NAME OF THE COURSE WORK
VIROLOGY

UNIT-IV
ANIMAL VIRUSES

NAME OF THE COURSE TEACHER
Dr. V. RAJESH KANNAN

- **Myxovirus,** any of a group of viruses of the families Orthomyxoviridae (agents of influenza) and Paramyxoviridae, members of which can cause the common cold, mumps, and measles in humans.
- The virus particle is enveloped in a fatty membrane; is variable in shape, from spheroidal to filamentous, and in size, from 60 to 300 nanometres (1 nanometre = 10^{-9} metre) in longest dimension; is studded with spike like protein projections; and contains ribonucleic acid (RNA).
- These viruses react with mucin (mucoprotein) on the surface of red blood cells many of them cause red cells to clump together (agglutinate).

General Characteristics

Characteristic	Orthomyxoviridae	Paramyxoviridae
Representatives	**One genus** **Influenzavirus:** Influenza virus A Influenza virus B Influenza virus C	**Three genera** **Paramyxovirus:** Parainfluenzavirus 1-4, Senai virus (mouse), mumps, Newcastle disease virus Morbillivirus: Measles, rinderpest, canine distemper Pneumovirus: Respiratory syncytial virus (RSV)
Size	Particle: 80-120nm (highly pleiomorphic) Core diameter: 9nm	Particle: 125-250nm (somewhat pleiomorphic) Core diameter: 18nm
Replication	Nuclear	Cytoplasmic
Genome	Segmented (-) sense RNA	Non-segmented (-)sense RNA

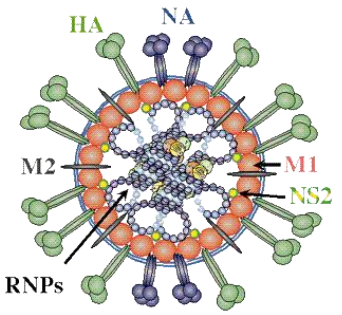
Orthomyxoviridae

- Family- **Orthomyxoviridae** (the influenza viruses)**.**
- Orthomyxoviruses contain a **single stranded**, negative **RNA** genome divided into 8 segments**.**
- The viruses have a lipid bilayer envelope with surface glycoproteins (**hemagglutinin** and **neuraminidase**).
- There are 3 viral antigens of importance: the nucleoprotein antigen that determines the virus type (**A, B or C**).
- The hemagglutinin (**H**) antigen, and the neuraminidase (**N**) antigen. The H and N antigens are variable.
- There are about 13 different H antigens and 9 different N antigens found in birds. This provides a total of 117 (13 x 9) possible combinations, 71 of which have been observed. There are only about 3 combinations that affect humans.

- The segmented genome of the influenza virus allows rearrangements to occur in simultaneously infected cells.
- This accounts for the periodic appearance of new variants. The new variants are responsible for the process of antigenic shift.
- **Influenza** commonly referred to as the **flu**, is an infectious disease, that affects **birds** and **mammals.**
- Which can be **fatal**, particularly for the young and the elderly.
- Influenza is transmitted through the air by **coughs** or **sneezes**, creating aerosols containing the virus, also be transmitted by **direct contact** with bird droppings or nasal secretions, or through contact with contaminated surfaces.
- Transmission is most important is not absolutely clear.
- Influenza viruses can be inactivated by sunlight, disinfectants and detergents.
- Virus can be inactivated by soap, frequent hand washing reduces the risk of infection.

Morphology

- Influenza virus-are highly pleiomorphic (variable), spherical/ovoid, 80-120nm diameter, but many forms occur, including long filamentous particles (up to 2000nm long x 80-120nm diameter).
- The outer surface of the particle consists of a lipid **envelope** from which project prominent **glycoprotein spikes** of two types:
  - **haemagglutinin** (**HA**), a 135Å trimer
  - **neuraminidase** (**NA**), a 60Å tetramer
- The inner side of the envelope is lined by the **matrix protein**.
- The genome segments are packaged into the core. The **RNP** (RNA + nucleoprotein, N) is in a helical form with the **3 polymerase polypeptides** associated with each segment.



Influenza virus A

- In virus classification influenza viruses are RNA viruses that make up three of the five genera of the family Orthomyxoviridae.
  - **Influenza virus A**
  - **Influenza virus B**
  - **Influenza virus C**
- These viruses are only distantly related to the human Para influenza viruses.

Influenza virus A

- Influenza A viruses are the most virulent human pathogens among the three influenza types and cause the most severe disease.

Influenza virus B

- Influenza B almost exclusively infects humans and is less common than influenza A.
- This type of influenza mutates at a rate 2–3 times slower than type A and consequently is less genetically diverse, with only one influenza B serotype.

Influenza virus C

- infects humans, dogs and pigs, sometimes causing both severe illness and local epidemics. However, influenza C is less common than the other types and usually only causes mild disease in children.

Host Range

- **Influenza A viruses** infect a wide variety of mammals, including man, horses, pigs, ferrets **and** birds.
- The main human pathogen, associated with epidemics and pandemics. There are 15 known haemagglutinin (H) serotypes and 9 known neuraminidase (N) serotypes.
- Important reservoirs - Pigs and birds.
- Get transferred back to the human population via close contact between humans and animals.
- **Influenza B viruses** infect mammals only and cause disease, but generally not as severe as A types.
- **Influenza C viruses** also infect mammals only, but rarely cause disease. They are genetically and morphologically distinct from A and B types.
- Different forms of sialic acid present on the virus glycoproteins.
- This property depends predominately (but not exclusively) on the amino acid at position 226 of the haemagglutinin protein.
- **Human viruses: $HA226_{leu}$** **Avian viruses: $HA226_{gln}$**
- Species barrier between birds and humans which is not easily overcome.
- However, pigs provide a "mixing pot" - able to be infected by both types of virus & thus allowing the passage of avian viruses to humans.

Pathogenesis

- Airborne. Spread is by aerosols - very efficient.
- The viruses deposit in lower respiratory tract, their primary site is the tracheobronchial mucosa.
- Necrosis of these cells results in the usual symptoms of the acute respiratory infection (fever, chills, muscular aching. headache, prostration, anorexia).
- Infection usually lasts 3-7 days.
- Neuraminidase produces liquefaction, which leads to viral spread.
- Respiratory symptoms include a cough, sore throat and nasal discharge. There is no viremia but systemic symptoms such as fever and muscle aches do occur.
- Severe complications include pneumonia (viral or bacterial).

