AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lecture 7.

Introduction to basic virology. The causative agents of acute respiratory viral infections (families of Orthomyxoviridae, Paramyxoviridae, Adenoviridae, Coronaviridae, Rhinovirus genus) and smallpox (Poxviridae family)

FACULTY: ***General Medicine***
SUBJECT: ***Medical microbiology - 2***

Discussed questions:

Lecture plan:

1. Introduction to specific virology
2. Viruses that cause acute respiratory infections
3. Orthomyxoviridae family. General features, classification.

- Influenza viruses. Virion structure, structural features, cultivation, resistance. Influenza virus antigens. Classification of influenza A viruses by neuraminidase and hemagglutinin, antigen variability, ecology. Pathogenesis of influenza. Complicating effects of bacterial flora, immunity, microbiological diagnostics. Principles of specific treatment and prevention (vaccines, immunoglobulin, interferon, chemicals)

4. Paramyxoviridae family. General features, classification. Structure and chemical composition of virion, cultivation. Hemolysis, hemagglutination and hemadsorption properties. Resistance.

- Parainfluenza viruses, their role in human pathology, features of immunity
- Mumps virus, cultivation. Pathogenicity features. Immunity. Specific prevention.
- Respiratory syncytial viruses, cultivation. Pathogenicity features. Immunity.
- Morbillivirus genus. Measles virus. Pathogenicity features. Immunity. Semi-acute sclerosing panencephalitis. Microbiological diagnosis, specific prophylaxis.

5. Adenoviridae family, classification. Virion structure, cultivation, sustainability. Serotypes, pathogenicity features. Persistence. Microbiological diagnosis
6. Coronaviridae family, classification. Virion structure, serotypes, cultivation problems, pathogenicity features. Severe acute respiratory syndrome (SARS), COVID-19 infection.
7. Rhinovirus genus. Human pathogenicity
8. Poxviridae family, classification. Virion structure, cultivation, sustainability. Monkeypox virus. Pathogenicity features. Microbiological diagnosis.

SPECIAL VIROLOGY

- ***Virology*** – studies the morpho-biological characteristics of clinically important viral pathogens, the pathogenesis, diagnosis and treatment of the infections caused by them.
- Viruses are divided into the following groups according to their structural characteristics:
- Naked DNA viruses
- Envelope DNA viruses
- Naked RNA viruses
- Envelope RNA viruses

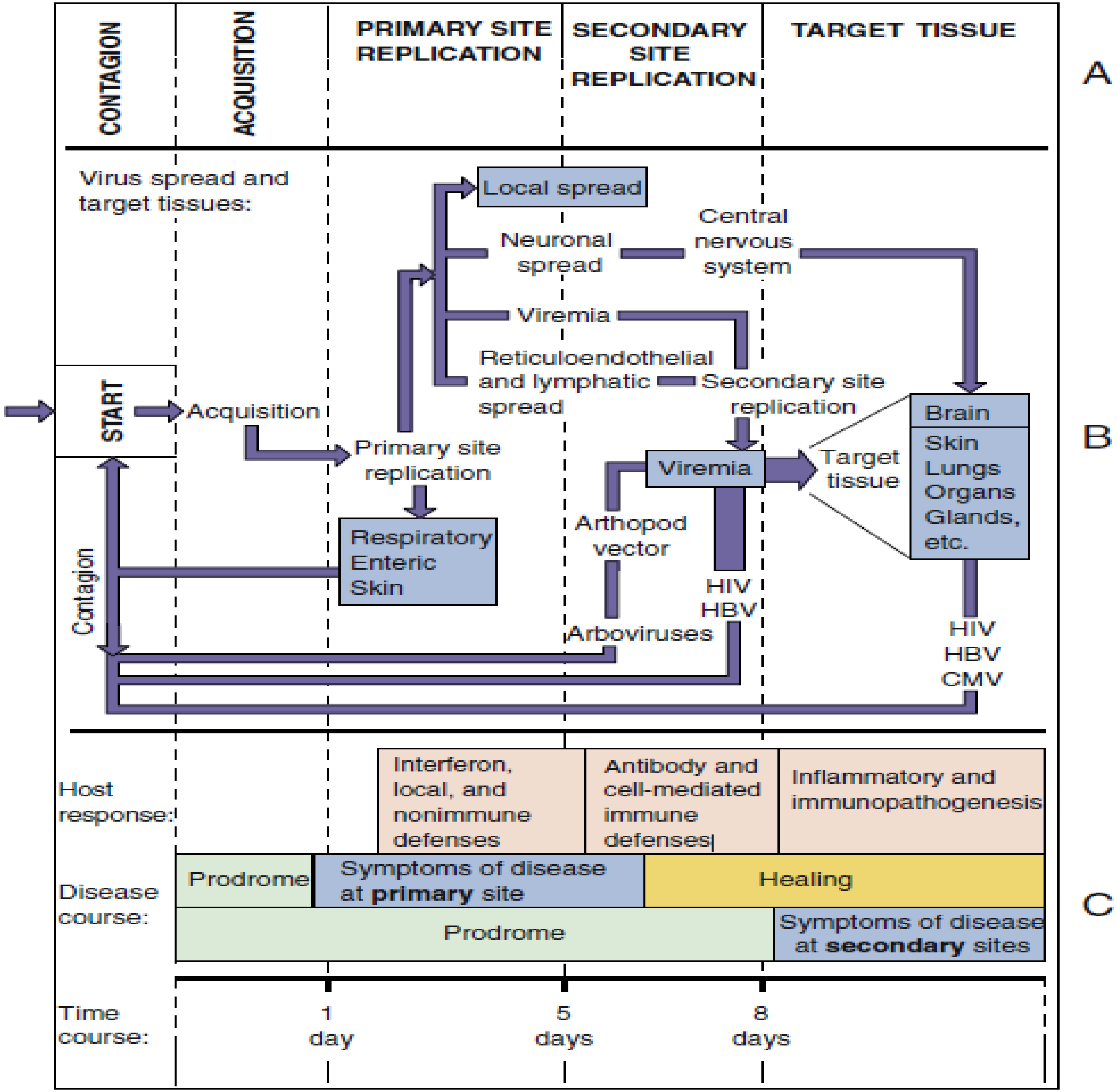
The study of some viruses is based not on their structure, but on their biological properties:

- Arboviruses
- Latent viral infections, etc.

Viral Transmission

Mode	Examples
Respiratory transmission	Paramyxoviruses, influenza viruses, picornaviruses, rhinoviruses, varicella-zoster virus, B19 virus
Fecal-oral transmission	Picornaviruses, rotavirus, reovirus, noroviruses, adenovirus
Contact (lesions, fomites)	HSV, rhinoviruses, poxviruses, adenovirus
Zoonoses (animals, insects)	Togaviruses (alpha), flaviviruses, bunyaviruses, orbiviruses, arenaviruses, hantaviruses, rabies virus, influenza A virus, orf (pox)
Transmission via blood	HIV, HTLV-1, HBV, HCV, hepatitis delta virus, cytomegalovirus
Sexual contact	HSV, human papillomavirus, molluscum contagiosum, Zika, HIV, HTLV-1, HBV, HCV
Maternal-neonatal transmission	Rubella virus, cytomegalovirus, B19 virus, echovirus, HSV, varicella-zoster virus, HIV
Genetic	Prions, retroviruses

HBV, Hepatitis B virus; *HCV*, hepatitis C virus; *HSV*, herpes simplex virus; *HTLV-1*, human T-cell lymphotropic virus 1.



1 (A) Stages of viral infection. The virus is released from one person, is acquired by another, replicates, and initiates a primary infection at the site of acquisition. Depending on the virus, it may then spread to other body sites and finally to a target tissue characteristic of the disease.

(B) The cycle starts with acquisition, as indicated, and proceeds until the release of new virus. The thickness of the arrow denotes the degree to which the original virus inoculum is amplified on replication. The boxes indicate a site or cause of symptoms.

(C) Time course of viral infection. The time course of symptoms and the immune response correlate with the stage of viral infection and depend on whether the virus causes symptoms at the primary site or only after dissemination to another (secondary) site. *CMV,* Cytomegalovirus; *HBV,* hepatitis B virus; *HIV,* human immunodeficiency virus.

Specimens Appropriate for Laboratory Diagnosis of Various Clinical Syndromes

Syndrome	Specimen
Respiratory	Nasal or throat swab; nasopharyngeal aspirate; sputum
Enteric	Feces
Genital	Genital swab, urine
Eye	Conjunctival (and/or corneal) swab
Skin	Vesicle fluid/swab/scraping; biopsy solid lesion
Central nervous system	Cerebrospinal fluid; feces (enteroviruses)
Generalized	Throat swab[a]; feces[a]; blood leukocytes[a]
Autopsy/biopsy	Relevant organ
Any	Blood for serology[b]

METHODS OF MICROBIOLOGICAL DIAGNOSIS OF VIRAL INFECTIONS

Rapid (express) methods

Direct detection of antigens or nucleic acid of the pathogen in clinical material obtained from the patient.

Virological method

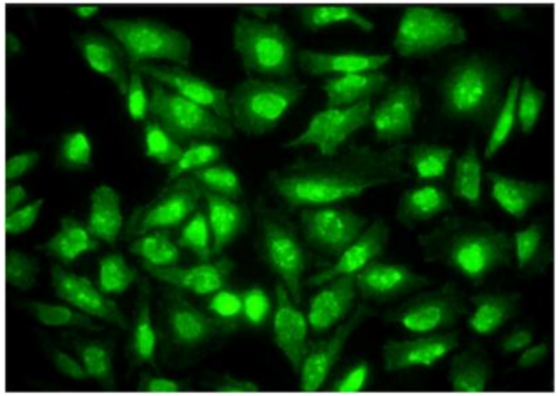
Isolation of virus from clinical material and its indication and identification.

