virus is typically not detected in the blood of patients with hemorrhagic cystitis or ureteral stenosis.

**Prognosis:** PML is a fatal disease, particularly among HIV-positive patients, but the introduction of HAART therapy has significantly improved one-year survival rates, now reaching up to 50%. Prognostic markers for PML include lower JCV burden in CSF, detectable JCV-specific immune response in blood and CSF, and an inflammatory immune response in the CNS. While positive JCV serology is common in PML patients, it alone doesn't prevent viral reactivation. However, a robust cellular immune response, notably cytotoxic T lymphocytes targeting specific VP1 epitopes, is associated with better outcomes.

BK virus-induced nephropathy poses a risk of irreversible graft failure in 1% to 10% of cases. Histological signs of mild viral cytopathic changes with minimal inflammation or fibrosis indicate a better prognosis for transplanted kidneys. Early screening for BK viruria and viremia, followed by adjustments in immunosuppressive therapy, has reduced nephropathy incidence. In renal transplant recipients with BKV nephropathy, a strong BKV-specific cellular immune response correlates with decreased viruria and viremia, while the humoral immune response primarily affects viremia levels.

**Treatment:** No specific treatment is available for PML. Cytarabine, cidofovir showed effective in controlling JCV replication in certain studies, clinical trials have yielded uncertain results. In cases of inflammatory PML with associated brain swelling, short-term corticosteroid therapy may be used to mitigate inflammation.

Treatment for BK virus-induced nephropathy primarily involves reducing immunosuppression, which can sometimes lead to resolution of the infection. Various antiviral agents like cidofovir, leflunomide, quinolones, and intravenous immunoglobulin have shown mixed success in small clinical studies. In cases of ureteral stenosis,, reducing immune suppression can be beneficial. Further treatments primarily involve surgical interventions aimed at relieving the obstruction. For hemorrhagic cystitis, symptomatic treatment includes continuous bladder irrigations, pain management, hydration, diuresis promotion, and transfusions to maintain adequate platelet and hematocrit levels.

**Other polyomaviruses:** In 2007, two novel viruses, **KI** and **WU**, were characterized within the polyomavirus genus via phylogenetic analysis. Initially detected in respiratory samples through high-throughput screening, subsequent molecular studies unveiled their widespread presence in children's respiratory secretions. Notably, both KI and WU viruses displayed genetic and protein similarities with BKV and JCV. Despite consistent detection of their DNA in patients with respiratory diseases, no serological assay or isolation of mature viral particles has been achieved, leaving their infectious nature uncertain.

In 2008, **Merkel cell polyomavirus (MCV)** was identified, primarily found in up to 80% of tissues with Merkel cell carcinoma, a highly aggressive neuroectodermal tumor affecting immunocompromised individuals. In contrast to KI and WU, MCV shares greater resemblance with the African green monkey lymphotropic polyomavirus. Notably, all three newly discovered polyomaviruses lack a gene encoding the agnoprotein, with its implications yet to be fully understood.

Recent years have witnessed the unveiling of multiple new human polyomaviruses, including **HPyV6**, **HPyV7**, **TSPyV**, **HPyV9**, and **MWPyV**. The roles of these viruses in various conditions, including respiratory and urinary tract infections and potential oncogenic properties, remain under investigation.

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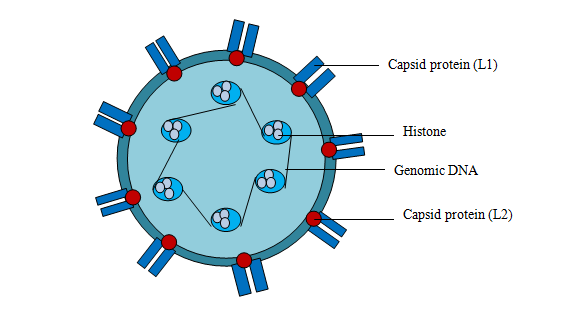
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**Papillomavirus**

The term 'papova' is derived from the names of viruses within this group, namely the papilloma and polyoma viruses. The family Papovaviridae comprises two genera: Polyomavirus, which includes simian vacuolating virus (SV40) and human polyomaviruses JC and BK, and Papillomavirus, which includes several genera, five of which cause human infections. Human papillomaviruses (HPVs) are widespread, have been found in higher vertebrates and are associated with epithelial tumors of the skin and mucous membranes. The infectious nature of human warts was recognized in the late 19th century, HPVs were determined as the causative agent. However, HPVs have proven challenging to study using standard virological techniques due to difficulties in propagation in tissue culture or laboratory animals.