Replication

Replication cycle of influenza A virus.

Binding and entry of the virus.

↓

Fusion with endosomal membrane and release of viral RNA.

↓

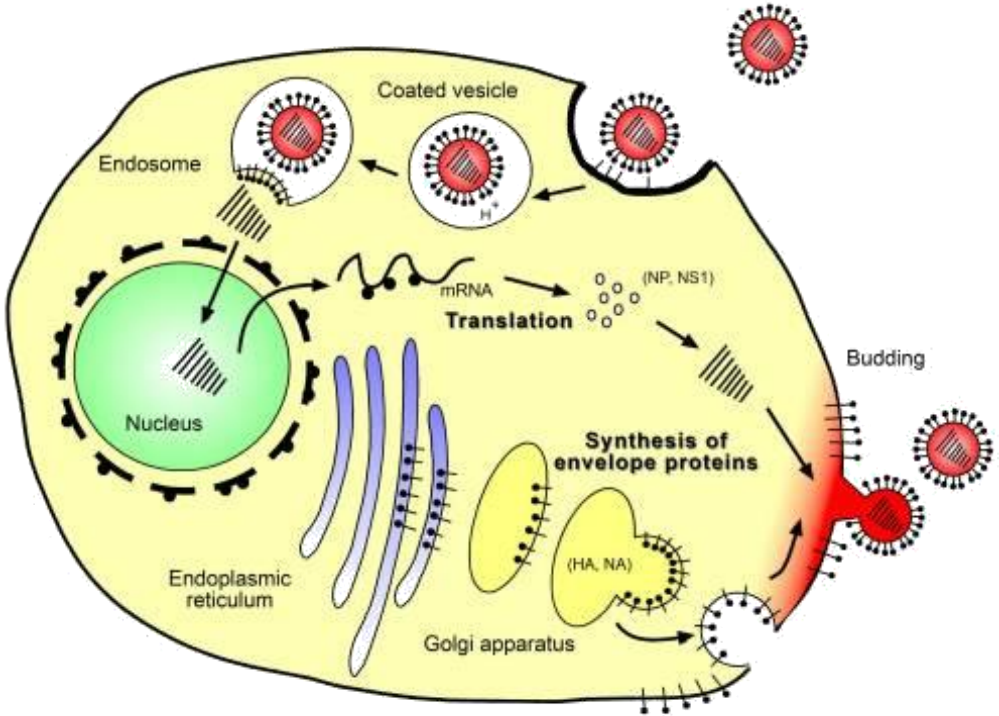
Replication within the nucleus.

↓

Synthesis of structural and envelope proteins.

↓

Budding and release of virions capable of infecting neighboring epithelial cells.



Host Defenses

- **Interferon is one non-specific defense.**
- **IgA in upper respiratory tract.**
- **IgG in lower respiratory tract.**
- **These antibodies are directed primarily against the hemagglutinin and neuraminidase.**
- **Cell-mediated (i.e. CTL) defenses are important in recovery.**

Epidemiology

- School children ⟶ home and infect siblings and parents.
- 3-6 weeks and the highest attack rates are for 5-19 year olds (generally Type A).
- **Antigenic Shift:**
  - Changes in the surface antigens; major changes are referred to as antigenic shift. These changes are responsible for pandemics and they result from rearrangement of the viral genome segments.
- **Antigenic Drift:**
  - Minor changes and these are responsible for many epidemics. They result from mutation in the viral RNA.
- Antigenic drift occurs every 2-3 years while antigenic shift only occurs every 10 years.
- 20th century, new strains of Influenza A - resulted in three influenza pandemics.
- **Spanish Flu (1918-1919);** H1N1 caused an estimated **20-50 million** deaths worldwide.
- **Asian Flu (1957-58);** H2N2 started in China in February 1957; by June 1957 it spread to United States, causing **70,000** deaths.
- **Hong Kong Flu (1968-1969);** H3N2 started in Hong Kong in early 1968, it spread to the United States and caused **34,000** deaths.

Diagnosis

- **Clinical:** Influenza usually displays a sudden onset with fever, malaise, headache, muscle aches, sore throat, cough and rhinorrhea, generally in winter. The presence of disease in the community (i.e. epidemiology) is helpful in diagnosis.
- **Laboratory:** Serology on the patient's serum can be performed or the viruses may be isolated in chick embryos if necessary.

Prevention/Treatment

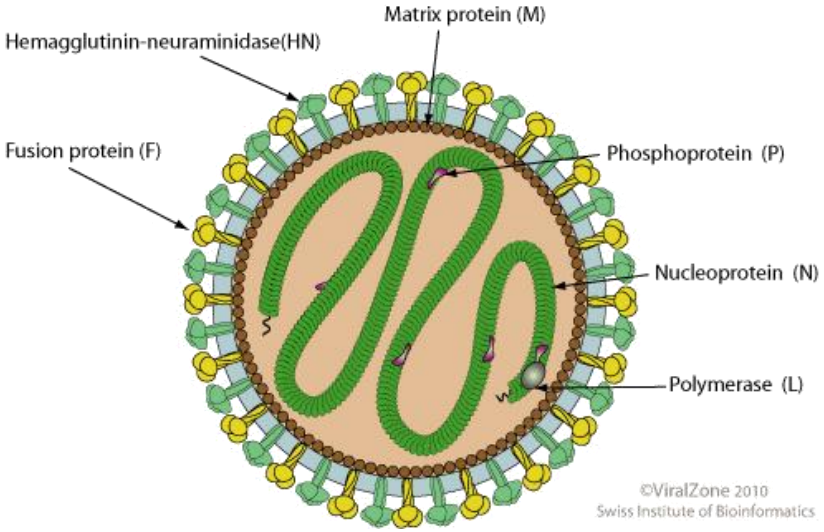
- Amantadine and Rimantadine are active against influenza A viruses (but not B viruses).
- The action of these closely related agents is complex and incompletely understood, but -believed to block cellular membrane ion channels.
- The target for both drugs is the matrix protein (M2), but resistance to the drug maps to the haemagglutinin (HA) gene.
- **Neuraminidase inhibitors -** These drugs work against all strains of influenza A and B.
- Hoffman-La Roche's **Tamiflu** (oseltamivir phosphate, GS4104)
- Glaxo Wellcome's **Relenza** (Zanamivir) must be inhaled.
- Both drugs are now approved for use in the UK.