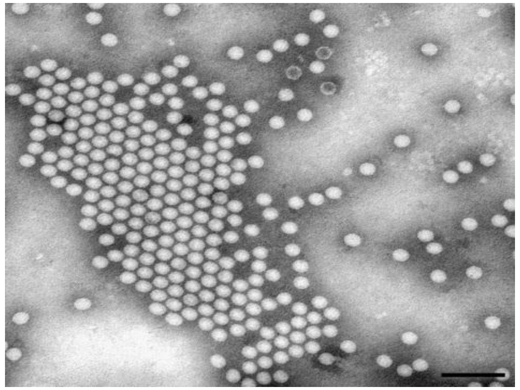
Serological method

Determination of the titer of antibodies against the virus in the paired sera of the patient.

RAPID (EXPRESS) METHODS

- **Rapid detection** is the identification of the virus and its antigens in bio substrates (biopsy samples, sediment epithelium, leukocytes, histological sections, etc.).
- **Rapid methods include:**
- **serological method** - determination of viral antigen using diagnostic antiviral sera: immunofluorescence, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay, immunoelectrophoresis, immunoelectron microscopy, direct and indirect hemagglutination reaction, reversed indirect hemagglutination inhibition (RIHAI) test;
- **microscopic method** - detection of inclusions formed by viruses in cells using light, luminescent or electron microscopy:
- **molecular-genetic method** - molecular hybridization, PCR





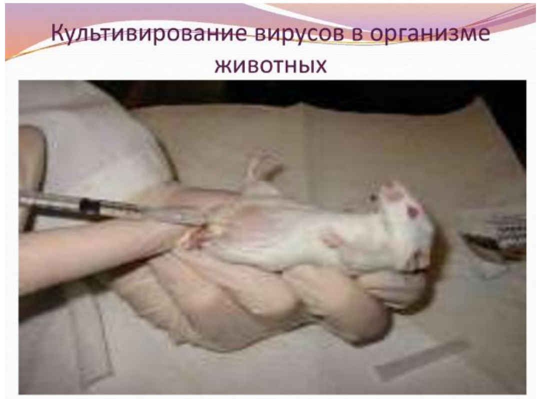
VIROLOGICAL METHOD

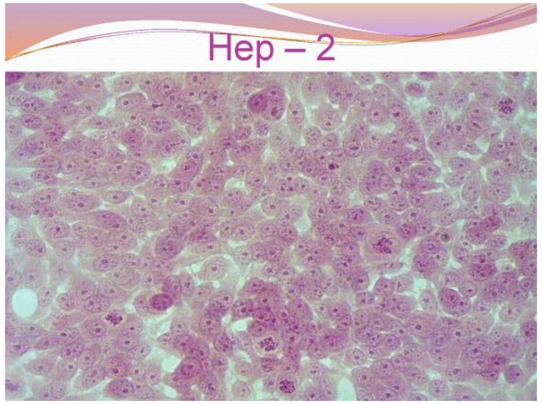
The virological method is based on the cultivation of viruses in sensitive biological objects (cell cultures, chicken embryos, laboratory animals).

It consists of the following stages:

- *Collection of pathological material*
- *Selection of a sensitive test system.*
- *Injection based on the principle of cytotropism.*
- *Indication (detection) of the virus.*
- *Virus identification (type determination).*

- **Indication** of viruses, that is, non-specific detection of infection, is based on the determination of the biological properties of viruses and their interaction with sensitive cells.
- **Identification** means identifying the type and variant of the virus.





CULTİVATİON OF VİRUSES

LABORATORY ANİMALS

CELL (TISSUE) CULTURES

EMBRYONATED CHICKEN EGGS

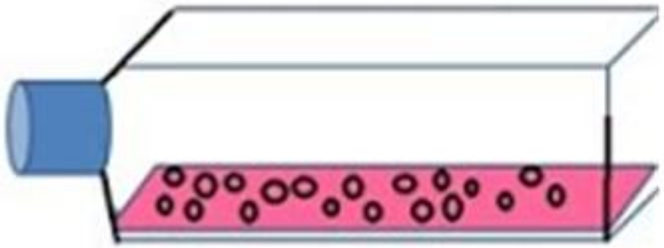
Monolayer

Primary

Diploid cell strain

Continuous cell lines

Cell Line - Types

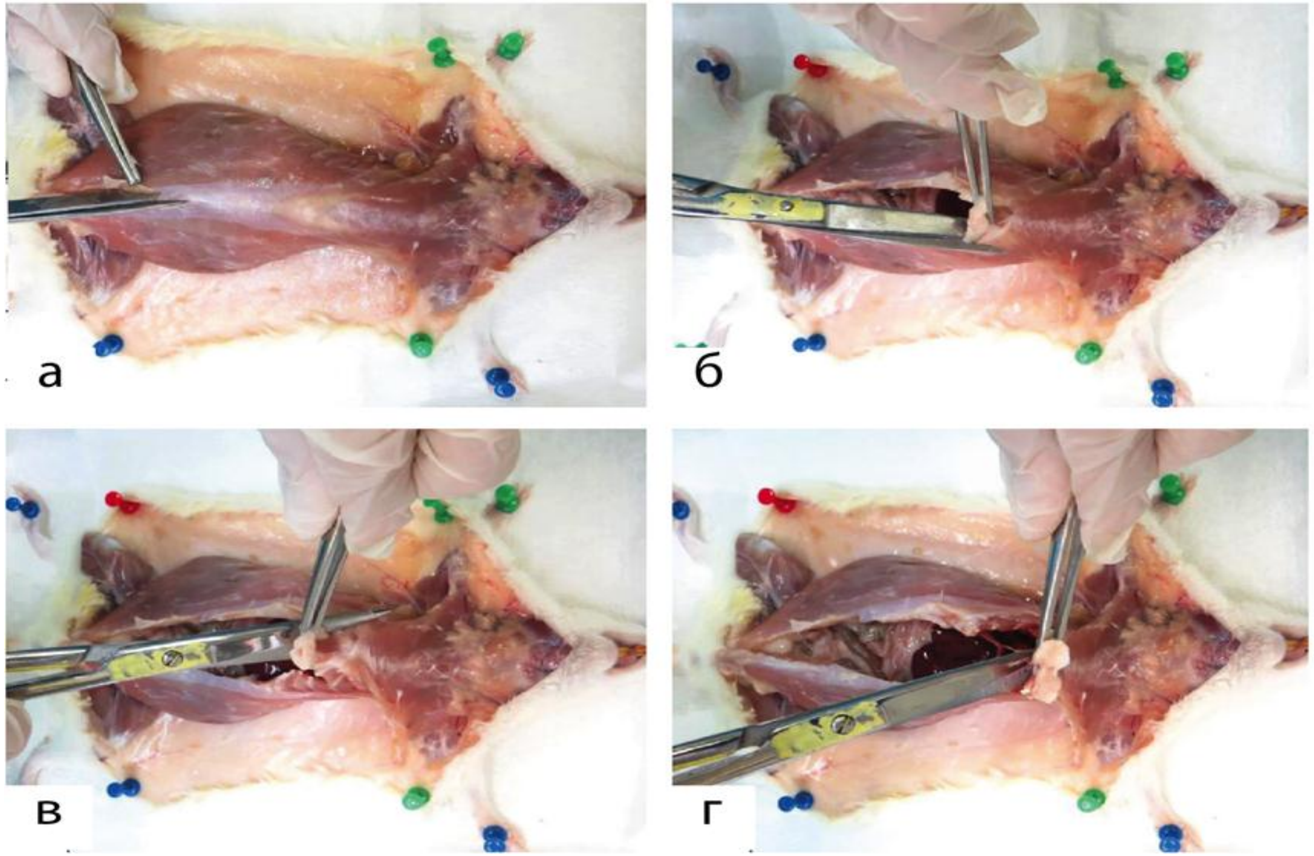


- Primary cell lines – 5-10 times can divide
  - Monkey Kidney cell lines – Myxoviruses, Enteroviruses, Adenoviruses
  - Human Amnion cell line
  - Chick embryo cell line
- Secondary or diploid cell lines – 10 to 50 times can divide
  - Human Fibroblast cell line – for recovery of CMV
  - Human embryonic lung cell strain - MRC-5, WI-38 – for Vaccines, growth of virus
- Continuous cell lines – cancerous , immortal, haploid
  - HeLa cell line – from cervix
  - HEp-2 cell line - from larynx
  - KB cell line
  - McCoy
  - Vero cell line
  - BHK