**Structure:** HPVs are small nonenveloped viruses (Figure 56) with an icosahedral capsid composed of 72 capsomeres enclosing a double-stranded circular DNA genome. This genome comprises approximately 7900 base pairs and is organized into three functional (Table 25) regions: an upstream regulatory region, early region (E1-E7), and late region (L1 and L2).



**Figure 56: Structure of human papillomavirus**

**Table 25: HPV functional regions and their functions**

|  |  |  |
| --- | --- | --- |
| Functional regions | Functions | |
| Upstream regulatory region [long control region (LCR) or the non-coding region] | Located upstream of the early genes. Contains regulatory elements essential for the initiation of viral DNA replication and transcriptional regulation of early and late viral genes. | |
| Early region | E1 | E1 helicase activity unwinds the DNA helix to initiate viral replication. |
| E2 | A transcriptional regulator that controls viral gene expression and replication. It  also plays a role in viral genome maintenance and segregation |
| E3 | Plays a crucial role in the viral life cycle by facilitating viral evasion of the host immune response. E3 achieves this immunoevasion by interfering with the transport of I MHC-I molecules to the cell surface. E3 is not present in all HPV types, and its function may vary depending on the HPV genotype. |
| E4 | Contributes to viral replication and cytoskeletal alterations during the viral life cycle. |
| E5 | Encoded by high-risk HPV types, E5 contributes to cellular transformation by interfering with cellular signaling pathways, such as the epidermal growth factor receptor (EGFR) pathway. |
| E6 | Encoded by high-risk HPV types, E6 binds to cellular proteins such as p53, leading to its degradation and inhibition of apoptosis. E6 also promotes cellular proliferation and transformation |
| E7 | Encoded by high-risk HPV types, E7 binds to and degrades the retinoblastoma protein (pRb), leading to cell cycle dysregulation, increased cellular proliferation, and inhibition of apoptosis. E7 also interacts with other cellular factors involved in cell cycle control and apoptosis regulation. |
| Late region | L1 | Encodes the major capsid protein, which forms the viral capsid and is essential for virion assembly. |
| L2. | Encodes the minor capsid protein, which plays a role in virion assembly and possibly in viral DNA packaging |

**Pathogenesis:** HPV infects basal cells of stratified squamous epithelium, with various gross histologic appearances depending on the site and virus type. Replication of the virus in standard cell culture is challenging due to it requires keratinocyte differentiation and epithelial stratification. HPV infection likely begins with mild abrasion of the epithelium, with α6-integrin (HPV-6 receptor). Once inside the host cell, HPV DNA replicates as cells differentiate, establishing episomal DNA in basal layers and switching to rolling-circle replication in suprabasal layers. HPV employs host cell factors to regulate transcription and replication, particularly through E6 and E7 gene products, which interfere with cellular growth regulation by targeting tumor suppressor proteins. High-risk HPV types can lead to malignancy by disrupting cell growth regulation and gene expression, with integration of HPV DNA into the host genome and subsequent mutations contributing to cancer development over 10 to 20 years.

HPV DNA presence is observed even after treatment, with integration of viral DNA associated with malignant disease. The immune system plays a crucial role in resolving HPV infection, with clinical observations indicating severe disease in immunocompromised individuals. Genetic mutations and molecular changes in the immune response influence HPV infection and disease progression. The adaptive and innate immune systems, along with cellular responses, contribute to controlling HPV-related diseases. Immunogenetic factors, including HLA alleles, also play a role in the development of HPV-associated genital cancer.

**Clinical presentation:**

**1. Cutaneous Warts-** Cutaneous warts encompass various types and each type presents with distinct morphological features and locations. Despite being usually asymptomatic, they can cause discomfort and pain, especially when located over pressure points. Rarely, they may transform into verrucous carcinomas. The types are-

* **Deep Plantar Warts (Verrucae Plantaris)-** Deep plantar warts, also termed myrmecia, predominantly affect adolescents and young adults. They present as raised bundles of soft keratotic fibers, often causing pain and sometimes located on the palms of the hands.
* **Common Warts (Verrucae Vulgaris)-** Common warts are well-demarcated, hyperkeratotic papules with a rough surface. They can occur on various parts of the body, including the hands, feet, and mucous membranes. Morphological variants such as mosaic warts and filiform warts are observed.
* **Plane Warts (Verrucae Planae)-** Plane warts, commonly found in children, appear as slightly elevated papules with a smooth surface. They are typically distributed on the face, neck, and hands, sometimes forming intermediate warts when more protuberant.