Control

- **Sanitary:** Avoid contacts.
- **Immunological:** Every year, inactivated vaccines are prepared using the most likely types and antigenic characters expected for any particular season. These vaccines are given parenterally in the fall, primarily for those at risk (older persons or those with chronic disease). Protection against disease is variable (50-90%).
- **Chemotherapeutic:** The drug amantadine HCl can be used for influenza type A but not type B in patients with other disease conditions. Generally, however, pain relievers (e.g. acetaminophen) are more generally employed

Paramyxoviruses

- Viruses of the *Paramyxoviridae* family of the *Mononegavirales* order.
- Negative-sense single-stranded RNA viruses responsible for a number of human and animal diseases.
- *Paramyxoviridae* family initiate infection via respiratory tract.



Group: Group V (- ssRNA)

Order: ***Mononegavirale***

Family: ***Paramyxoviridae***

- Contain viral glycoproteins (G,H, or HN), fusion (F) glycoprotein very fragile.
- Antigenically stable, Particles are labile yet highly infectious.
- Cytoplasm; particles bud from plasma membrane

Properties

- Spherical, pleomorphic, 150 nm or more in diameter (helical nucleocapsid, 13-18nm).
- RNA 1%, Protein-73%, Lipid-20%, Carbohydrate6%.
- SsRNA, linear, non segmented, negative sense, non-infectious, about 15kb. In length and containing 6-10 genes.
- Nucleocapsid – Phosphoprotein – Matrix – Fusion – Attachment – Large (polymerase)
- **N** - Nucleocapsid protein- Protects the RNA from nuclease digestion.
- **P** – Phosphoprotein - forms part of the RNA polymerase complex.
- **M** – Matrix protein - organizes and maintains virion structure.
- **F** – Fusion protein - Mediates cell entry by inducing fusion between the viral envelope and the cell membrane.
- **H/HN/G** – the cell attachment proteins span the viral envelope and project from the surface as spikes.
- **L** – Large protein - catalytic subunit of RNA dependent RNA polymerase (RDRP).
- Accessory proteins – Not essential for replication but may aid in survival in vitro or may be involved in mRNA to antigenome synthesis.

Replication

- Virus attachment, penetration, and Uncoating
- Transcription, translation, and RNA replication
- Maturation
- Budding
- Release of virus

Pathogenesis

- **Mumps Virus**
  - Group: Group V ((-)ssRNA)
  - Order: Mononegavirales
  - Family: *Paramyxoviridae*
  - Genus: *Rubulavirus*
- Acute, affecting children and characterized by non suppurative enlargement of the parotid glands.
- Closely related to parainfluenza viruses.
- ssRNA, enveloped virus of helical symmetry.
- Single RNA molecule of 16 - 18 kbp.
- Only one serotype of the virus exist.
- Antigenic cross-reaction occurs with other members of the paramyxovirus genus.

- Transmitted by droplet spread or by direct contact.
- The primary site of viral replication of the epithelium of the upper respiratory or the GI tract or eye.
- Virus multiplication in target organs leads to a secondary viraemia Parotitis.
- Occasionally, meningitis may precede parotitis by a week.
- It is not known whether virus actually multiplies in renal tissues or whether the virus is of haematogenous origin
- Life-long immunity is the rule after natural infection, but reinfections can occur and 1 - 2% of all cases are thought to be reinfections.

Clinical Features

- The incubation period is 16 - 18 days but may vary from 14 - 25 days.
- Parotid swelling develops in 95% of those with clinical illness.
- Typically, a prodromal illness consisting of headache, malaise, myalgia and low grade fever occurs 1 - 2 days before the onset of parotid enlargement.
- The parotid swelling starts to subside after 4 to 7 days.
- Virus shedding into the saliva begins a couple of days before the onset of parotitis and ends 7 to 8 days later.
- All the other manifestations of mumps:
  - Meningitis, Encephalitis, Hearing Loss , Orchitis and oophoritis, Pancreatitis, Arthralgia, Myocarditis, Transient Renal Dysfunction, Insulin Dependent Diabetes, Abortion, Thyroiditis.

Management and Prevention

- No specific treatment is available for mumps.
- Some authorities recommend a short course of corticosteriods in severe cases.
- An inactivated vaccine was first used in the 1940s successfully. This has now been replaced by a live attenuated vaccine.
- Mumps vaccine is now routinely given as part of the **MMR** regimen in many countries to all infants.

Measles Virus

- Measles is one of the typical viral diseases of childhood.
  - Group: Group V ((-)ssRNA)
  - Family: Paramyxovirus
  - Genus: Morbillivirus
  - Species: Measles virus
- ssRNA enveloped virus, helical symmetry.
- Envelope consists of haemagglutinin protein and the fusion protein embedded in the lipid bilayer.
- M protein (membrane or matrix protein) lies immediately below the membrane.
- ssRNA is encased in a helix of N (nucleocapsid protein). The ssRNA molecule is of negative sense.
- Measles is an antigenically stable virus.
- There is one serotype only and there are very little differences between different isolates.

Epidemiology

- The virus spreads by the respiratory route via aerosol droplets and respiratory secretions.
- In tropical regions epidemics are less.
- The maximum incidence was seen in children aged 5 - 9 years.
- By the age of 20, approximately 99% of subjects have been exposed to the virus.
- Here the disease is a serious problem with a high mortality (10%) with malnutrition being an important factor.
- In general measles mortality is highest in children < 2 years and in adults. In contrast to the influenza virus measles does not have an animal reservoir.

Clinical Manifestations

- After an incubation period of 10 - 11 days, the patient enters the prodromal stage with fever, malaise, sneezing, rhinitis, congestion, conjunctivitis and cough.
- Koplik's spots, which are pathognomonic are measles, appear on the buccal and lower labial mucosa opposite the lower molars.
- **Atypical measles** infection may be seen in people who have been incompletely vaccinated.
- The majority of cases develop a pneumonia. Occasionally, marked hepatosplenomegaly, hyperaesthesia, numbness or paraesthesia are also found.