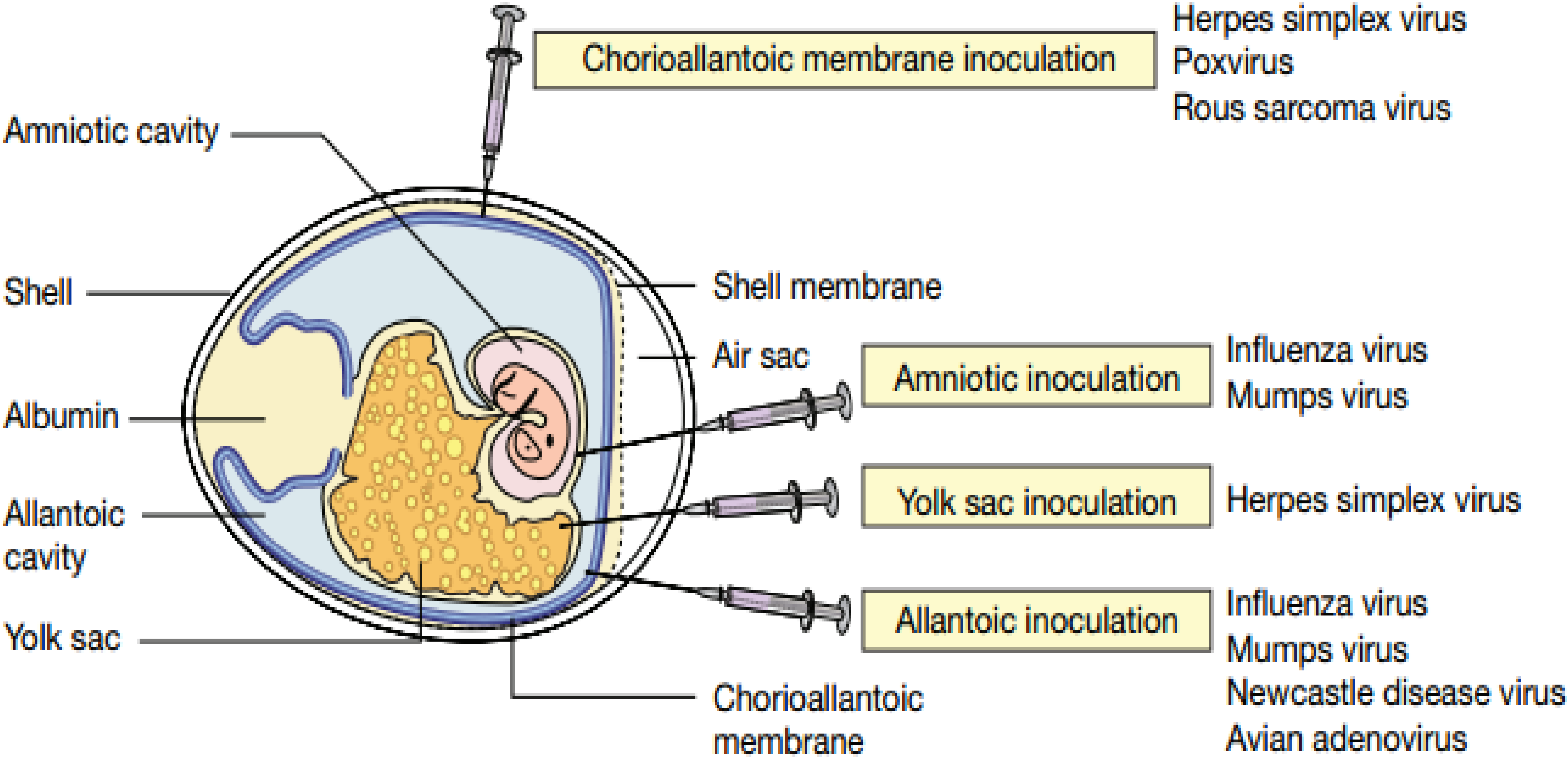
INDICATION OF VIRUSES IN THE BODY OF LABORATORY ANIMALS

The indication is based on the following characteristics

- *typical symptoms of the disease*
- *pathomorphological changes in animal organs and tissues*



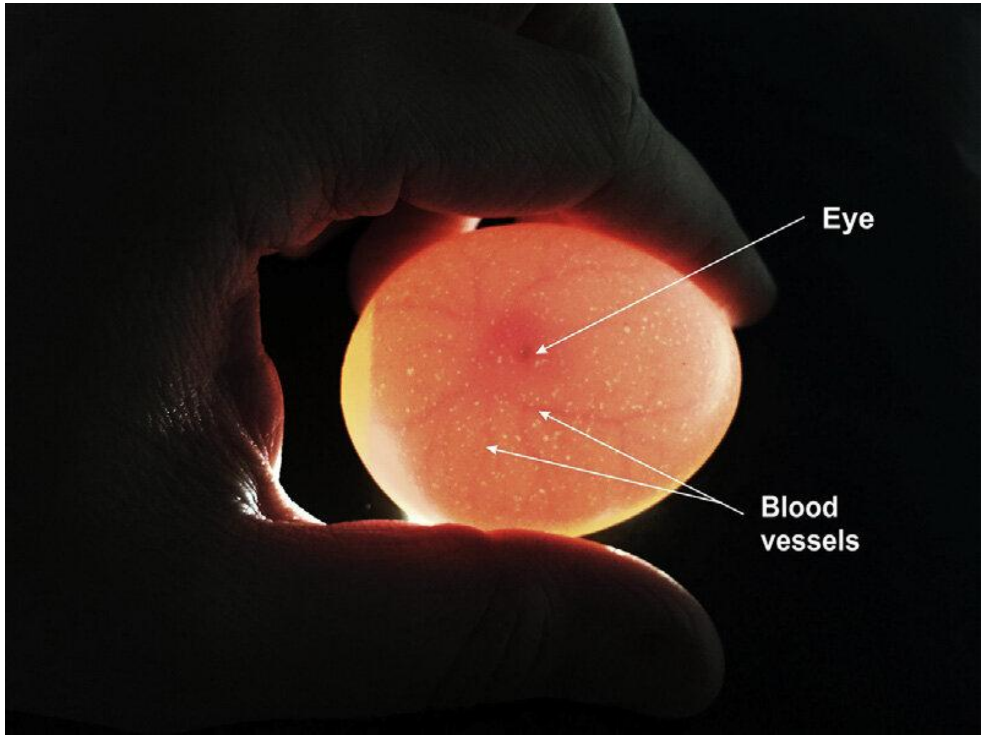
CULTIVATION OF VIRUSES IN EMBRYONATED EGG

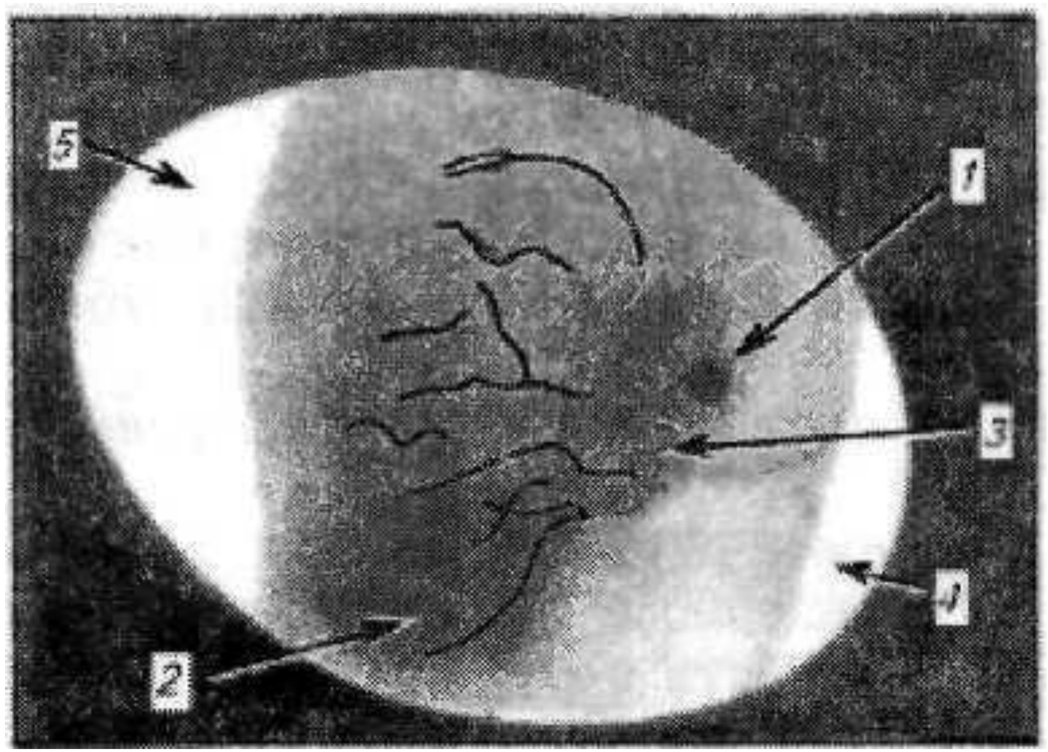


INDICATION OF VIRUSES IN INFECTED CHICKEN EMBRYOS

The development of viruses in an infected chicken embryo is determined by the following:

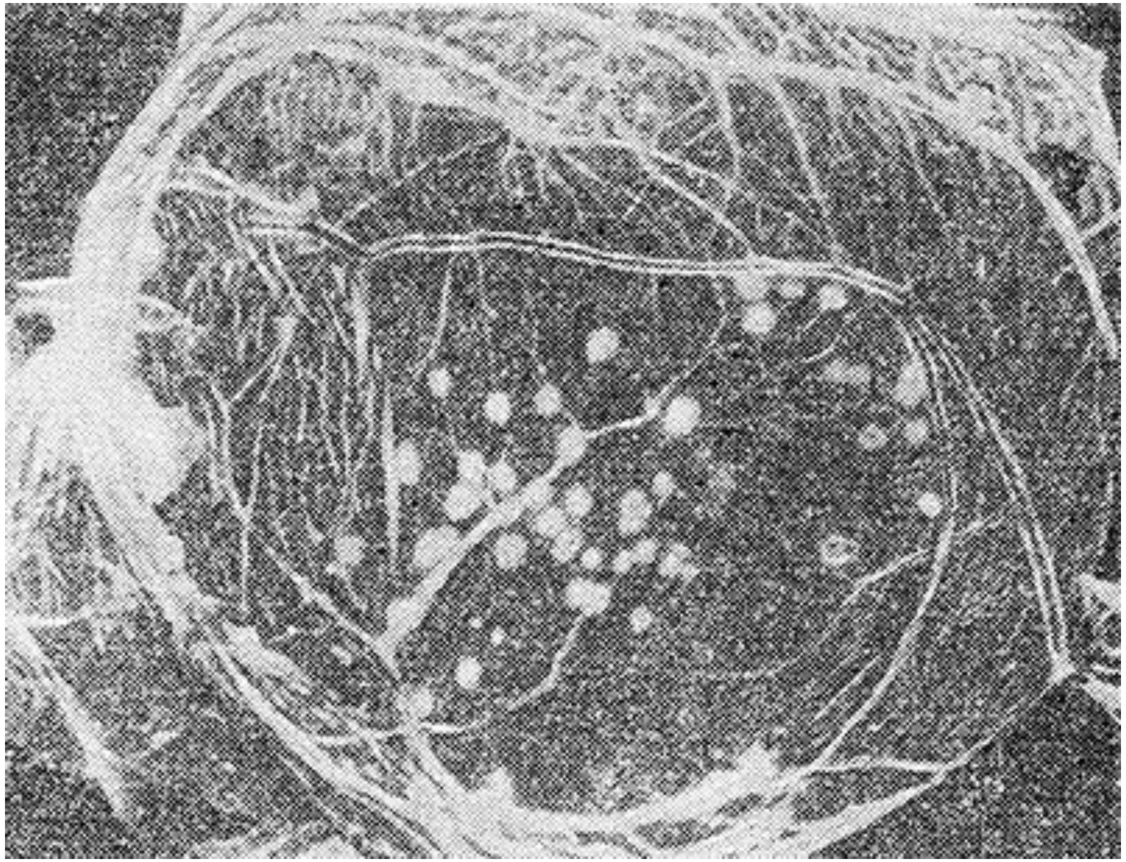
- *the death of the embryo,*
- *areas of necrosis caused by some viruses in the chorion-allantois membrane,*
- *hemagglutination reaction with amniotic and allantoic fluids,*





