LECTURE-9 *Lect. Dr. Ahmed Yaseen Abed*

Orthomyxoviruses

The term myxovirus was coined for a group of **enveloped RNA viruses** that have the ability to adsorb onto mucoprotein receptors on erythrocytes, causing hemagglutination.

It included influenza, mumps, parainfluenza, and Newcastle disease viruses

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

Differences between orthomyxoviruses and paramyxoviruses

Influenza Viruses:

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

Properties of the Virus:

Influenza viruses show following features:

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

■ Influenza virus is composed of a characteristic segmented single-stranded RNA genome, a nucleocapsid, and anenvelope.

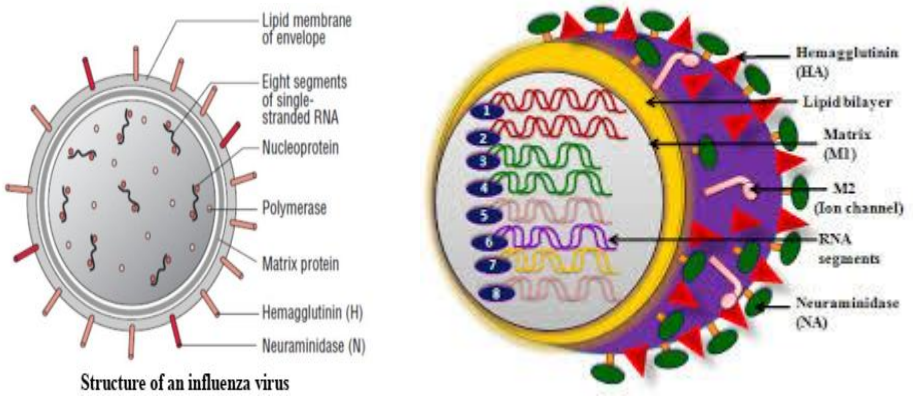
■ The viral genome is a single-stranded antisense RNA. The genome consists of an **RNA-dependent RNA polymerase**, which transcribes the negative-polarity genome into mRNA**. The genome, therefore, is not infectious**. The viral RNA has a molecular weight of 5 million daltons and a length of 13,600 nucleotides. Characteristically, it is segmented and consists of seven or eight segments.

These segments code for different proteins which are NS1, NS2, NP, M1, M2, **M3**, HA, and NA.

■ The genome is present in **a helically symmetric nucleocapsid** surrounded by a lipid envelope. The envelope has an inner membrane protein layer and an outer lipid layer. The membrane proteins are known as matrix or M protein and are composed of two components M1 and M2.

■ Two types of spikes or peplomers project from the envelope:

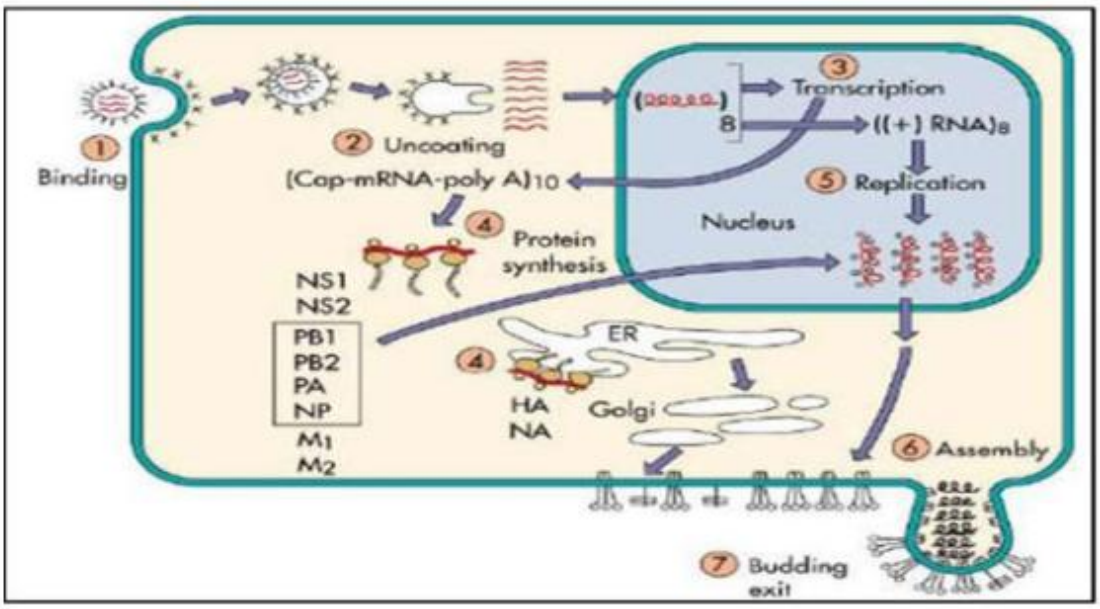
(a) **the triangular** hemagglutinin (HA) peplomers and (b) **the mushroom-shaped** neuraminidase (NA) peplomers.