Pathogenesis

- Measles first gains access to the body via the upper respiratory tract or the conjunctiva.
- Virus quickly spreads to the immediate lymph nodes.
- Destruction of the lymphoid tissues leads to a profound **leucopenia.**
- A primary viraemia ensues spreading of virus to the R-E system and the respiratory system.
- A secondary viraemia follows whereby the virus is further spread to involve the skin, the viscera, kidney and bladder.
    - Acute Measles Panencephalitis.
    - Subacute Measles Encephalitis.
    - Subacute Sclerosing Panencephalitis (SSPE).
- Natural immunity to measles is known to last at least 65 years.

Diagnosis

- **Microscopy -** such cells are detectable in the NPS (nasopharyngeal secretions). This is more rapid and practical than virus isolation
- **Immunofluorescence -** Direct and indirect immunofluorescence have been used extensively to demonstrate MV antigens in cells from NPS specimens
- **Virus isolation -** measles virus can be isolated form a variety of sources, e.g. throat or conjunctival washings, sputum, urinary sediment cells and lymphocytes.
- **Serology -** The methods that can be used include HAI, CF, neutralization and ELISA tests.

Management

- Such patients can be protected by the administration of human anti-measles gammaglobulin if given within the first 3 days after exposure.
- Alternatively, the exposed individual can simply be vaccinated within 72 hours of exposure.
- **Pneumonia** - antibiotics may be indicated in cases of secondary bacterial pneumonia or otitis media.
- **Encephalitis** - treatment of acute measles encephalitis is only symptomatic and supportive.

Prevention

- **Inactivated Vaccine** - live vaccination is now generally recommended and individuals previously immunized with the killed vaccine should be reimmunized with the live vaccine
- **Live vaccine** - **MMR** vaccine

Canine Distemper

- Canine distemper affects **dogs** at any age.
- Cats, skunks, and binturong are some other animals that are also susceptible to the canine distemper virus.
- It is caused by a paramyxovirus, a type of virus that causes measles in humans and rinderpest in hoofed-animals such as cattle.

Transmission

- An infected animal can easily shed the virus through exhalation implying that the virus is transmitted via air.
- The virus is also shed through other bodily secretions and excretions such as urine and feces.
- Younger dogs are more vulnerable canine distemper than older dogs because of their under developed immune systems.

Signs and Symptoms

- Gastrointestinal (such as vomiting, decrease in appetite, and diarrhea) or respiratory in nature (difficulty in breathing).
- Transient fever or dramatic and sporadic increases in body temperature is a telltale sign of the infection.
- Behavioral changes include lethargy, weakness, and depression.
- Neurological signs may also be seen such as muscle twitching especially near the mouth and legs.
- Seizures and **paralysis** may occur in severe cases.
- Sudden death is not uncommon with this disease.

Diagnosis

- Such as blood tests are recommended to detect and confirm the disease and its severity.
- Actual viral isolation and identification is also possible depending on the capacity of a laboratory.

Prevention

- Basic proper hygiene and sanitation such as the use of standard disinfectants is sufficient to kill the canine distemper virus.
- Infected animals should also be quarantined from other animals.
- Once a dog has been infected, a dog owner can only offer supportive treatment and hope for the best.
- Antibiotic therapy may also be prescribed to lessen any detrimental effects of opportunistic secondary bacterial infections.
- Recovery may be absolute.

Herpes Simplex Virus I (HSV I)

General Properties

Group : Group I (dsDNA)

Family : *Herpesviridae*

Subfamily: Alphaherpesvirinae

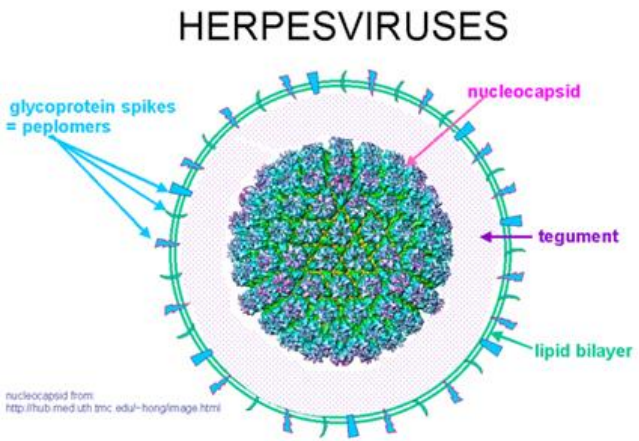
Genus : *Simplexvirus*

Species : Herpes Simplex Virus I

- Herpes viruses are large viruses.
- Virion is spherical,150-200nm in diameter
- Icosahedral capsid.
- Linear double standard DNA,124-235 Kbp.
- More than 35 proteins are available in virion.
- Replication is in nucleus.
- Genome is large enough to code for atleast 100 proteins.

Structure

- It contains three main structural components.
- A **central core** holds the viral DNA, an **inner core** is surrounded by an envelope that is made of viral glycoproteins and host cell membranes, and a capsid.
- The envelope is joined to the capsid by means of a Tegument.
- This complete particle is known as the virion envelope.



Epidemiology

- Worldwide rates of HSV infection are between 65% and 90%.
- HSV1 is more common than HSV2 with rates of both increasing as people age.
- Rates of infection are determined by the presence of antibodies against either viral species.
- In the US 17.2% of the population is HSV-2 seropositive with only 14.5% of the seropositive population aware that they are infected.

Replication

Virion binds to the extracellular protein through gB and gC receptor

↓

Another viral protein gD interests with a second cellular receptor

↓

The virus is uncoated, liberating tegument proteins and nucleocapsid into the cytoplasm

↓

Viral nucleocapsid docks at the nuclear pore and release viral DNA into the nucleus, where the DNA circularies

Pathogenesis

- HSV is transmitted by contact of a susceptible person with an individual excreting virus and encounter the mucous membrane.
- After the contact with mucous membrane or a break in the skin, HSV undergoes replications in the parabasal and intermediate epithelial cells, which lyses and invoke inflammatory response.
- HSV causes cytolytic infections.
- Characteristic histopathological changes include ballooning of infected cells, production of cow dry type.
- Cell fusion provides an efficient method for cell-to-cell spread.
- Edema fluid accumulates between the epidermis and dermal layer.
- In mucous membranes, the vesicle rupture rapidly forms ulcers.
- Primary infections are usually mild and most of them are symptomatic.