**2. Epidermodysplasia Verruciformis-** Epidermodysplasia verruciformis is an autosomal recessive genodermatosis characterized by a large array of HPV types, often leading to lesions resembling flat warts or pityriasis versicolor. Malignant transformation into invasive squamous cell carcinomas may occur, particularly in sun-exposed areas, despite patients having normal resistance to other pathogens.

**3.** **Anogenital Warts-** Anogenital warts are flesh-colored to gray-colored papules, occurring predominantly in the anogenital area. They can range from small papules to larger plaques and may cause various symptoms. These warts may also transform into squamous cell carcinomas or be associated with intraepithelial neoplasias.

**4. Recurrent Respiratory Papillomatosis-**Recurrent respiratory papillomatosis is characterized by lesions in the upper respiratory tract, often leading to respiratory distress or obstruction. While less aggressive in adults, it can be life-threatening in children and may undergo malignant transformation, particularly in cases with lung involvement.

**5. Other HPV infections-** Other HPV infections manifest as oral squamous cell papillomas, oral condyloma acuminatum, or focal epithelial hyperplasia in the oral cavity. Conjunctival and periungual squamous cell carcinomas, along with various skin lesions, have also been associated with HPV infection, although the prevalence varies among different lesions.

**Epidemiology:** HPV infections manifest across a spectrum from clinical to subclinical, with genital/mucosal cases most common among those aged 15-44. Skin-to-skin contact primarily drives transmission, with sexual activity playing a significant role, especially in individuals with multiple partners or a history of STIs. Fomites also facilitate nonsexual transmission. Cutaneous warts spread through close personal contact, while anogenital warts mainly transmit sexually, though children can acquire them through contact with non-genital lesions. In children, respiratory papillomatosis likely occurs during birth, indicating intrauterine and perinatal transmission. HPVs, particularly high-risk types, are strongly linked to various cancers, notably cervical cancer. Coinfection with HSV-2 might influence cervical cancer initiation, although evidence is inconclusive. Factors like steroid hormone exposure, smoking, and genetics elevate the risk of cervical neoplasia. Coinfection with adeno-associated virus could potentially lower this risk by disrupting HPV oncogenes.

**Laboratory diagnosis:**

Cervical swabs, biopsies, and liquid-based cytology samples are used for sample collection. Samples should be handled according to guidelines to prevent degradation and contamination, ensuring reliable results. HPV cannot be cultured from clinical specimens, and immunologic assays are inadequate for detection. Cytology and histology are primary diagnostic tools, with molecular methods for detecting HPV DNA recently introduced.