Structure of an influenza virus

Influenza virus life cycle:

Replication of influenza A virus include after binding to sialic acid-containing receptors, influenza is endocytosed which fuses with the vesicle membrane and uncoats mediated by the M2 proteins and is facilitated by the low pH within the endosome/vesicle. The viral nucleocapsid enters the cytoplasm and migrates to the nucleus where the genome RNA (8 segments) gets transcribed into mRNA by the viral RNA polymerase (transcriptase). Unlike for most other RNA viruses, transcription and replication of the genome occur in the nucleus were Viral proteins synthesized Most RNA's move to cytoplasm, some remain in the nucleus to serve as a template for the synthesis of negative polarity strand RNA genomes for the progeny, by a different subunit of viral RNA polymerase (replicase). Helical nucleocapsid segments form and associated with the M1 protein-lined membranes containing M2 and the HA and NA glycoprotein's. The virus **buds** from the plasma membrane.



Influenza virus life cycle

Antigenic Changes of Orthomyxoviruses:

Changes in the antigenicity of hemagglutinin and neuraminidase **confers** on the Influenza A virus the ability **to cause pandemics**.

Two types of antigenic changes are known

1- **Antigenic drift** refer to a minor change based on accumulate mutations during virus replication in the genome RNA. Thus, Influenza viruses have many **serotypes**.
2- **Antigenic shift** that involves a major change based on the reassortment of segments of the genome RNA. Antigenic shifts can result from mechanisms Genetic reassortment between subtypes. Reassortment is possible whenever two different influenza viruses infect a cell simultaneously; **when the new viruses (the progeny)** are assembled, they may contain some genes from one parent virus and some genes from the other.

Types of influenza viruses

There are **four types** of influenza viruses: A, B, C and D
1. **Influenza A viruses Influenza A viruses** include the avian, swine, equine and canine influenza viruses, aswell as the human influenza A viruses. Influenza A viruses are classified into subtypes based on two surface antigens, the **hemagglutinin (H)** and **neuraminidase (N)** protein. There are **18** different known H antigens (H1 to H18) and **11** different known N antigens (N1 to N11). H1N1, H1N2, and H3N2 are the only known influenza A virus subtypes currently circulating among humans.

2. **Influenza B viruses** Influenza B viruses are mainly found in humans. These viruses can cause epidemics in human population, **but have not**, to date, been responsible for pandemics.

3. **Influenza C viruses** Influenza type C infections generally cause mild illness and are not thought to cause human flu epidemics.

4- **Influenza D viruses** primarily affect **cattle** and are not known to infect or cause illness in people. Viral Transmission Influenza viruses are transmitted in **aerosols** created by coughing and sneezing, and **by contact** with nasal discharges, either directly or **on fomites**. Close contact and closed environments favor transmission. Person-to-person transmission occurs with the **H1N1** virus that is currently circulating in humans.