Signs and Symptoms

- Gingiva stomatitis
- Fever
- Sore throat
- Lesions in oral cavity
- Malaise
- Submandibular lymphadenopathy
- Anorexia
- Pharygotonsilitis
- Keratoconjuctivies

Lab Diagnosis

Culture

- The virus can be cultured. This is a labor intense methodology, which requires expertise and will take on average 3-7 days from the time the specimen was collected.

Rapid detection

- Immunofluorescence testing (IF) and enzyme linked immunosorbent assays (ELISA), which will give same day results.

Detection of HSV DNA (=PCR)

- Polymerase chain reaction (PCR) is a technique which allows the detection and multiplication of selected parts of the viral DNA so that thousands of copies are made. These can then be detected by standard measurement techniques.

Treatment

- **Acyclovir** targeting HSV infected cells and viral DNA polymerase
- Topically applied **Idoxuridine, Trifluridine, Vidarabine** and Acyclovir have been used for Hepertic keratitis.
- Certain dietary supplements and alternative remedies are claimed to be beneficial in the treatment of herpes.
- There is however insufficient evidence to support use of many of these compounds including **Echinacea, eleuthero, L-lysine, zinc,** bee products and **Aloe vera.**

Herpes Simplex Virus II (HSV II)

General Properties

Group: Group I (dsDNA)

Family: *Herpesviridae*

Subfamily: Alphaherpesvirinae

Genus: *Simplexvirus*

Species: Herpes simplex virus 2

- HSV type 2(HSV 2) is responsible for the majority of genital tract infections and is commonly transmitted venerally.
- They are heat labile and have to be stored at -70°C.
- HSV-2 rarely causes complications or spreads to other parts of the body.
- Oral HSV-2 infections are rare. But even when an infection does occur, recurrent oral outbreaks are uncommon

Structure

- Large linear **dsDNA** genome encased within an icosahedral protein cage called the capsid which is wrapped in a lipid layer.
- The envelope is joined to the capsid by means of a **tegument.**
- HSV-1 and HSV-2 each contain at least **74 genes** (or open-reading frames, ORFs) within their genomes, although speculation over gene crowding allows as many as 84 unique protein coding genes by **94** putative **ORFs**.
- Transcription of HSV genes is catalyzed by **RNA polymerase II** of the infected host.

Epidemiology

- Worldwide rates of HSV infection are between **65%** and **90%**. HSV1 is more common than HSV2 with rates of both increasing as people age.
- Rates of infection are determined by the presence of antibodies against either viral species.
- In the US **17.2%** of the population is **HSV-2** seropositive with only **14.5%** of the seropositive population aware that they are infected.

Transmission

- **HSV-1** and **HSV-2** are transmitted horizontally during **close contact** with an infected person who is shedding virus from the **skin**, often in **saliva** or in secretions from the genitals.
- HSV-2 is primarily a **sexually transmitted** infection.
- May also be transmitted vertically from **mother to child** before or during childbirth.
- Some forms of HSV can be **fatal to** the **infant**, as the developing immune system of the child is unable to defend against the virus, resulting in inflammation of the brain (encephalitis) that may cause **brain damage**.

Pathogenesis

- Primary infection is usually acquired in early childhood, between two and five years of ago.
- Humans are the only natural hosts and the sources of infection are saliva, skin lesions or respiratory secretion.
- HSV 2 establishes latency in sacral ganglion cells and becomes quiescent with little or no activity.
- Occasionally, virus replicate in the ganglion cells and migrates down a sensory nerve to cause an outbreak on the skin in the innervated area.
- The control mechanism for reactivation and recurrence is unknown.

Clinical Manifestation

- **Cutaneous infections** -The most common site is the face-on the cheeks, chin, around the mouth or on the forehead. Lesions may also appear on the buttocks in infants as napkin rash
- **Mucosal** -The buccal mucosa is the site most commonly affected.
- **Opthalmic** - Acute keratoconjunctivitis may occur by itself or by extention from facial herpes.
- **Visceral** - HSV esophagitis may cause dyphagitis, substernal pain and weight loss
- **Genital** - In men, the lesions occur mainly on the penis, or in the urethra causing urethritis. In women, the cervix, vagina, vulva and perineum are affected.
- **Congenital** -Transplacental infection with HSV 2 can lead to congenital malformations, but this is rare.

Lab Diagnosis

- MICROSCOPY - The Tzanck smear is a rapid, fairly sensitive and inexpensive diagnostic method.
- PCR based DNA detection has replaced brain biopsy.
- VIRUS ISOLATION - Inoculation in mice and on chick embryo CAM is insensitive and has been replaced by tissue culture for virus isolation.
- SEROLOGY- ELISA, neutralization or complement fixation tests.

Prevention & Treatment

- Vaccination for herpes would be the ideal preventive method.
- Avoidance of direct contact with lesions or infected secretions is the primary means of prophylaxis.
- I**doxyuridine** used topically in eye and skin infections was one of the first clinically successful antiviral agents.
- The introduction of acyclovir and **vidarabine** enabled the effective management of deep and systemic infections.
- Early treatment with intravenous **acyclovir** has improved the outcome of encephalitis. Oral and topical use may help in less serious conditions.
- **Valaciclovir** and **famciclovir** are more effective oral agents.

ADENO VIRUSES

- Adenoviruses are a frequent cause of acute **Upper Respiratory Tract** (**URT**) infections. Also other type of infections.
- Widespread in nature, infecting birds, many mammals and man.
- Can undergo latent infection in lymphoid tissues, becoming reactivated some time later.
- Several types have oncogenic potential.
- There are at least 51 human adenovirus serotypes (genus Mastadenovirus) which have been divided into subgroups- (A to F).
- All human adenoviruses s.tudied so far can transform primary rodent cells in culture

Taxonomy

Family	Genus	Types of Species	Host
Adenoviridae	Atadenovirus	Ovine adenovirus D	Vertebrates
	Aviadenovirus	Fowl adenovirus A	Vertebrates
	Mastadenovirus	Human adenovirus C	Vertebrates
	Siadenovirus	Turkey adenovirus B	Vertebrates

Structure

- Adenoviruses are **double-stranded DNA** viruses.
- They have **icosahedral capsids** with twelve vertices and seven surface proteins.
- The virion is **non-enveloped**, spherical and about seventy to ninety nm in size.
- The genome encodes about thirty proteins. Both strands of adenovirus DNA encode genes.
- Transcription occurs in three stages -- immediate early, early and late.