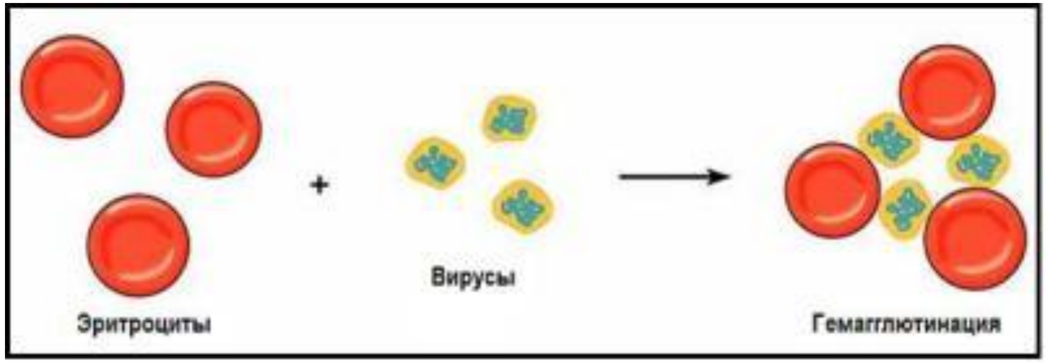
CHANGES IN THE CHORION-ALLANTOIS MEMBRANE

- During the study of changes in the chorionic-allantois membrane, it is cut with scissors and its contents are poured into a Petri dish.
- The chorion-allantois membrane remains inside the shell. It is removed with tweezers, placed in a Petri dish, washed with physiological solution, and ***the characteristics of focal lesions*** is studied on a dark background.



HEMAGGLUTINATION REACTION WITH AMNIOTIC AND ALLANTOIC FLUIDS

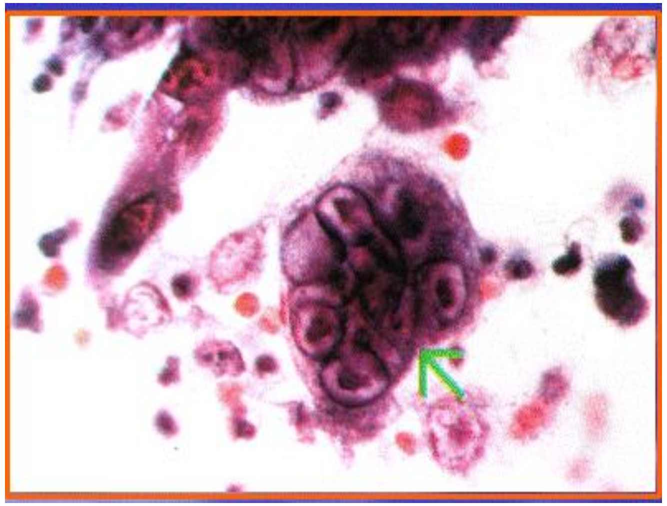
- The presence of the virus in the allantois and amniotic fluids of an infected embryo is determined by the hemagglutination reaction.



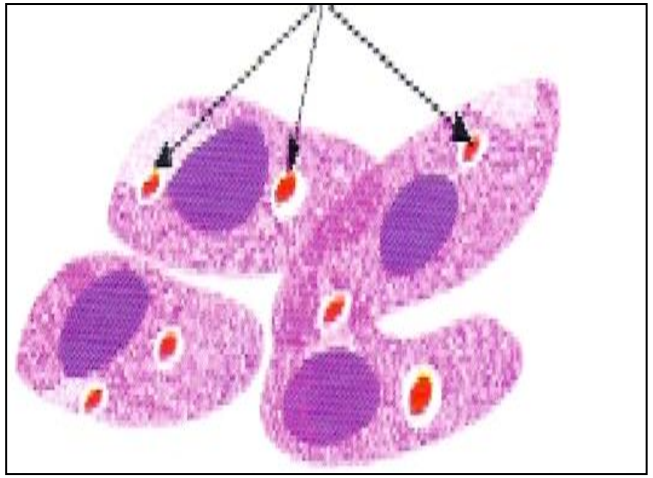
- This reaction is based on the ability of the hemagglutinin antigens of some viruses to agglutinate the erythrocytes of various animals and is used in the ***indication of viruses.***

METHODS OF INDICATING VIRUSES IN CELL CULTURES:

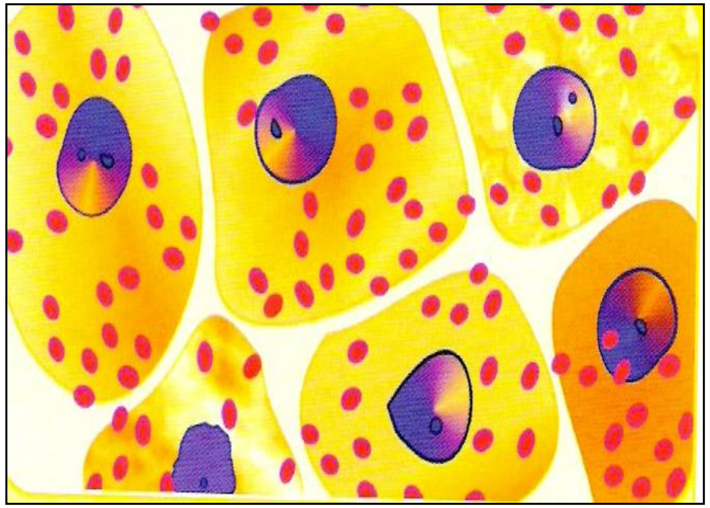
1. cytopathic effect (CPE)
2. intracellular inclusions
3. the formation of plaques
4. hemadsorption phenomenon
5. "color" test



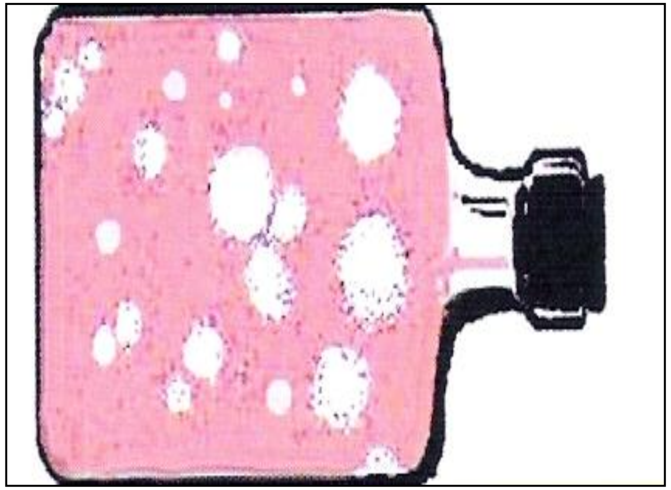
cytopathic effect (CPE)