Clinical findings:

Incubation Period : The incubation period for human influenza is usually short; most infections appear after **one to four days**. The incubation period for the novel **H1N1** virus circulating in humans appears to **be 2 to 7 days**. Clinical Signs &Pathogenicity Uncomplicated infections with human influenza **A or B** viruses are usually characterized by **upper respiratory symptoms**, which may include fever, chills, anorexia, headache, myalgia, weakness, sneezing, rhinitis, sore throat and a nonproductive cough. Nausea, vomiting and otitis media are common **in children,** and **febrile seizures** have been reported in **severe cases**. Most people recover in one to seven days, but in some cases, the symptoms may last up to two weeks or longer. More severe symptoms, including pneumonia, can be seen in individuals with chronic respiratory or heart disease. Secondary bacterial or viral infections may also occur.

Laboratory Diagnosis of Human Influenza:

Specimen collection

Respiratory specimens: Respiratory specimens obtained within four days of onset of symptoms and different types of respiratory specimens can be used such as nasal washes and nasopharyngeal aspirates tend to **be more sensitive** than pharyngeal swabs.

Blood specimens: Acute and convalescent serum samples **14 - 21** days should be collected to demonstrate a significant (at least **fourfold**) rise in strain-specific antibody titer.

Laboratory Tests

1- **Isolation methods** (Viral Culture) - Embryonated egg culture - Cell culture: - Various cell-lines are utilized to isolate influenza viruses, most commonly primary monkey kidney cells. Infection of cells gives a visible cytopathic effect (CPE).

2- **Direct methods**

- Immunofluorescence
- Enzyme immuno assays
- Reverse transcription polymerase chain reaction (RT-PCR).

3- Serology Different serological techniques are available for influenza diagnosis includes **haemagglutination inhibition** (HI), complement fixation (CF), enzyme immunoassays (EIA) and indirect immunofluorescence.

LECTURE 10 **Lect. Dr. Ahmed Yaseen Abed**

Paramyxoviruses

Paramyxoviruses are the major respiratory pathogens in this age group. The paramyxoviruses include the most important agents of respiratory infections of **infants** and **young children** (**respiratory syncytial virus** [RSV] and the **parainfluenza viruses**) as well as the causative agents of two of the most common contagious diseases of childhood (mumps and measles). All members of the Paramyxoviridae family initiate infection **via** the respiratory tract. Whereas replication of the respiratory pathogens is limited to the respiratory epithelia, **measles** and **mumps** become disseminated throughout the body and produce generalized disease.

Classification

The family Paramyxoviridae consists of three important genera

1-**Paramyxovirus** includes parainfluenza and mumps virus.

2- **Morbillivirus** includes the measles virus.

3-**Pneumovirus** includes respiratory syncytial virus (RSV), which is responsible for majority of acute respiratory infections in **infants** and **children**.

Paramyxovirus Family

GENUS	MEMBERS	GLYCOPROTEINS
Paramyxovirus	mumps human parainfluenza viruses (HPIV 1-4)	HN, F
Morbillivirus	Measles	H, F
Pneumovirus	Respiratory syncytial virus	G, F

Properties of Paramyxo viruses

Virion: Spherical, pleomorphic,150 nm or more in diameter (helicalnucleocapsid,13–18nm)

● **Composition**: RNA(1%),protein (73%),lipid (20%), carbohydrate(6%)

● Genome: Single- stranded negative RNA, linear, non-segmented, about15kb, no reassortment .

●Proteins: Six to eight structural proteins.

●Envelope: Contains viral glycoprotein (**G ,H, or HN**)(which sometimes carries hemagglutinin or neuraminidase activity) and fusion(F) glycoprotein

● **Replication**: Cytoplasm: particles bud from plasma membrane. . A large excess of nucleocapsids are produced in infected cells, which form characteristic cytoplasmic inclusion bodies.

● **Outstanding characteristics**: **Antigenically stable Particles** are labile yet highly infectious.