Adenovirus-Associated Human Disease

- Pharyngitis
- Acute Respiratory Disease
- Pneumonia
- Pharyngoconjunctival Fever
- Epidemic Keratoconjuntivitis
- Genitourinary Infections (cervicitis, urethritis, hemorrhagic cystitis)
- Gasteroenteritis
- Some asymptomatic and persistent infection
- Adenovirus oncogenically tranforms rodent cells but not human cells.

Transmission

- Ingestion/Fecal-Oral Route
- Respiration (through respiratory droplets)
- Contact/Hand-to-eye transfer
- Venereal

Pathogenesis

Primarily attacks mucoepithelial cells of the conjunctiva, respiratory tract, gastrointestinal and genitourinary tracts

↓

Attachment to host cell receptor occurs via fiber protein

↓

The virus replicates in the cytoplasm of host cells

But viral DNA replicates within the host cell nucleus

↓

Early and late phases of replication, followed by assembly and release of virions

↓

Inefficient (error-prone) replication of the virus results in many excess antigenic components

↓

These are liberated into the culture fluid in vitro as soluble antigens and lead to formation of intra-nuclear inclusion bodies within infected cells

Epidemiology

- Endemic, epidemic and sporadic infections.
- Outbreaks have been noted in military recruits, swimming pool users, residential institutions, hospitals, and day care centers.
- Respiratory disease mainly occurs in late winter through early summer.
- GI disease does not seem to be seasonal.

Clinical Syndromes

- **Upper Respiratory Infections-** Common cold (rhinitis), Pharyngitis (with or without fever), Tonsillitis.
- **Lower Respiratory Infections -** Bronchitis, Pharyngoconjunctival fever, acute respiratory disease, Pertussis-like syndrome, Pneumonia.
- **Eye -** Epidemic Keratoconjunctivitis (EKC), acute follicular conjunctivitis, pharyngoconjunctival fever.
- **Gastrointestinal -** Gastroenteritis, mesenteric adenitis, intussusception, hepatitis, appendicitis, Diarrhea.
- **Fatal disease** in immunocompromised patients.

Diagnosis

- **Clinical Specimens**, such as swabs (nasopharyngeal, conjuncticval, rectal, or other) and washings, corneal scrapings, stool, urine or biopsy and autopsy materials etc,.
- **Viral Isolation** in cell cultures. Human Embryonic Kidney (HEK) and Human Fetal Diploid Cells (HDFL), A549 cells lines are used for types 1-39.
- Rapid detection of enteric types (serotypes 40, 41) by ELISA, IF antibody for rapid detection.

Prevention

- Good hand washing.
- Contact precautions, respiratory precautions in health care settings .
- Adequate chlorination of swimming pools.
- Sterilization and disinfection of ophthalmologic equipment and use of single dose vials of ophthalmic medications.
- **Vaccine-** live, enteric coated, oral vaccine (types 4, 7, 21) was available in past, but is no longer being manufactured.

Treatment

- There are no antiviral drugs to treat adenoviral infections.
- So treatment is largely directed at the symptoms (such as acetaminophen for fever).
- Antibiotic eye drops for conjunctivitis, help to prevent secondary bacterial infections.

Adeno Associated Viruses

- **Adeno-associated virus (AAV)** is a small virus which infects humans and some other primate species.
- AAV can infect both dividing and non-dividing cells and may incorporate its genome into that of the host cell.
- These features make AAV a very attractive candidate for creating viral vectors for gene therapy.

Classification

Group : Group II (ssDNA)

Family : *Parvoviridae*

Subfamily : *Parvovirinae*

Genus : *Dependovirus*

Species : *Adeno associated virus*

AAV genome, Transcriptome and Proteome

- The adeno-associated virus genome is built of **ssDNA** either positive- or negative-sensed which is about 4.7 kb long.
- The genome comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): rep and cap.
- The former is composed of four overlapping genes encoding Rep proteins required for the adeno-associated virus life cycle, and the latter contains overlapping nucleotide sequences of capsid proteins: VP1, VP2 and VP3, which interact together to form a capsid of an icosahedral symmetry.

ITR Sequences

- The Inverted Terminal Repeat (ITR) sequences comprise 145 bases each.
- The ITRs were also shown to be required for both integration of the adeno-associated virus DNA into the host cell genome.
- Many methods were established for efficient production of recombinant adeno-associated virus (rAAV) vectors containing a reporter or therapeutic gene.
- Cis Acting Rep-dependent Element (CARE) inside the coding sequence of the rep gene.
- CARE was shown to augment the replication and encapsidation when present in cis.

Serotypes, Receptors and Native Tropism

- 11 adeno-associated virus serotype described the 11th in 2004. All of the known serotypes can infect cells from multiple diverse tissue types.
- Tissue specificity is determined by the capsid serotype and pseudo typing of adeno-associated virus vectors to alter their tropism range will likely be important to their use in therapy.

Serotype 2

- Three cell receptors have been described for AAV2: heparan sulfate proteoglican (HSPG), avβ5 integrin and fibroblast growth factor receptor 1 (FGFR-1).
- The first functions as a primary receptor, while the latter two have a co-receptor activity and enable AAV to enter the cell by receptor-mediated endocytosis.

Serotype 2 and cancer

- Studies have shown that serotype 2 of the virus (AAV-2) apparently kills cancer cells without harming healthy ones.

Other Serotypes

- Adeno-associated virus **6** appears much better in infecting **airway epithelial cells**.
- Adeno-associated virus **7** presents very high transduction rate of **murine skeletal muscle** cells (similarly to Adeno-associated virus 1 and Adeno-associated virus 5)
- Adeno-associated virus **8** is superb in transducing hepatocytesand adeno-associated virus 1 and 5 were shown to be very efficient in gene delivery to **vascular endothelial cells**. Adeno-associated virus **6** a hybrid of Adeno-associated virus 1 and Adeno-associated virus 2 also shows lower immunogenicity than adeno-associated virus 2.