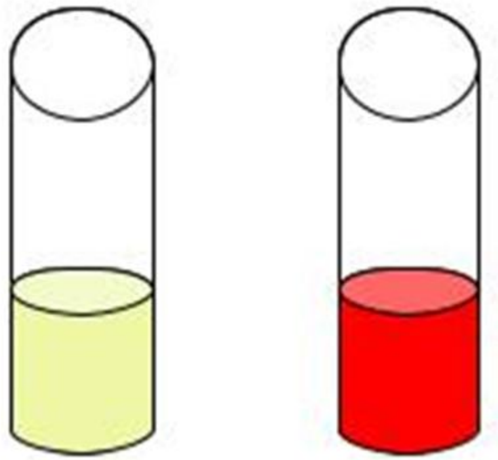
intracellular inclusions



hemadsorption phenomenon



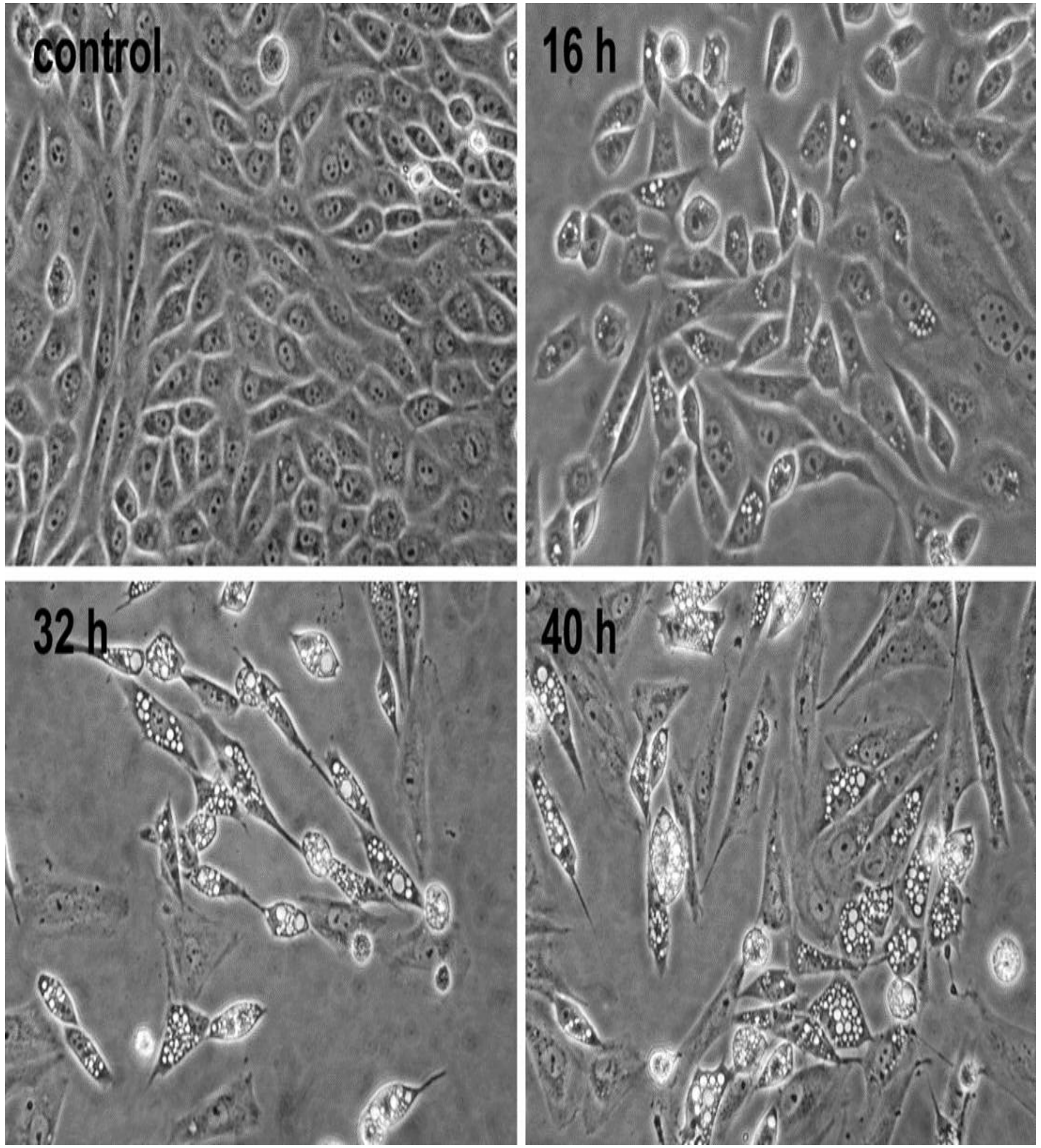
Negative colonies



«Color test»

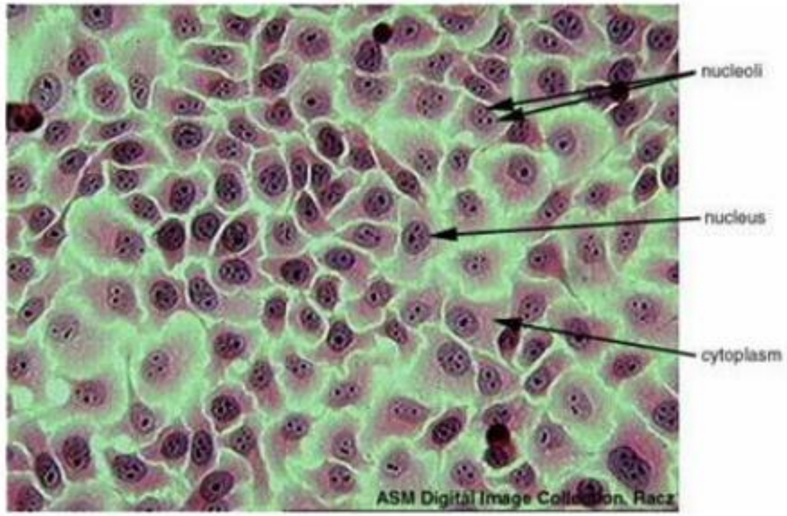
CYTOPATHIC EFFECT (CPE)

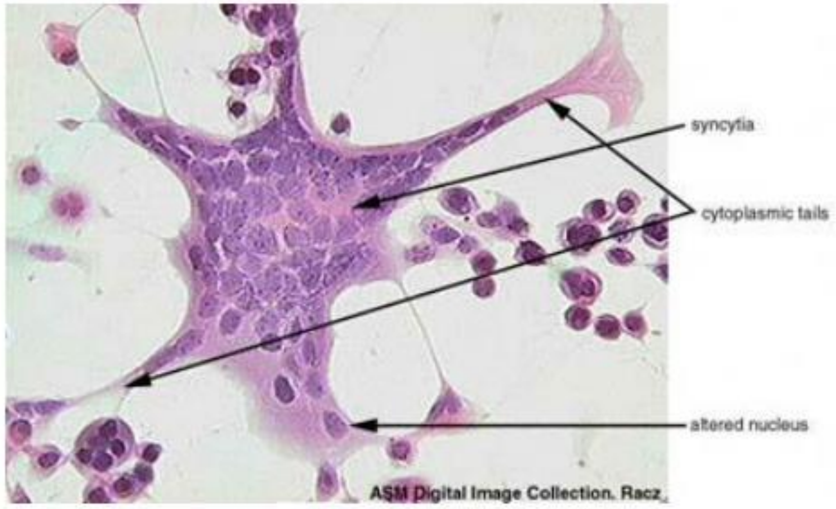
- During reproduction in cell culture, some viruses cause their degeneration, that is, cytopathic effect (CPE).
- After CPE virus infection, the tissue culture is evaluated in dynamics by studying it under a microscope at different times. CPE detection is one of the methods of virus **indication and identification.**
- Some viruses can be detected and identified by the **inclusions** they form in the cytoplasm and nucleus of infected cells.
- The shape of the inclusions varies, and their sizes vary from 0.25 μm to 25 μm.
- They represent the places of accumulation of virus particles and are detected by the Giemsa method and in preparations stained with fluorochrome.
- ***Different CPE are specific for different viruses.***



Examples of Cytopathic Effects of Viral Infection

- Nuclear shrinking (pyknosis)
- Proliferation of nuclear membrane
- Vacuoles in cytoplasm
- Syncytia (cell fusion)
- Margination and breaking of chromosomes
- Rounding up and detachment of cultured cells
- Inclusion bodies



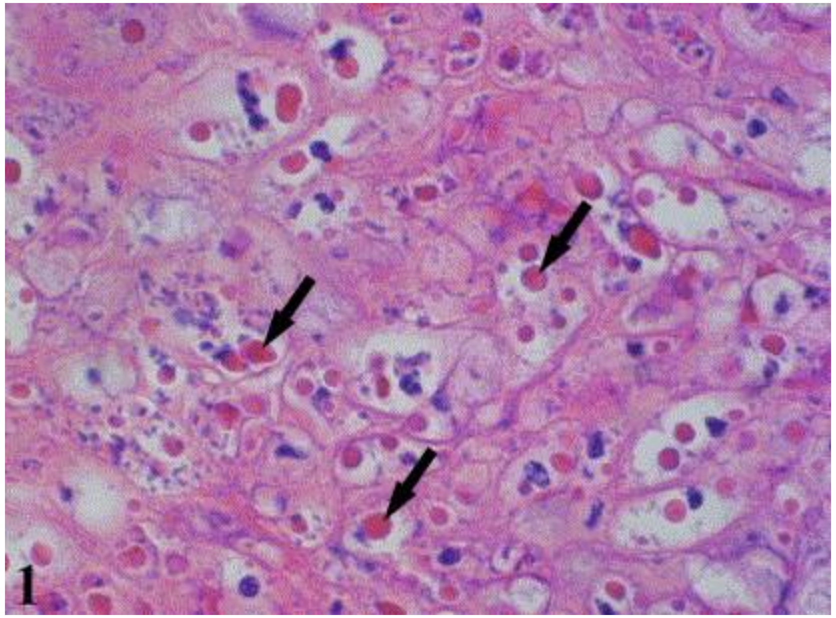


Cytopathic Effect (CPE)

Types of Cytopathic elfect (CPE)	Virus
Rapid crenation(leaf like) and degeneration of the entire cell sheet	Enteroviruses – Eg. Polio,
Syncytium or multinucleated giant cell formation	Measles, RSV, HSV
Diffuse roundening and ballooning of the cell line	HSV
Cytoplasmic vacuolations	SV 40 (Simian vacuolating virus-40)
Large granular clumps resembling bunches of grapes	Adeno virus

INTRACELLULAR İNCLUSİONS (BODIES)

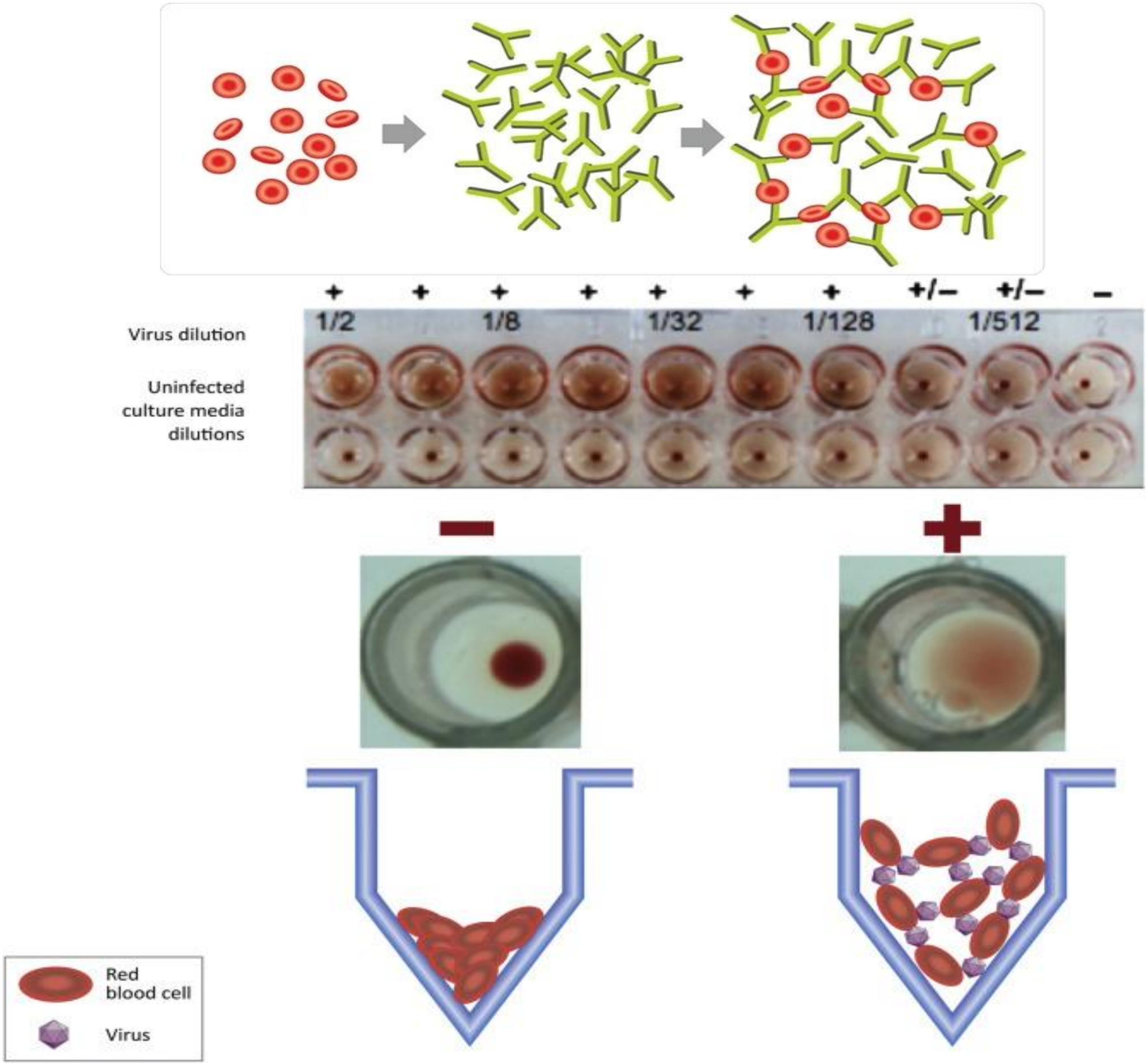
- Some viruses can be detected and identified by the inclusions they form in the cytoplasm and nucleus of infected cells.
- The shape of the inclusions varies, and their sizes vary from 0.25 μm to 25 μm.
- They represent the places of accumulation of virus particles and are detected by the Giemsa method and in preparations stained with fluorochrome.