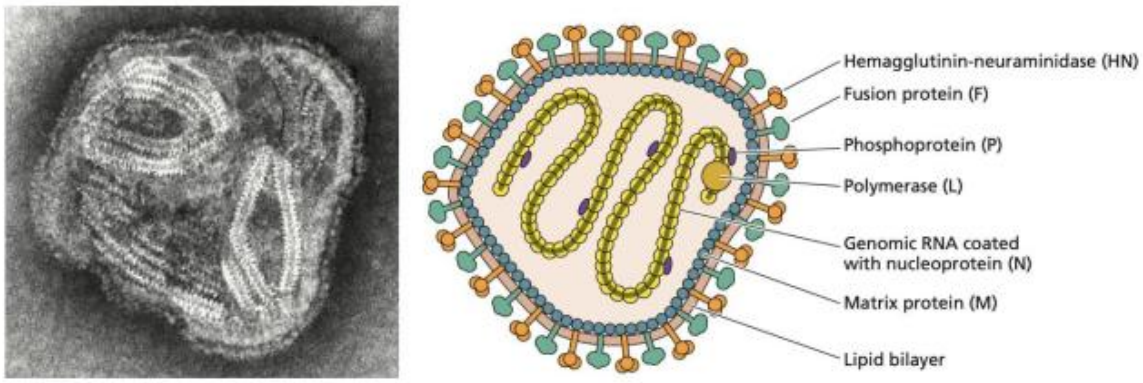


Figure: Paramyxovirus structure

Transmission :-

spread by droplets from the nose and mouth to close contacts. Many of them are highly infectious and go around the community in epidemics- often seasonal, eg. Winter coughs and colds. Fomites might also assist spread

Human parainfluenza viruses (HPIVs)

HPIVs are single-stranded, enveloped RNA viruses of the Paramyxoviridaie family. There are four **serotypes**(1-4) which cause respiratory illnesses in

children and **adults**. HPIVs bind and replicate in the ciliated epithelial cells of the upper and lower respiratory tract and the extent of the infection correlates with the location involved. Seasonal HPIV epidemics result in a significant burden of disease in children and account for 40% of pediatric hospitalizations for lower respiratory tract illnesses and **75%** of **croup cases**.

Pathogenesis of human parainfluenza virus (HPIV) infection

The virus adsorbs to the respiratory epithelial cells by specifically combining with neuraminic acid receptors in the cell through its **hemagglutinin**. Subsequently, the virus enters the cells following fusion with the cell membrane, mediated by **F1 and F2 receptors**. The virus replicates rapidly in the cell **cytoplasm** and causes formation of multinucleated giant cells. The virus also causes the formation of single and multilocular cytoplasmic vacuoles and basophilic or eosinophilic inclusions. The virus causes inflammation of the respiratory tract, leading to secretions of high level of inflammatory cytokines, usually 7–10 days after initial exposure. Airways inflammation, necrosis, and sloughing of respiratory epithelium, edema, and excessive mucus production are the noted pathological features associated with HPIV infections.

Clinical feature

Human parainfluenza viruses cause croup (a heterogeneous group of illnesses that affects the larynx, trachea, and bronchi. The condition manifests as fever, cough, laryngeal obstruction), pneumonia, bronchiolitis and tracheobronchitis, and Otitis media, pharyngitis, conjunctivitis. The severity of the disease occurred in infant **less than 6 months**.

Laboratory Diagnosis

Respiratory specimens include nasopharyngeal aspirations nasal washings, and nasal aspirations.

1- **Direct antigen detection** The ELISA, immunofluorescence assay are used to detect HPIV antigen

2-**Molecular Diagnosis** : polymerase chain reaction (PCR) has been developed for detection of HPIV-1, HPIV-2, and HPIV-3 genome in clinical specimens.