Adeno Associated Virus Immunology

- **Innate -** Produce innate responses lasting 24 hours or longer. soluble factor levels and cell infiltration appear to return to baseline within six hours
- **Humoral -** The virus is known to instigate robust humoral immunity in animal models and in the human population where up to **80%.**
- **Cell-mediated -** The cell-mediated response to the virus and to vectors is poorly characterized and has been largely ignored in the literature as recently as 2005.

Infection Cycle

Attachment to the cell membrane

↓

Endocytosis

↓

Endosomal trafficking

↓

Escape from the late endosome or lysosome

↓

Translocation to the nucleus

↓

Formation of double-stranded DNA replicative form of the adeno-associated virus genome

↓

Rep genes expression

↓

Genome replication

↓

Cap genes expression, synthesis of progeny ssDNA particles

↓

Assembly of complete virions, and

↓

Release from the infected cell.

Gene Therapy

- AAV vectors are used to deliver genes to the brain. This is possible because AAV viruses can infect non-dividing (quiescent) cells, such as neurons in which their genomes are expressed for a long time.

Adeno-Associated Viral Vectors

- ITRs seem to be the only sequences required in cis next to the therapeutic gene: structural (**cap**) and packaging (**rep**) genes can be delivered in trans.
- With this assumption many methods were established for efficient production of recombinant AAV (rAAV) vectors containing a reporter or therapeutic gene.

Tumor Viruses of Human

- **Tumor viruses** are those viruses that are able to infect cells and **cause changes** within the **cell's** operating machinery such that the cell's ability to regulate its growth and division is destroyed and the cells become cancerous.
- Viruses with a **DNA** genome, such as adenovirus, and
- Viruses with an **RNA** genome, like the Hepatitis C virus (HCV) can cause cancers, retroviruses having both **DNA** and **RNA** genomes (Human T-lymphotropic virus and hepatitis B virus, which normally replicates as a mixed double and single-stranded DNA virus but also has a retroviral replication component).

Classes of Tumor Viruses

- DNA tumor viruses
- RNA tumor viruses, the latter also being referred to as Retroviruses.

Family	Associated Cancer
RNA Viruses	
Flaviviridae	Hepatocellular carcinoma
Hepatitis c virus	Hematopoietic cancers, sarcomas,
Retroviridae	carcinomas.
DNA viruses	
Adenoviridae	Various solid tumors
Hepadnaviridae	Hepatocellular carcinoma
Herpesviridae	Lymphomas, Sarcomas,
Papilomaviridae	Carcinomas
Polyoma viridae	Papillomas and carcinomas
	Various solid tumors

DNA Tumor Viruses

- In permissive cells, all parts of the viral genome are expressed. This leads to viral replication, cell lysis and cell death.
- In cells that are non-permissive for replication, viral DNA is usually, but not always, integrated into the cell chromosomes at random sites.
- Only part of the viral genome is expressed.
- This is the early, control functions (e.g. T antigens) of the virus.
- Viral structural proteins are not made and no progeny virus is released

DNA Tumor Viruses Involved in Human Cancers

Papillomaviruses

- Papilloma viruses have a genome size about 8 kilobases.
- They cause **warts** and also human and animal cancers. **Warts** are usually benign but can **convert to malignant** carcinomas.
- Epidermodysplasia verruciformis, which is associated with a high risk of skin carcinoma, is typically associated with HPV types 5 and 8 (but other types may also be involved).
- These infect most people (up to 80% of the population) and are usually asymptomatic.
- Papilloma viruses are also found associated with human penile, uterine, cervical and anal carcinomas and are very likely to be their cause; moreover, genital warts can convert to carcinomas.

- There are more than **100 types** of **human papilloma viruses** but, clearly, not all are associated with cancers; however, papillomas may cause **16%** of female cancers worldwide and **10%** of all cancers.
- The most common genital human papilloma viruses (HPV) are types 6 and 11.
- All infected persons develop a cancer, there are probably **co-factors** in stimulating the disease.
- Such co-factors have been identified in alimentary tract carcinomas in cattle where a diet containing bracken fern is associated with the disease.
- In the case of human cervical cancer, the efficacy of the anti-HPV vaccine makes the contention that HPV does cause cervical cancer very compelling.

Polyoma Viruses

- Small viruses with genomes of about 5 kilobases.

Murine Polyoma virus

- It causes a wide range of tumors in a number of animal species at many different sites. It was originally isolated from AK mice and is fully permissive for replication in mouse cells. It causes leukemia's in mice and hamsters.

Simian virus 40

- SV40 virus was initially discovered in the rhesus monkey kidney cells that were used to make inactivated Salk polio vaccine virus.
- SV40 replicates in rhesus monkey kidney cells but has no cytopathic effect on them.
- Many early recipients of the Salk polio vaccine received contaminating SV40 since anti-SV40 antibodies (against a protein called the large tumor antigen (T-antigen)) could be detected in their blood.
- No elevated incidence of cancer has been found in these people.

Human Polyoma Viruses

- The first two human polyoma isolates, known as BK and JC were discovered in 1971.
- 70 to 80% of the human population is seropositive for JC. This virus is known to be the cause of (progressive multifocal leukoencephalopathy) PML, a disease associated with immunosuppression.
- In **1979**, the rate of occurrence of this disease was **1.5 per 10 million** population.
- **Polyoma viruses** are usually **lytic** and when transformation occurs, it is because the transforming virus is **defective**.

Adenoviruses

- These viruses are somewhat larger than polyoma and papilloma viruses with a genome size of about **35 kilobases**.
- They were originally isolated from human tonsils and adenoids, are highly oncogenic in animals and only a portion of the virus is integrated into the host genome.
- This portion codes several T antigens that carry out early functions.
- Tumor-bearing animals make antibodies against the T antigens.
- No humans cancers have been unequivocally associated with adenoviruses

Complex Tumor Viruses

- Herpes viruses are much larger than the DNA viruses described above and have a genome size of **100** to **200** kilobases.
- Because of their large size, a lot remains to be discovered concerning the way in which these viruses transform cells.
- These large enveloped viruses in human cancers and they are highly tumorigenic in animals.
- Herpes viruses are often co-carcinogens. They may have a hit and run mechanism of oncogenesis, perhaps by expressing proteins early in infection that lead to chromosomal breakage or other damage.