1. **Clinical examination for warts-** Typically relies on clinical examination. Differentiating warts from other skin lesions such as calluses or nevi can be challenging. For example, deep plantar warts might be mistaken for calluses, but paring can reveal typical punctate, thrombosed capillaries.
2. **Colposcopy with acetic acid application-** Colposcopy is a diagnostic procedure used to closely examine the cervix, vagina, and vulva for signs of disease, particularly after abnormal results from a Pap smear or HPV test. This procedure is typically performed in a gynecologist's office and involves the use of a colposcope, an instrument with a magnifying lens and a light, which provides an illuminated and magnified view of the cervical tissues. Acetic acid helps highlight abnormal cells by turning them white, making them more visible.
3. Biopsy is essential for lesions with concerning features or to confirm diagnoses, especially for epidermodysplasia verruciformis.
4. **Cytology (Pap smear)-** Screens for cervical abnormalities. Examination of cells scraped from the cervix under a microscope to detect precancerous or cancerous changes. Subject to interpretation variability and less specific for HPV types. The Papanicolaou-stained (Pap) smear, introduced in 1949, remains the primary method for detecting high-risk HPV. It has significantly reduced cervical cancer incidence and mortality. The Bethesda System, updated in 2001, classifies squamous cell abnormalities into four categories: ASC, LSIL, HSIL, and squamous cell carcinoma. However, the Pap smear has limitations, including a high false-negative rate due to sample inadequacies and human error.
5. New specimen collection and processing methods, such as the ThinPrep and PrepStain systems, improve the accuracy of Pap smears by preserving cell structure and reducing contaminants. These methods have shown statistically significant improvements in detecting precancerous lesions and are better at predicting dysplasia presence compared to conventional Pap smears. Automated systems like the AutoPap 300QC and PapNet assist in consistent and objective Pap smear evaluation. These systems reduce human error but add expense.
6. **HPV DNA testing-** Detects the presence of high-risk HPV types associated with cervical cancer. Techniques like immunohistochemistry and in situ hybridization detect HPV antigens or nucleic acids. PCR-based methods, including type-specific and general primer PCRs. The FDA-approved Hybrid Capture assay is widely used for clinical HPV DNA detection, although it is not recommended for general population screening.
7. **HPV mRNA testing-** The In-Cell viral load test detects mRNA of HPV's E6 and E7 genes, indicating active genes causing malignant changes. This test can be automated and offers high sensitivity but lower specificity compared to Pap smears.
8. Positive HPV DNA or mRNA test Indicates the presence of high-risk HPV. Positive results require further clinical evaluation, such as colposcopy and possible biopsy. Negative tests suggest a low risk of significant cervical disease, allowing for longer screening intervals.
9. **Hybrid capture II-** uses RNA probes to hybridize with HPV DNA and detects the virus via chemiluminescence. It identifies a group of high-risk HPV types.
10. **PCR-** Real-time PCR provide quantitative data on viral load. High sensitivity and specificity able to detect multiple HPV types and are suitable for primary screening and triage.
11. **Genotyping and sequencing-** Genotyping identifies specific HPV types, crucial for understanding epidemiology and patient management. Sequencingdetermines the exact sequence of HPV DNA, providing precise genotyping. Next-Generation Sequencing (NGS- High-throughput sequencing) provides comprehensive data on HPV types present in a sample.

**Treatment:** Noninvasive lesions are typically treated with cryotherapy or laser therapy, while loop electrosurgical excision procedures (LEEP) are preferred for cost-effectiveness and tissue preservation. Imiquimod (topical), cidofovir (antiviral) and podophyllin (immunomodulator), have shown promise in treating HPV-associated warts.

**Cervical cancer and high-risk HPV infection:** Decades of research have established that high-risk HPV types are a precursor to cervical cancer. The progression of cervical cancer typically follows a continuous disease process from mild cervical intraepithelial neoplasia (CIN1) to more severe neoplasias (CIN2 or CIN3) and eventually to invasive cancer. . CIN1 is viewed as a self-limited sexually transmitted HPV infection, while CIN2 and CIN3 are considered true precursors to cervical cancer. High-risk HPV infection often occurs early in life, persists, and, combined with other factors, can lead to a gradual progression to severe disease. Early detection and treatment of HPV in precancerous lesions are crucial for preventing cancer progression.

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**Parvovirus**

"Parvum" means small in Latin, and the Parvoviridae family comprises some of the smallest DNA viruses that infect mammalian cells. Parvoviridae is divided into two subfamilies: Parvovirinae, which infect vertebrates, and Densovirinae, which infect invertebrates. The Parvovirinae subfamily is further divided into five genera: Parvovirus, Dependovirus (human adeno-associated viruses), Erythrovirus, Bocavirus, and Amdovirus, based on their transcription maps, replication methods, and sequence homology. The most studied is Parvovirus B19 (B19V), classified under the Erythrovirus genus.

**Parvovirus B19**

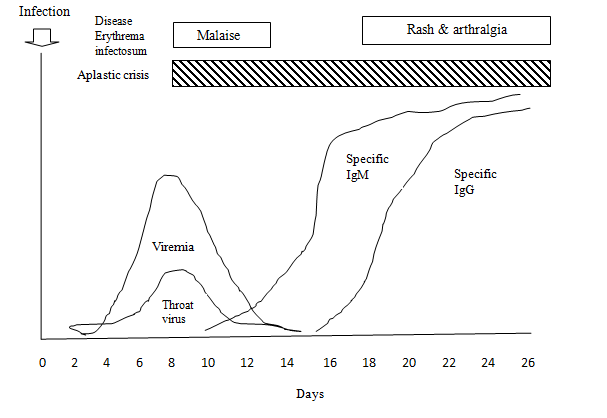
B19 virus was discovered in 1974 during hepatitis B surface antigen tests, when sample 19 in panel B (hence the name B19) showed an unexpected "false positive" result.