3- **Isolation and identification Nasal wash** are good specimens, culture in monkey kidney cell line , the diagnosis depending on hem adsorption

Prevention and Control

Currently there is **no vaccine** against infection by HPIV, However, researchers are trying to develop one

Mumps

Mumps is an acute contagious disease characterized by **nonsuppurative enlargement** of one or both salivary glands. Mumps virus mostly causes a mild childhood disease, but in adults complications including **meningitis** and **orchitis** are fairly common. More than one-third of all mumps infections are asymptomatic

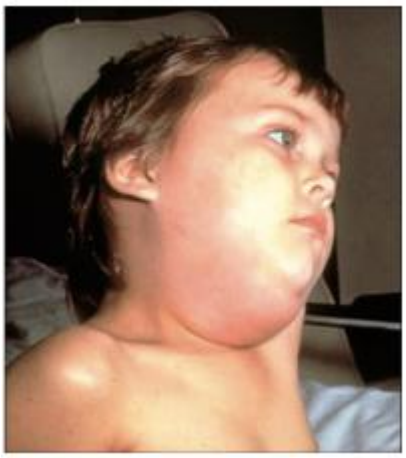


Figure: Child with mumps (swollen parotid gland)

Pathogenesis & Pathology

Humans are the **only natural** hosts for mumps virus. **Primary replication** occurs in nasal or upper respiratory tract epithelial cells. **Viremia** then disseminates the virus to the salivary glands and other major organ systems. Involvement of the **parotid gland** is not an obligatory step in the infectious process. The incubation period may range from 2 to 4 weeks but is typically about 14– 18 days. Virus is shed in the saliva from about 3 days before to 9 days after the onset of salivary gland swelling. About one-third of infected individuals do not exhibit obvious symptoms (in apparent infections) but are equally capable of transmitting infection. Virus frequently infects the kidneys and can be detected in the urine of most patients**. Viruria** may persist for up to 14 days after the onset of clinical symptoms. The central nervous system is also commonly infected and may be involved in the absence of parotitis.

Clinical Findings

Fever, **malaise** followed by **rapid enlargement of the parotid gland** and it is **painful** . mumps may be associated with aseptic meningitis . testis and ovaries may be infected especially after puberty and it may pass to **sterility** in man but it **is rare** (not more than 1%).

Laboratory diagnosis of Mumps virus

1) Clinical feature

2) Isolation and identification

The most appropriate clinical samples for viral isolation are saliva, cerebrospinal fluid, and urine collected within a few days after onset of illness. Virus can be recovered from Culture in monkey kidney cells and diagnosis by using the urine for up to 2 weeks. mumps specific antiserum by immunofluorescence method, hemadsorption test can also be used.
3) **Nucleic acid detection:-** by PCR test.

4) **Serology IgM and IgG Abs** detection by ELISA and Heamagglution inhibition test.

Treatment and Prevention

● There is no specific therapy .

● Immunization with attenuated live mumps virus vaccine is the best approach to reducing mumps-associated morbidity and mortality rates. Mumps vaccine is available in combination with measles and rubella (**MMR**) live-virus vaccines.

Measles

Measles is an acute, **highly infectious disease** characterized by fever, respiratory symptoms, and a **maculopapular rash**. Complications are common and may be quite serious.

Pathogenesis & Pathology

Humans are the only natural hosts for measles virus. The virus gains access to the human body via the respiratory tract, where it multiplies locally; the infection then spreads to the regional lymphoid tissue, where further multiplication occurs. **Primary viremia disseminates** the virus. Finally, a **secondary viremia seeds the epithelial surfaces of the body**, including the skin, respiratory tract, and conjunctiva, where focal replication occurs. The described events occur during the incubation period, which typically lasts 8–12 days but may last up to 3 weeks in adults. involvement of the central nervous system is common in measles. Clinical Findings Infections in non immune hosts are almost always symptomatic. After an incubation period of 8–12 days, measles is typically a 7-11days illness. **The prodromal phase** is characterized by fever, sneezing, coughing, running nose, redness of the eyes, **Koplik spots**, and lymphopenia. The conjunctivitis is commonly associated with photophobia. **Koplik spots** are small, bluish-white ulcerations on the buccal mucosa opposite the lower molars. These spots contain giant cells and viral antigens and appear about 2 days before the maculopapular rash. . **The most common complication of measles is otitis media (5–9% of cases)**. Pneumonia is the most common life-threatening complication of measles, caused by secondary bacterial infections.

