

# *Virology*

*Lec (7)*

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## **Teaching Objectives:**

1. To define paramyxoviridae
2. To list different types of paramyxoviruses with general characteristic for each one.
3. To recognize the mechanism of entry and replication.
4. To describe methods of diagnosis, treatment and prevention.
5. To identify the differences and similarities between ortho and paramyxoviruses.

Paramyxovirus from Greek Para-beside, myxo-mucus or slim. The paramyxovirus family contains four important human pathogens, parainfluenza, respiratory syncytial virus (RSV), mumps and measles.

## ***Important properties of paramyxoviruses:***

- Spherical pleomorphic shape, about 150-300 nm in diameter.
- Single strand RNA, linear, non- segmented, negative sense totals 15Kb. The virion contains RNA-dependant RNA polymerase.
- Have three structural proteins, nucleoprotein (**NP**), phosphoprotein (**P**), and large protein (**L**). These protein found in Nucleocapsid (Complexed with RNA genome as part of the viral RNA-polymerase complex)
- Helical nucleocapsid.
- Matrix Protein **M** found Inside envelope have essential role in Assembly.
- Enveloped virus lipid bi-layer with associated 2 virus-specific glycoprotein's, hemagglutinin and neuraminidase protein on the same spike. Play important role in attachment to host cell receptors and diagnosis of viral diseases by hemagglutination and hemadsorption.

Fusion (F) protein spikes out from the envelope. It promotes the fusion of host and viral cell membranes, which is an initial step in infection. It also promotes membrane fusion between several host cells, leading to multinucleated giant cells

(syncytia cell). Fusion (F) protein, which is activated by extra-cellular proteolytic cleavage to an active form that has 2 subunits, F1 and F2, linked by a disulfide bond.

-Viruses replicate in cytoplasm of cell.

- Sensitive to heat and detergents, antigenically stable and hemadsorbing virus (the property of hemadsorption is due to interaction between viral hemagglutinin and specific erythrocyte receptors on guinea pig red cells).

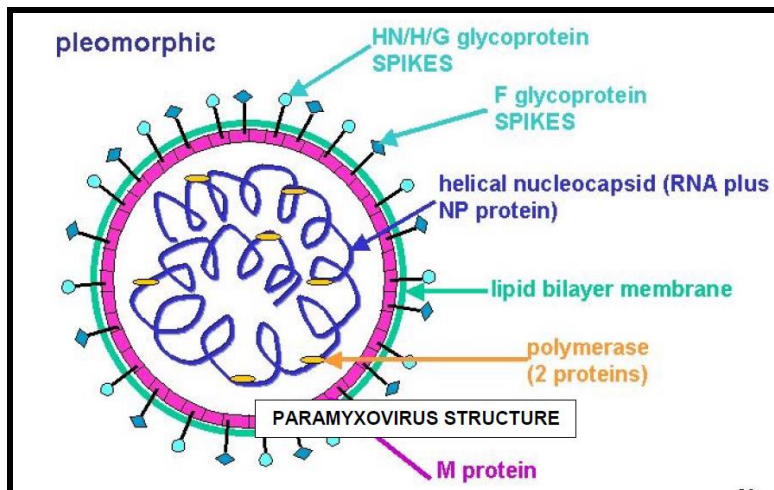


Figure (1): Morphology of paramyxovirus virus

### *Mode of transmission*

These viruses are transmitted via respiratory droplets (large droplets- person to person through close contact and aerosols of respiratory secretions) and fomites; they cause disease worldwide, primarily in the winter months.

### *Summary of replicative cycle*

**1-**Parainfluenza virus infects respiratory epithelium (initially the nasopharyngeal mucosa).

**2-**The first step in the infection cycle involves attachment of the virus to host cell sialic acid receptors. This is mediated by viral attachment protein, a function served by the HN glycoprotein. After attachment, the F protein catalyzes fusion of the viral envelope and host cell membrane, resulting in uncoating and release of the nucleocapsid structure into the host cell cytoplasm without internalization in endosomes and under neutral pH.

3-Transcription and protein synthesis to occur, first multiple mRNA are formed with the help of RNA- dependent RNA polymerase (3 structural proteins form this complex). Viral proteins are synthesized in the cytoplasm.

4-The genome is replicated by formation of a full-length positive sense RNA template onto which a negative sense RNA is then transcribed.

5-Assembly of the nucleocapsid occurs and M proteins link nucleocapsid to viral glycoprotein-modified cell membranes.

6-Mature virions are released from host cell membranes by budding figure (2).

7-Following infection in normal hosts, virus is shed for approximately a week. Prolonged viral shedding is seen in immunocompromised hosts.