**Structure:** Parvoviruses are small viruses, approximately 20 nm in size, with a single-stranded DNA genome. They rely on the host cell's DNA machinery for replication. Among parvoviruses, only Parvovirus B19 is medically significant for humans. These nonenveloped viruses are about 22 nm in diameter and exhibit icosahedral symmetry. The major capsid protein VP2 forms most of the icosahedral capsid, with a small proportion of the larger VP1 protein. VP1 is not necessary for capsid formation but is involved in viral infectivity due to a phospholipase A2 motif.

**Resistance:** Due to the lack of an envelope and minimal DNA content, B19 virus is resistant to physical inactivation. It can be inactivated by heat at low protein concentrations but withstands 56°C for over 60 minutes and 80°C for 72 hours at high concentrations. B19 virus is stable in lipid solvents like ether and chloroform but can be inactivated by formalin, β-propiolactone, oxidizing agents, and γ-irradiation.

**Pathogenesis:** Parvovirus B19 targets human erythroid progenitor cells and CD36-positive erythroblasts in the bone marrow (Figure 57). The virus is difficult to culture outside of primary erythroblasts. Humans are only known hosts, as primates are resistant to B19 virus but have related erythroviruses. The specificity of the virus is due to the presence of globoside (P antigen) on erythroid lineage cells, endothelial cells, and fetal myocytes. Individuals lacking P antigen are resistant to B19 virus. The infection is cytotoxic and infection leads to a temporary cessation of red cell production. Fetal infection occurs when B19V crosses the placenta, infecting fetal erythroid precursors and cardiac myocytes, leading to severe anemia and potentially myocarditis and heart failure.

**Transmission:** Transmission of B19V is primarily via the respiratory route, though blood-borne transmission has been reported. Virus particles are detectable in throat secretions about one week after infection. The initial phase of infection involves viremia and flu-like symptoms, followed by the immune response phase with rash and arthralgia as the virus becomes undetectable. Chronic B19 virus infection and anemia can occur in immunodeficient patients, such as those with leukemia or AIDS. Early detection involves identifying the virus in the blood.



**Figure 57: Pathogenesis of Parvo B19**

**Immune response:** In patients with fifth disease, high-titer viremia is absent, as symptoms arise from immune complex formation. IgM antibodies can remain in the serum for several months after exposure, while IgG antibodies persist for life and increase with reexposure. IgA antibodies specific to B19V may help protect against nasopharyngeal infection.

Initially, the antibody response in immunocompetent individuals targets the major capsid protein VP2, but as the immune response matures, it increasingly targets the minor capsid protein VP1. Patients with persistent B19V infection typically have antibodies to VP2 but not VP1. The cellular immune response to B19V infection involves significant CD8+ and CD4+ responses, both essential for viral clearance.

**Clinical presentation:** The virus causes various diseases depending on the host's immunologic and hematologic status.

**1. Erythema Infectiosum (fifth disease)-** It presents in two phases.

* **Prodromal phase-** About 7 to 8 days after infection, a prodromal phase with flu-like symptoms such as headache, malaise, chills, and fever occurs. This is followed by an asymptomatic week.
* **Rash phase-**The second phase, 17 to 18 days post-infection, involves a mild fever and a characteristic maculopapular rash. The rash (Figure 58a) starts with pronounced redness on the cheeks ("slapped-cheek" appearance) and spreads to the trunk and limbs (Figure 58b), becoming reticular and disappearing within 1 to 3 weeks.
* **Arthropathy-** In adults, acute arthralgia can be observed with mild flu-like symptoms, joint involvement particularly symmetric arthritis in the hands, wrists, ankles, and knees, usually resolving within 2 to 4 weeks.
* **Complications-** Complications like transient lymphopenia, neutropenia, and thrombocytopenia are rare.