Guarnieri bodies

Inclusion body	Virus
Intracytoplasmic inclusion bodies	
Negri bodies	Rabies virus
Molluscum bodies	Molluscum contagiosum virus
Guarnieri bodies	Vaccinia virus
Bollinger bodies	Fowl pox virus
Perinuclear cytoplasmic acidophilic bodies	Reovirus
Intranuclear inclusion bodies	
Owl's eye inclusion bodies	Cytomegalovirus
Cowdry type A inclusion bodies	Herpes simplex virus and measles virus
Intranuclear basophilic	Adenovirus
Acidophilic inclusion bodies	Papovavirus

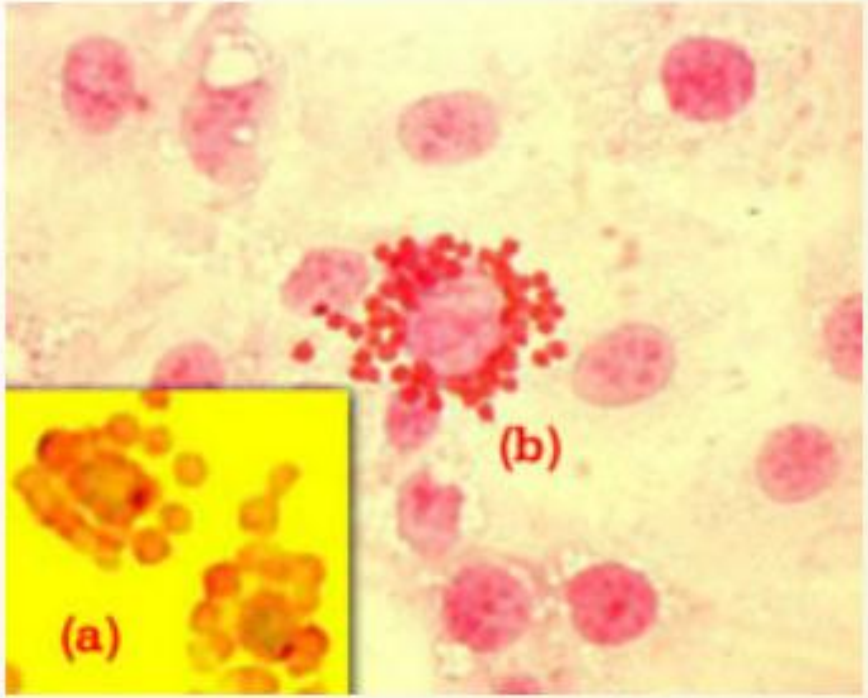
- **HEMAGGLUTINATION** is a reaction that causes clumping of red blood cells in presence of some **enveloped** viruses, such as the influenza virus. A glycoprotein on the viral surface, namely hemagglutinin, interacts with red blood cells, leading to the clumping of red blood cells and the formation of a lattice.
- In **absence of an enveloped** virus, red blood cells precipitate at the bottom of a well, forming a red-colored dot. However, in presence of a virus, red blood cell clumps are dispersed, forming no red-colored dot. This is the basic principle of a hemagglutination assay.



Hemadsorption

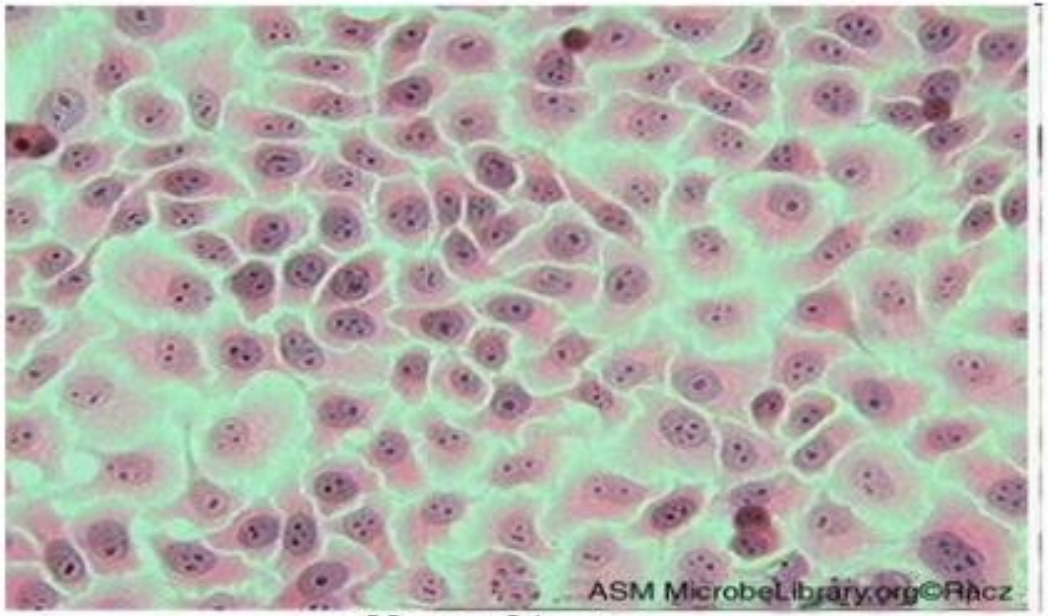
- Infective cell produce some proteins which have the ability to adsorb erythrocytes phenomenon known as *Haemadsorption* .
- Incorporation of viral glycoprotein peplomers into the plasma membrane of infected cells where they serve as receptors for ligands on the surface of erythrocytes.

Eg. orthomyxoviruses, paramyxoviruses, and togaviruses,

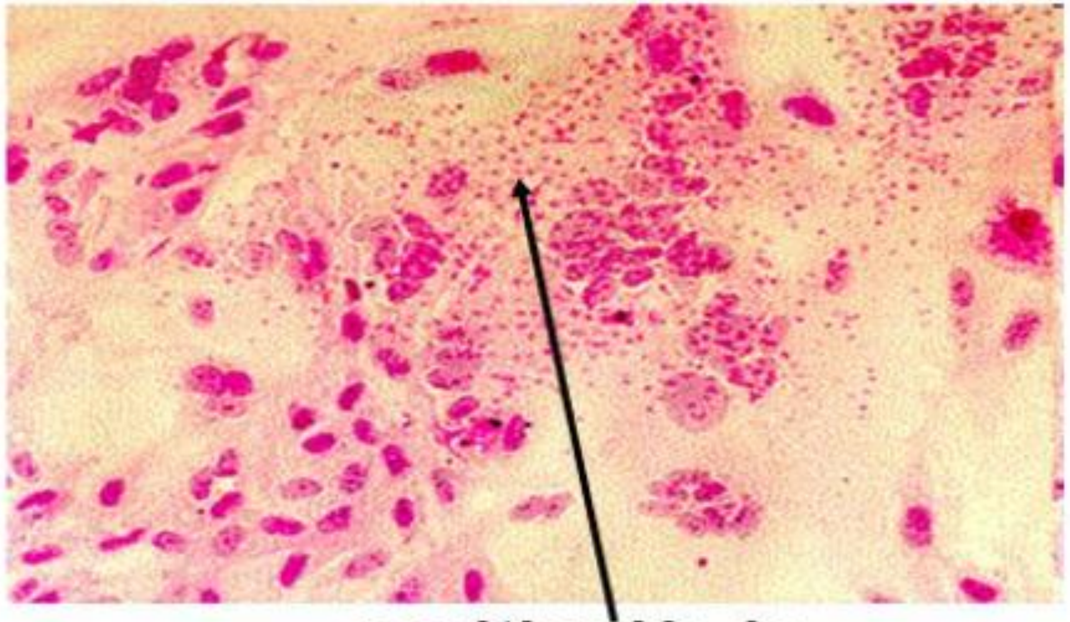


Hemadsorption (Hads)

- Virus growth in cell cultures is detected by testing for hemadsorption: red cells are added to the culture and adhere to virus budding from infected cells.
- If the culture tests positive, hemadsorption inhibition test with specific antisera is used to identify the virus.