Subacute sclerosing panencephalitis (SSPE) is a very rare, but fatal disease of the central nervous system that results from a measles virus infection acquired earlier in life SSPE generally develops 7 to 10 years after a person has measles.

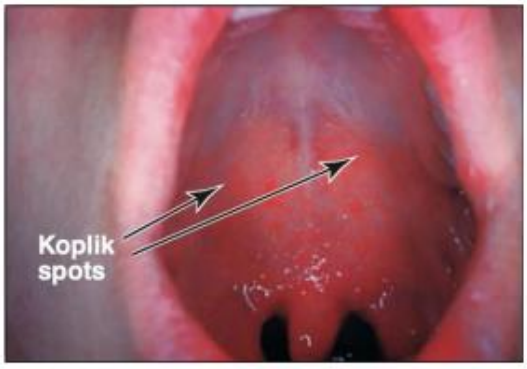


Figure: Koplik spots in the mouth caused by measles virus

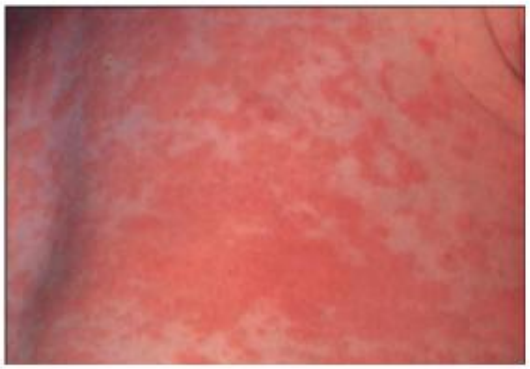


Figure: The measles skin rash (maculopapules)

Laboratory Diagnosis

1) Clinical feature

2) Isolation & Identification of Virus

- Nasopharyngeal and conjunctival swabs, blood samples, respiratory secretions, and urine collected from a patient during the febrile period are appropriate sources for viral isolation. culture in monkey and human kidney cells , diagnosis by cytopathic effect , multinucleated and intra nuclear and intra cytoplasmic inclusion bodies.

3- Antigen detection Measles antigen can be directly detected from specimen include respiratory secretion , nasopharynx and conjunctiva by Immunofluorescence test.

4- Serology IgM and IgG antibodies by ELISA and Heamagglution inhibition test (HI) test.

5- Detection of viral RNA by RT-PCR Is a sensitive method that can be applied to a variety of clinical samples for measles diagnosis. Treatment, Prevention, & Control No treatment . A highly effective and safe attenuated live measles virus vaccine has been available since 1963.

Respiratory syncytial virus(RSV)

It is the most common cause of lower respiratory tract illness in infant and young children.

Pathogenesis and pathology

Replication of the virus occurred initially in the **nasopharynx** , then the virus may spread to the lower respiratory tract and produce bronchiolitis and pneumonia. The incubation period 3-5 days and virus shedding for 1-3 weeks.

Clinical findings

Common cold, pneumonia in infant and may bronchitis and bronchiolitis which Life threatening disease in infant especially under 6, and can lead to chronic lung disease in later life. Reinfection is common in both children and adult with less severity. This virus are a common cause of otitis media about 30% of otitis media cause in infant .

Laboratory diagnosis of Respiratory syncytial virus(RSV)

1- Clinical feature

2- Antigen detection Nasal wash or aspirate are good sample . Virus antigens

detection by immunofluorescence test .

3- Isolation and identification of the virus By culturing the specimen into human heteroploid cell line (Hela) and Hep-2, the diagnosis is depend on the cytopathic effect and appearance of giant cells.

4- Nucleic acid detection Diagnosis by detection of the RNA of the virus by PCR.

5-Serology Detection of serum antibodies which include IgM and IgG Abs by using immunofluorescence test . Treatment Supportive care , Ribavirin may be used in the treatment of sever cases by aerosol for 3-6 dayes . No vaccine is available toda but passive immunization immunoglobulin can be given for infected premature infants.