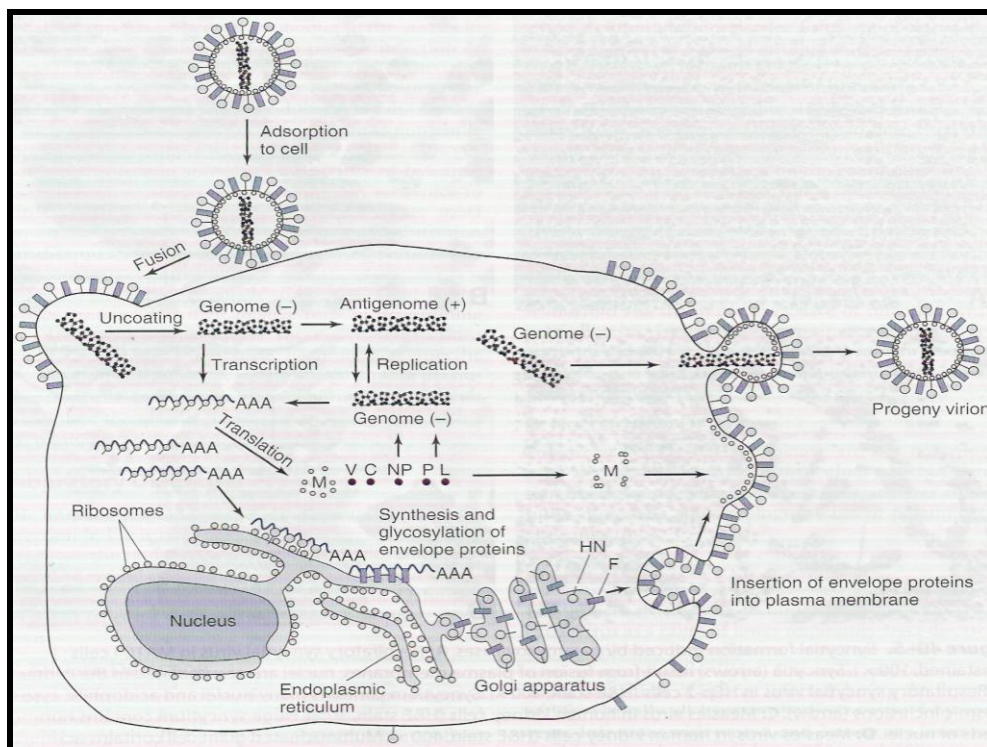


Figure (2): Replication cycle of paramyxovirus

### *Pathogenesis and clinical features*

These viruses cause upper and lower tract disease without viremia. Incubation period is usually 2-6 days. Most infections are asymptomatic, especially in older children and adults.

1- *Parainfluenza virus 1 and 2* may cause cold-like symptoms, along with croup in children, the infection more extensive and involved the larynx and upper trachea,

resulting in croup (laryngotracheobronchitis), croup is characterized by respiratory obstruction due to swelling of the larynx and related structures]. As well as rhinorrhea/rhinitis, cough and fever.

**2- Parainfluenza virus 3** is the most common parainfluenza virus isolated from children between 6 to 18 months, the virus spread deeper to the lower trachea and bronchi, culminating in pneumonia or bronchiolitis.

**3- Parainfluenza virus 4** is less common than the other types, and is known to cause mild to severe respiratory tract illnesses.

- ✓ Re-infections are clinically less severe; most commonly involve the upper respiratory tract, and occur throughout life. The complication of parainfluenza is otitis media, parotitis, aseptic meningitis occurs (rare).
- ✓ Human metapneumovirus, initially described in about 2001, is also implicated in bronchitis, especially in children. Though it was discovered in 2001 it was proven to be circulating in the population for at least 50 years and that by the age of five most children have already been exposed to it.

## *Diagnosis*

By laboratory diagnosis due to clinically similar to other viruses causes flu.

### **1. Isolation of virus in cell culture.**

Cell lines such as Primary Rhesus monkey kidney epithelial Cells (PRMK), and Human embryonic kidney cells are used. Then tested by

- ✓ Rapid method like Immunofluorescence using monoclonal antibodies.
- ✓ Hemadsorption with guinea pig erythrocytes can be observed at 4°C.
- ✓ Cytopathic effects such as syncytial formation. Parainfluenza viruses grow slowly and produce very little cytopathic effect.

### **2. Detection of viral antigen.**

Detection of viral antigen in a patient specimen by various techniques such as, enzyme linked immunosorbent assay (EIA or ELISA), immunofluorescence tests

(direct immunofluorescence test or indirect immunofluorescence test or latex agglutination (LA) tests.

**3. Nucleic acid detection by** polymerase chain reaction (PCR) assays is not commonly used this time outside of research settings because of difficulties of RNA detection in respiratory secretions.

### *Prevention and Treatment.*

- + Depends primarily on support care.
- + Removal of secretions.
- + -Administration of oxygen and humidification of air.
- + No specific antiviral therapy is available but can use ribavirin for treatment LRT infection especially in immunocompromised patients.

### *Respiratory syncytial virus [RSV]*

This virus is most commonly implicated in causing lower respiratory infections in infants, including bronchiolitis and pneumonia.

Two major groups of strains of human RSV exist- group A and B; strains of group A predominate.

### *Important properties of Respiratory syncytial virus*

- Spherical or pleomorphic, about (100-350 nm).
- Single-strand, negative sense linear RNA.
- Nucleocapsid is slightly smaller than other parainfluenza viruses (13 nm).
- Envelop contains two glycoproteins

A- **G-protein**, is a large, highly glycosylated protein, that is important for viral attachment to host cells. Antigenic variations in type of G protein determine the strain (A or B).