cell culture

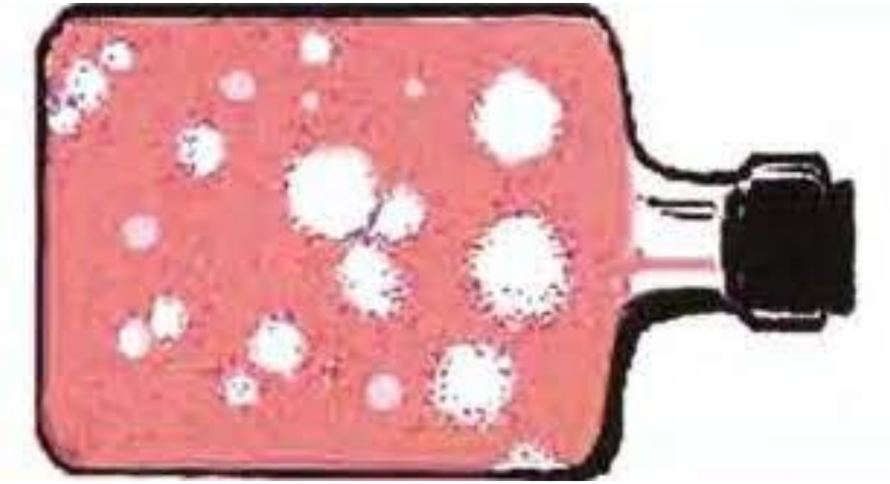

positive Hads

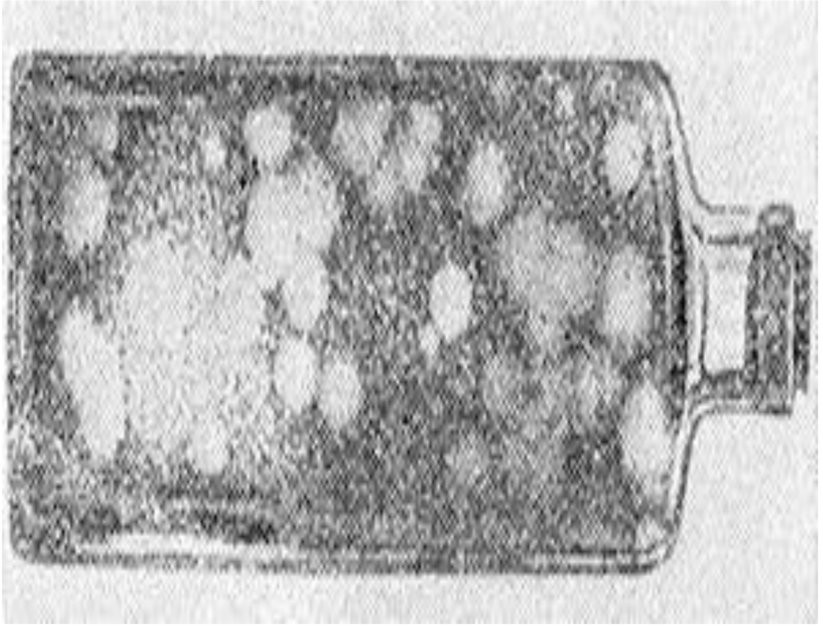
"NEGATİVE COLONİES"

•The growth of some viruses in cell cultures results in the destruction of cells in the corresponding region, which can be used to identify the viruses by revealing these areas ("negative colonies").

•After infecting the cell culture, adding an agar layer on top of it limits the areas of virus reproduction.

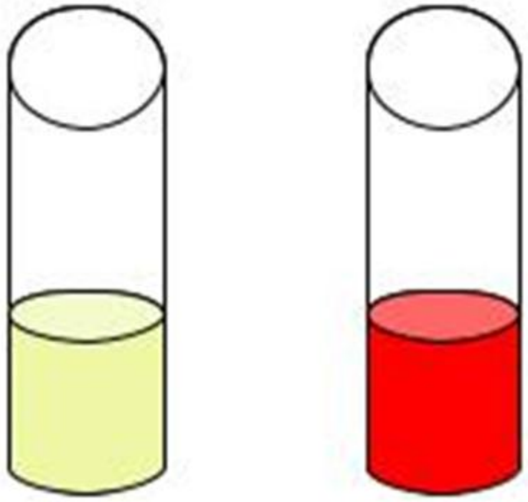
•As a result, the necrosis areas formed by them are isolated from each other.

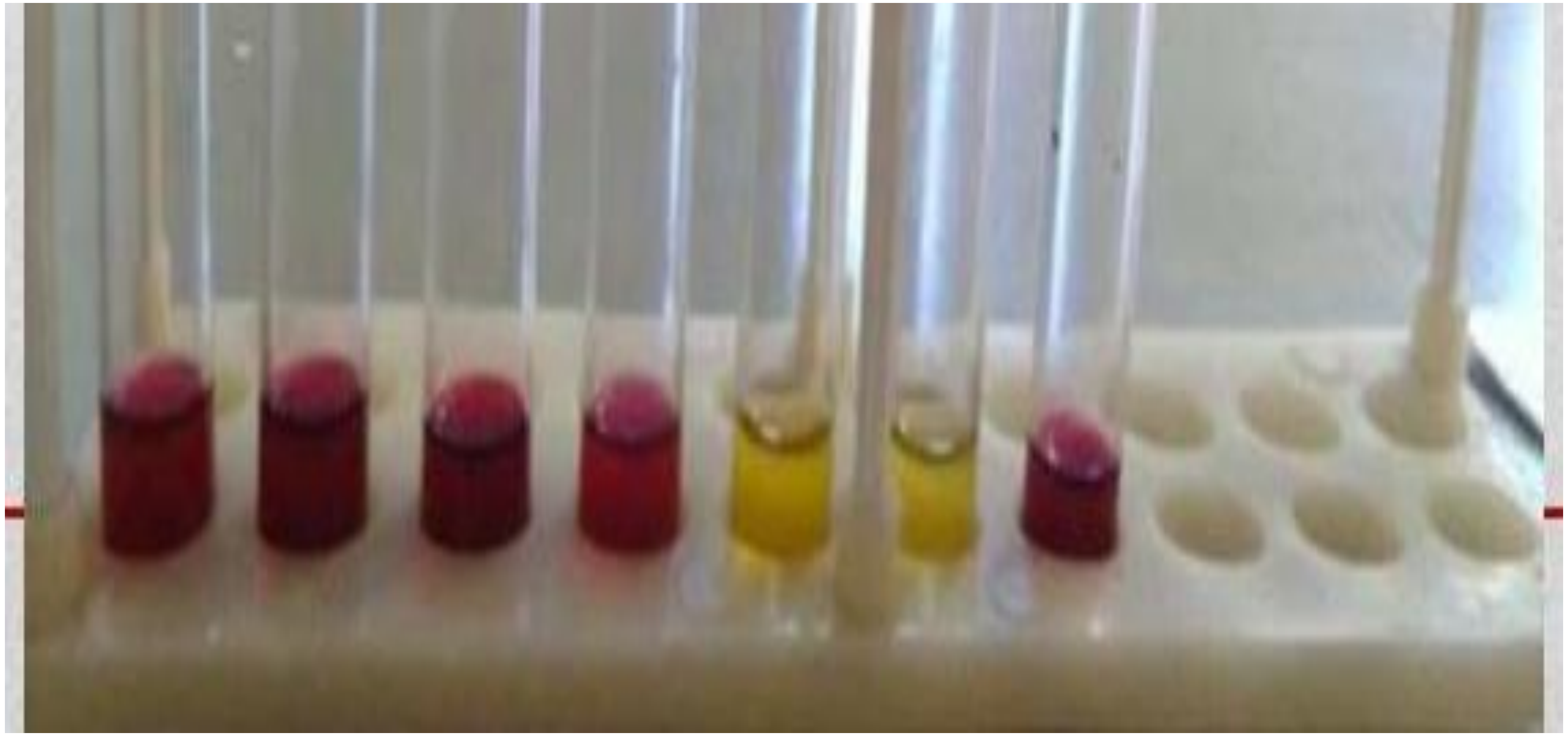




«COLOR TEST»

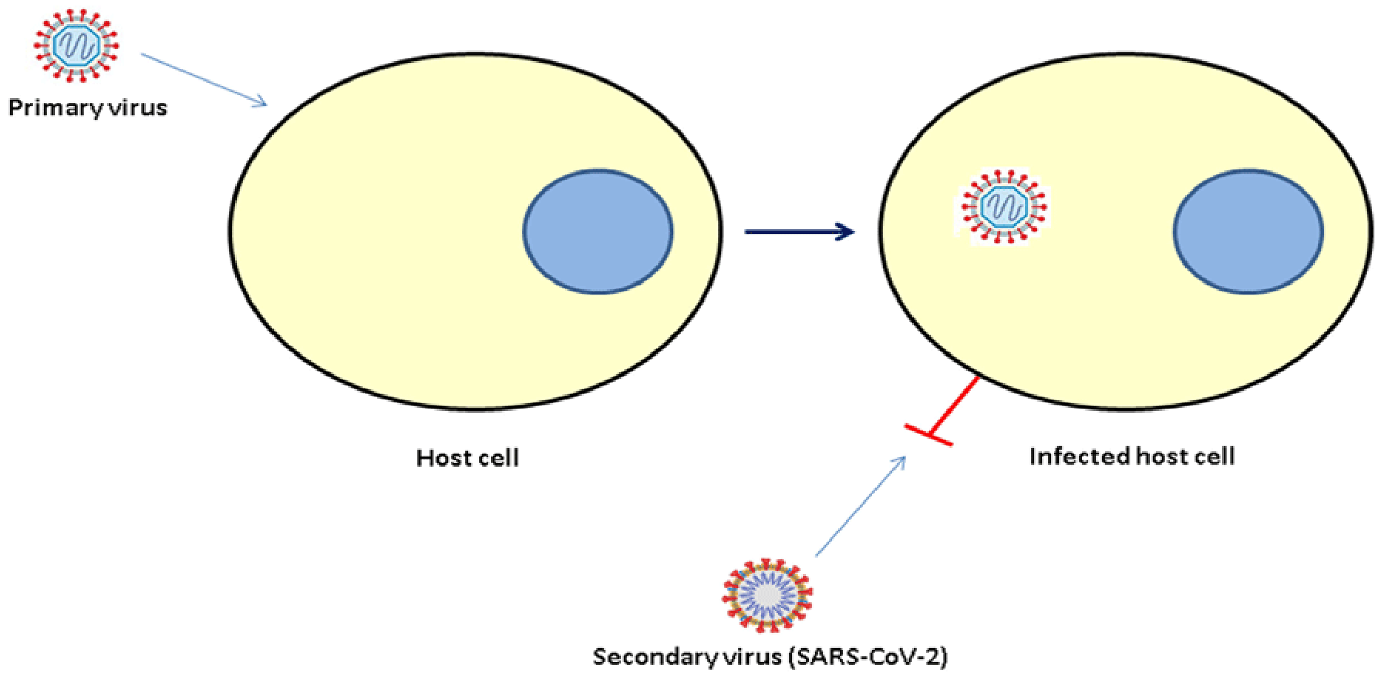
- The growth of viruses in cell cultures can be indicated by means of a "color test". For this, cell culture cultivated in a nutrient medium with an indicator (e.g., methyl red) is used.
- As the virus growth, the cells are destroyed, so the original color **(red)** of the medium remains unchanged.
- If the virus does not growth, a change in the color of the medium **(yellow)** is observed as a result of the effect of the metabolic products of the cells.