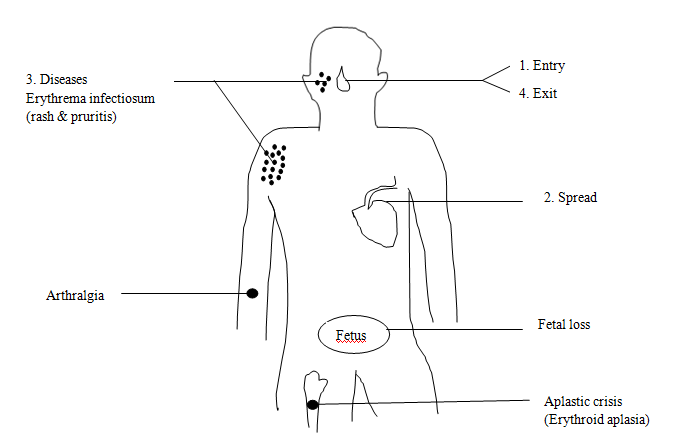
**(b)**

**(a)**

**Figure 58: Erythema Infectiosum - (a) Slap cheek appearance (b) rashes in arm** (Kostolansky and Waymack, 2024)

**2. Aplastic crisis-** An acute, self-limiting aplastic crisis can occur in individuals with hemolytic anemias, such as sickle cell anemia and β-thalassemia intermedia, following B19 virus infection. . In immunocompromised individuals, it can lead to persistent anemia. The disease is characterized by severe anemia, critically low hemoglobin, reticulocytopenia, and sometimes leukopenia and thrombocytopenia. Bone marrow examination reveals a complete absence of erythroid precursors. Although the anemia resolves on its own, blood transfusions are often necessary until the bone marrow recovers

**3. Fetal hydrops-** B19 virus infection (Figure 59) in pregnant women can lead to intense viremia, allowing the virus to cross the placenta and infect the fetus. The fetus, unable to effectively combat the virus, may experience prolonged viral replication. Infection during the first and second trimesters increases the risk of fetal abortion and nonimmune hydrops fetalis (fluid accumulation in tissues and cavities causing swelling) due to severe fetal anemia and edema. Third-trimester infections may lead to stillbirths.



**Figure 59: Clinical presentation and pathogenesis of Parvo B19**

**Laboratory diagnosis:**

* **Virus Detection-** The virus is challenging to isolate from clinical specimens, so detection relies on identifying viral DNA. High-titer B19 virus DNA can be detected in serum using dot-blot hybridization (sensitivity > 106 genome copies/mL) or quantitative PCR.
* **Antigen detection-** Antigen detection in serum is possible through countercurrent immunoelectrophoresis, radioimmunoassay, and enzyme immunoassay, with the latter two being sensitive enough for throat secretion detection.
* **Antibody detection-** B19 IgM persists for 2 to 3 months post-symptom onset, while IgG remains detectable much longer, likely for life, although it may fall below detection levels with current assays.
* **Detection of fetal infection-** Fetal infection can be confirmed via amniotic fluid sampling, fetal blood sampling, or postmortem tissue analysis.

**Treatment:** In most children and adults, the infection is benign, self-limiting, and results in lifelong immunity, requiring no treatment beyond symptomatic relief. Arthralgia and arthritis associated with the infection generally respond to nonsteroidal anti-inflammatory drugs. Specific treatment (blood transfusion or IV IgG) is necessary for patients with hematologic diseases or persistent infections.

**Other Human Parvoviruses**

**Human Dependoviruses (Adeno-associated viruses or AAVs)-** These are small non-enveloped icosahedral viruses that require helper viruses (adenovirus or herpesvirus) to replicate efficiently in host cells. They do not cause diseases in human or animal (except 9 strains such as 1, 2, 3, 8, 9) Due to their lack of pathogenicity and ability to integrate into the human genome, dependoviruses are widely used as vectors in gene therapy.

**Human Bocavirus-** Human bocavirus were first identified in respiratory samples from Swedish children. They can be detected by PCR in respiratory secretions, blood, and feces. It is a common early childhood infection causing respiratory illnesses in young children. Adult infections are rare, typically seen in immunocompromised individuals.

**Human Parvovirus 4 (PARV4)-** Associated with blood-borne transmission and often found in individuals with underlying risk factors such as injection drug use, haemophiliacs or HIV infection. The pathogenicity of Parv4 is still unclear, and it has not been cultured.

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