B- **F-protein** or Fusion protein is important for fusion of viral particles to target cells and also fusion of infected cells to neighboring cells to form syncytia.

-RSV lacks H/N proteins or hemagglutinating properties. Virus can **survive** on surfaces for about 6 hours, on gloves for  $\leq 2$  hours, and for about 30 minutes on hands.

-**Rapidly loses viability** with freeze-thaw cycles or in acidic conditions or with disinfectants.

### *Summary of replication cycle:*

1-Virus enters the host through mucosa of eye and nose. At a cellular level, attachment via G protein occurs to host cell membrane glycoside.

2-Localized infection of respiratory tract, with cell-to-cell transfer of virus leading to spread from upper to lower respiratory tract. Middle ear is also involved.

3-The virion RNA dependent RNA polymerase transcribes the negative-strand genome into mRNA then translated into the specific viral proteins.

4-The helical nucleocapsid is assembling, the matrix protein mediates the interaction with the envelope, and the virus is released by budding from the cell membrane.

### *Pathogenesis and clinical features*

Incubation period is usually 4-6 days (range 2 to 8 days). Infected cells undergo necrosis. Syncytia are formed through the fusion of adjacent cell membranes and contain many nuclei and acidophilic cytoplasmic inclusions.

1. Upper respiratory infection 'cold' in older children and adults. Clinical features are: fever, rhinitis, pharyngitis.
2. Lower respiratory infection (LRI) may occur after URI. Bronchiolitis and/or pneumonia is seen in infants with primary infection. Clinical features of LRI are cough (can persist for a few weeks), tachypnea, respiratory distress, hypoxemia, cyanosis, etc. In young infants, features of LRI include apnea, lethargy, irritability, and poor feeding.



- Host immune response also induces some of the pathological changes- mononuclear cell infiltration, local cytokine production and histamine release along with increased mucous production.

In bronchiolitis, smaller airways, especially the bronchioles are involved. Mucosal edema, cell necrosis and sloughing, increased mucin secretion and plugging of bronchiolar lumina with epithelial debris and mucin. Histological, features include peribronchial infiltration with lymphocytes. In pneumonia, the lungs show interstitial infiltration with mononuclear cells.

3. Severe infections occur in preterm infants (especially <35 weeks gestation and those with chronic lung disease), children with cyanotic congenital heart disease, and immunocompromised hosts.
4. Radiological features of bronchiolitis are atelectasis, hyperinflation and streaking.

Spread beyond respiratory epithelium (to heart, liver, kidneys) is seen in immunocompromised hosts (rare).

### *Laboratory diagnosis*

- 1- Clinical specimens. Nasal washings, nasal aspirates or nasopharyngeal swabs should be transported on ice and use for different test.
- 2- Viral culture. Cell lines such as HeLa, Hep-2, and Monkey Kidney cells. Cytopathic effects are usually seen in 2-5 days. Shell vial technique is useful along with immunofluorescent antibody staining of infected cells.
- 3- Antigen detection. Direct identification of viral antigens in clinical samples is rapid and sensitive or serology test such as neutralizing antibodies by Complement fixation test (CF), immunofluorescence are not very useful for clinical decision-making. Mainly useful for epidemiological studies.

### *Treatment.*

-Supportive treatment is the main of therapy for bronchiolitis fluids, oxygen, humidification of air, respiratory support, removal of secretions and possibly, bronchodilators use.

- Aerosolized ribavirin (Virazole) is recommended for severely ill hospitalized infants

- A combination of ribavirin and hyperimmune globulins against RSV may be more effective.

### *Prevention*

There is no vaccine. Previous attempts to protect with a killed vaccine resulted in an increase in severity of symptoms.

- ✚ Active immunization- formalin-inactivated vaccine is no longer used because it was associated with an increase in severity of disease. Other vaccine candidates are in trial phases (e.g. subunit vaccines).
- ✚ Passive immunization with a monoclonal antibody directed against the fusion protein of RSV (palivizumab, Synagis) can be used for prophylaxis in premature or immunocompromised infants.
- ✚ Hyperimmune globulins (RespiGam) are also available for prophylaxis in these infants and in children with chronic lung disease. Nosocomial outbreaks can be limited by hand washing and use of gloves.

Property	Orthomyxoviruses	Paramyxoviruses
Disease caused in humans	Influenza types A,B and C	Parainfluenza 1- 4, respiratory syncytial disease and mumps
Genome organization	Single- stranded RNA in eight pieces	Single- stranded RNA in single pieces
Inner ribonucleoprotein helix	9nm in diameter	18nm in diameter
RNA in nucleocapsid	RNase - sensitive	RNase - resistant
Fusion of virus with cell	Endosome	Plasma membrane
Transcription of viral RNA	Host cell nucleus	Host cell cytoplasm
Genetic reassortment	frequent	Rare
Rate of antigenic change	High	Low