Epstein-Barr virus (Human herpes virus 4)

- EBV is the herpes virus that is most strongly associated with cancer.
- It infects primarily lymphocytes and epithelial cells.
- In lymphocytes, the infection is usually non-productive, while virus is shed (productive infection) from infected epithelial cells.
- EBV can cause lymphoma in Marmosets and transform human B lymphocytes i*n vitro*.
- EBV also causes infectious mononucleosis, otherwise known as glandular fever.
- This is a self-resolving infection of B-lymphocytes which proliferate benignly.
- Often infection goes unnoticed (it is sub-clinical) and about half of the population in western countries has been infected by the

- EBV is causally associated with
- Burkitt's lymphoma in the tropics, where it is more common in malaria-endemic regions
- Nasopharyngeal cancer, particularly in China and SE Asia, where certain diets may act as co-carcinogens
- B cell lymphomas in immune suppressed individuals (such as in organ transplantation or HIV)
- Hodgkin's lymphoma in which it has been detected in a high percentage of cases (about 40% of affected patients)
- X-linked lymphoproliferative Disease (Duncan's syndrome).

Human Herpes Virus 8 (Kaposi's Sarcoma Herpes Virus)

- HHV-8 infects lymphocytes and **epithelial/endothelial** cells and is the causative agent of Kaposi's sarcoma.
- It has also been associated with hematologic malignancies, including primary effusion lymphoma, multicentric Castleman's (also Castelman's) disease (MCD), MCD-related immunoblastic/plasmablastic lymphoma and various atypical lymphoproliferative disorders.
- EBV and HHV-8 have been found to be associated with oral lesions and neoplasms in HIV-infected patients.
- Among these diseases is oral hairy leukoplakia (OHL) which is benign and causes white thickenings on the tongue epithelium in which these viruses proliferate.

Hepatitis B Virus

- Hepatitis B virus is very different from the other DNA tumor viruses.
- it is much more similar to the oncornaviruses (RNA tumor viruses) in its mode of replication.
- The DNA is transcribed into RNA not only for the manufacture of viral proteins but for genome replication.
- Genomic RNA is transcribed back into genomic DNA. This is called reverse transcription.
- The latter is not typical of most DNA tumor viruses but reverse transcription is a very important factor in the life cycles of RNA-tumor viruses.

RNA Tumor Viruses Involved in Human Cancers

Retrovirus

- Coat proteins (surface antigens) are encoded by *env* (envelope) gene and are glycosylated.
- One primary gene product is made but this is cleaved so that there are more than one surface glycoprotein in the mature virus (cleavage is by host enzyme in the Golgi apparatus).
- The primary protein (before cleavage) is made on ribosomes attached to the endoplasmic reticulum and is a transmembrane (type 1) protein.
- There are two molecules of genomic RNA per virus particle with a 5' cap and a 3' poly A sequence. Thus, the virus is diploid. The RNA is plus sense (same sense as mRNA).
- About 10 copies of reverse transcriptase are present within the mature virus, these are encoded by the *pol* gene.

❖ The pol gene products are:

- Reverse transcriptase (a polymerase that copies RNA to DNA).
- Integrase (integrates the viral genome into the host genome).
- RNase H (cleaves the RNA as the DNA is transcribed so that reverse transcriptase can make the second complementary strand of DNA).
- Protease (cleaves the polyproteins translated from mRNAs from the gag gene and the pol gene itself). This virally encoded protease is the target of a new generation of anti-viral drugs.

Group of Retroviruses

- **ONCOVIRINAE**
- These are the tumor viruses and those with similar morphology.
- The first member of this group to be discovered was **Rous Sarcoma Virus** (RSV) **-** which causes a slow neoplasm in **chickens**.
- Viruses in this group that cause tumors in humans are:
- **HTLV-1** (human T-cell lymphotropic virus-1) which causes Adult T-cell Leukemia (Sezary T-cell Leukemia).
- This disease is found in some Japanese islands, the Caribbean, Latin America and Africa. HTLV-1 is sexually transmitted.
- HTLV-2 (human T-cell lymphotropic virus-2) which causes Hairy Cell Leukemia.

LENTIVIRINAE

These have a long latent period of infection before disease occurs; they were mainly associated with diseases of ungulates (e.g. visna virus) but HIV (formerly HTLV-III) which causes AIDS belongs to this group. It is much more closely related to some Lentivirinae than it is to HTLV-I and HTLV-II which are Oncovirinae.

SPUMAVIRINAE

There is no evidence of pathological effects of these viruses.

They establish persistent infections in many **animal** species.

They have been isolated from primates (including humans), cattle, cats, hamsters, and sea lions.

Cells infected by spumaviruses have a foamy appearance (because of numerous vacuoles) and often form syncytia of giant multinucleate cells.

Type B Oncoviruses

- Type B and D particles develop from the budding of preformed cores known as type A particles.
- Only one member of the type B oncoviruses mouse mammary tumor virus (MMTV) has been clearly identified.
- This virus has a distinctive morphology and is produced when a preformed core buds through a cytoplasmic membrane.

Type C Oncoviruses

- The type C oncoviruses include the human and animal leukemogenic retroviruses.
- One group of type C oncoviruses includes human T-cell leukemia viruses types I and 2 (HTLV-l and HTLV-2), as well as bovine (BLV) and simian (STLV-l) leukemogenic viruses.
- The HTLV group of viruses carries a viral gene, tax, which is probably involved in neoplastic transformation.

Type D Oncoviruses

- Type D oncoviruses have been isolated only from nonhuman primates, in which they induce both immunosuppression and proliferative syndromes.

Vaccines in the Prevention of Virus-Associated Cancers

- Live attenuated virus vaccines have been used.
- Construct live recombinant virus vaccines that are specifically engineered to express a viral protein known to be immunogenic and to confer immunity to the virus.
- Such a vaccine is the vaccinia virus recombinant that contains a ***gp340 EBV*** glycoprotein.
- This vaccine prevents the cotton-top Tamar in from developing lymphomas when challenged with EBV.