INTERFERENCE PHENOMENON

- In some cases, the phenomenon of interference is used to indicate viruses that do not cause CPE, especially those that are cultivated. The essence of interference is that a cell infected with one type of virus becomes resistant to other viruses.
- For example, rubella virus does not cause CPE despite cultivation in different cell cultures. In primary cell cultures, this virus can be detected due to the interference phenomenon.
- For this, the cell culture infected with the rubella virus is also infected with an indicator virus that produces CPE, for example, with the vesicular stomatitis virus. CPE is not observed because the growth of rubella virus in cell culture inhibits the replication of the indicator virus. However, when the rubella virus does not growth in cell culture, the indicator virus begins to multiply, and this is manifested by CPE.

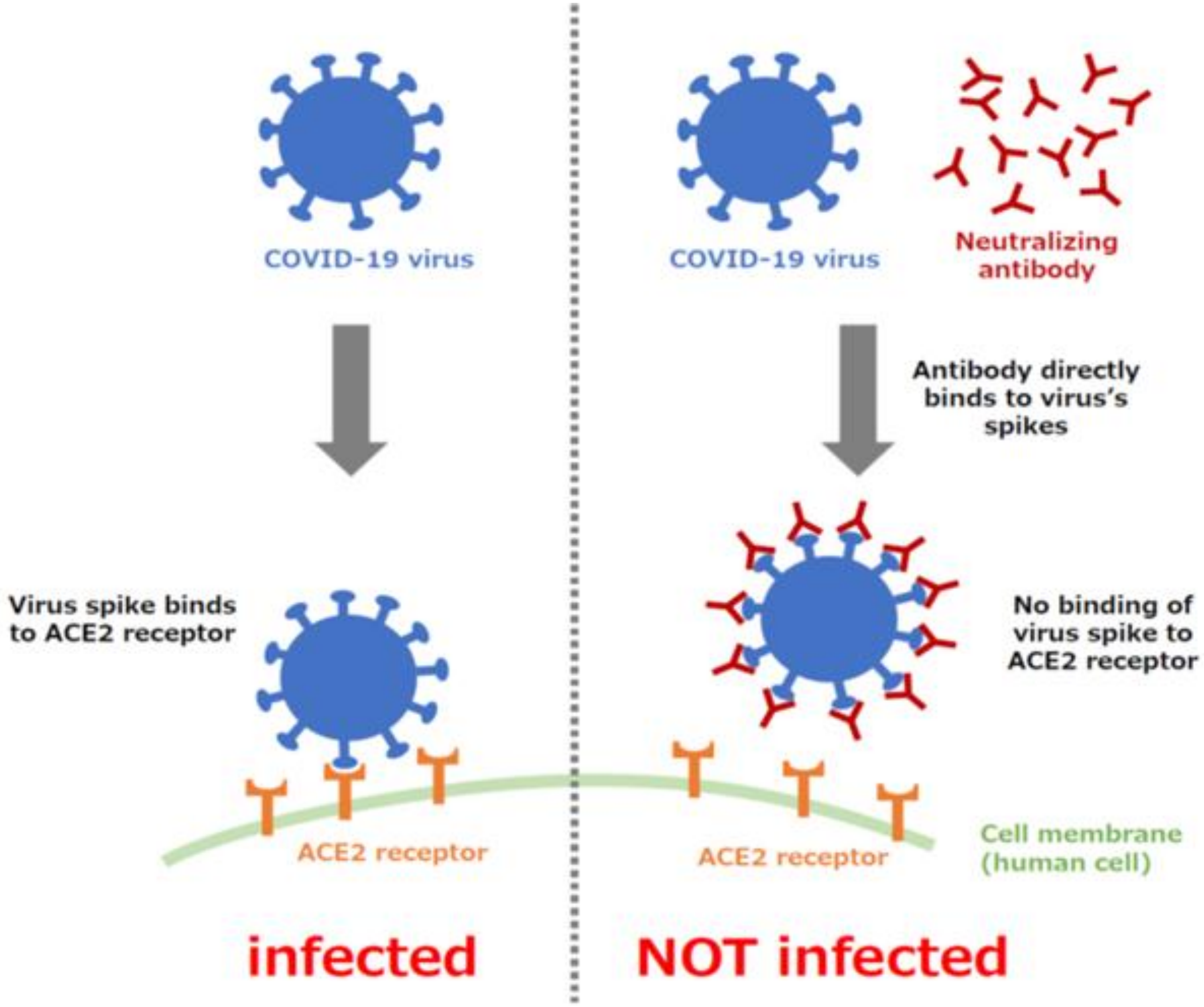


IDENTİFİCATİON OF VİRUSES

- Identification of viruses is the determination of their **variant, species, genus and family affiliation.**
- Virus identification is based on this principle: identifying the **unknown based on the known.**
- For the identification of viruses, serological reactions are performed using a known component - specific antiviral sera.
- These reactions include: neutralization reaction (**NR**), hemagglutination inhibition reaction (**HIR**), (hemadsorption inhibition reaction (**HAdsIR**), passive hemagglutination reaction (**PHAR**), complement fixation test (**CFT**), immunofluorescence reaction (**IFR**), enzyme-linked immunosorbent assay (**ELISA**)) and so on.
- These sera containing specific antivirus antibodies are called **diagnostic**.

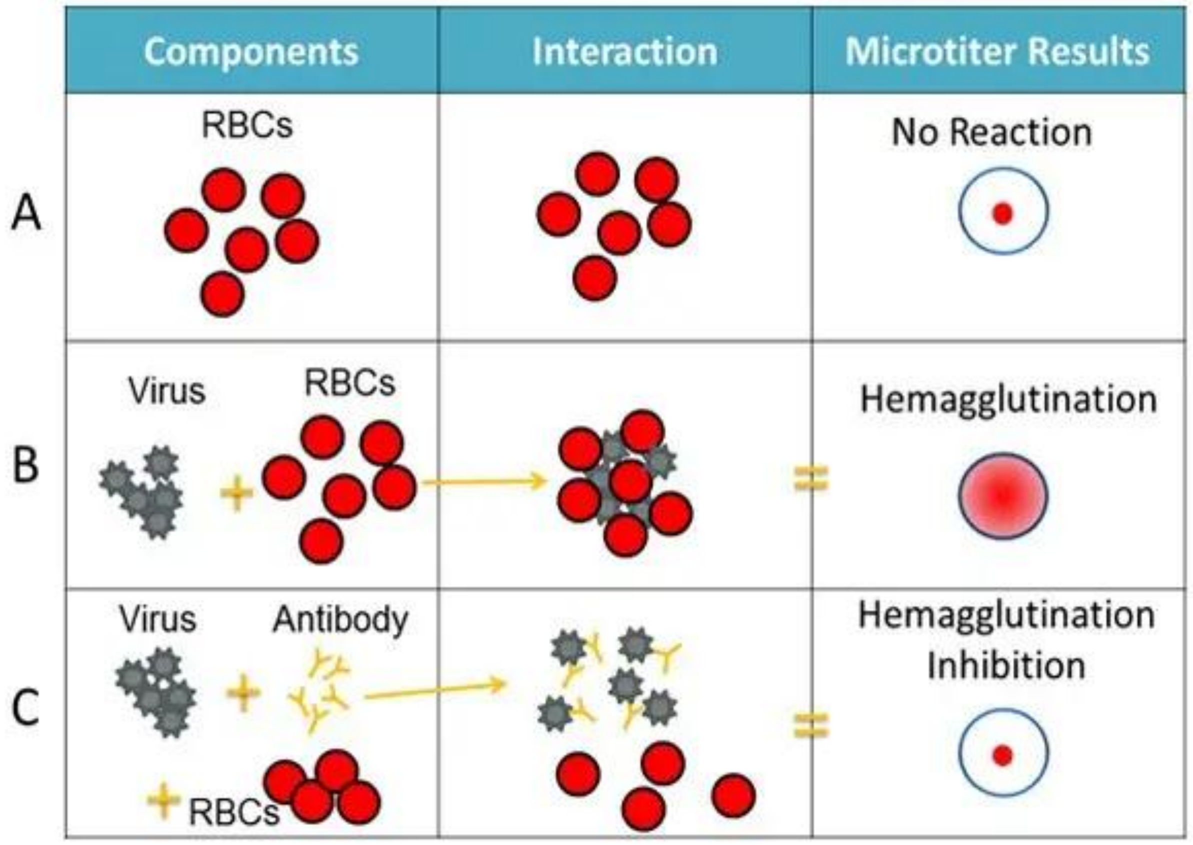
NEUTRALİZATİON REACTİON OF VİRUSES

- Virus neutralization reaction (biological neutralization reaction) allows identification of viruses.
- Due to the effect of appropriate antibodies, viruses do not cause disease in sensitive laboratory animals, do not have a cytopathic effect on cell and tissue cultures, and do not multiply in chicken embryos.



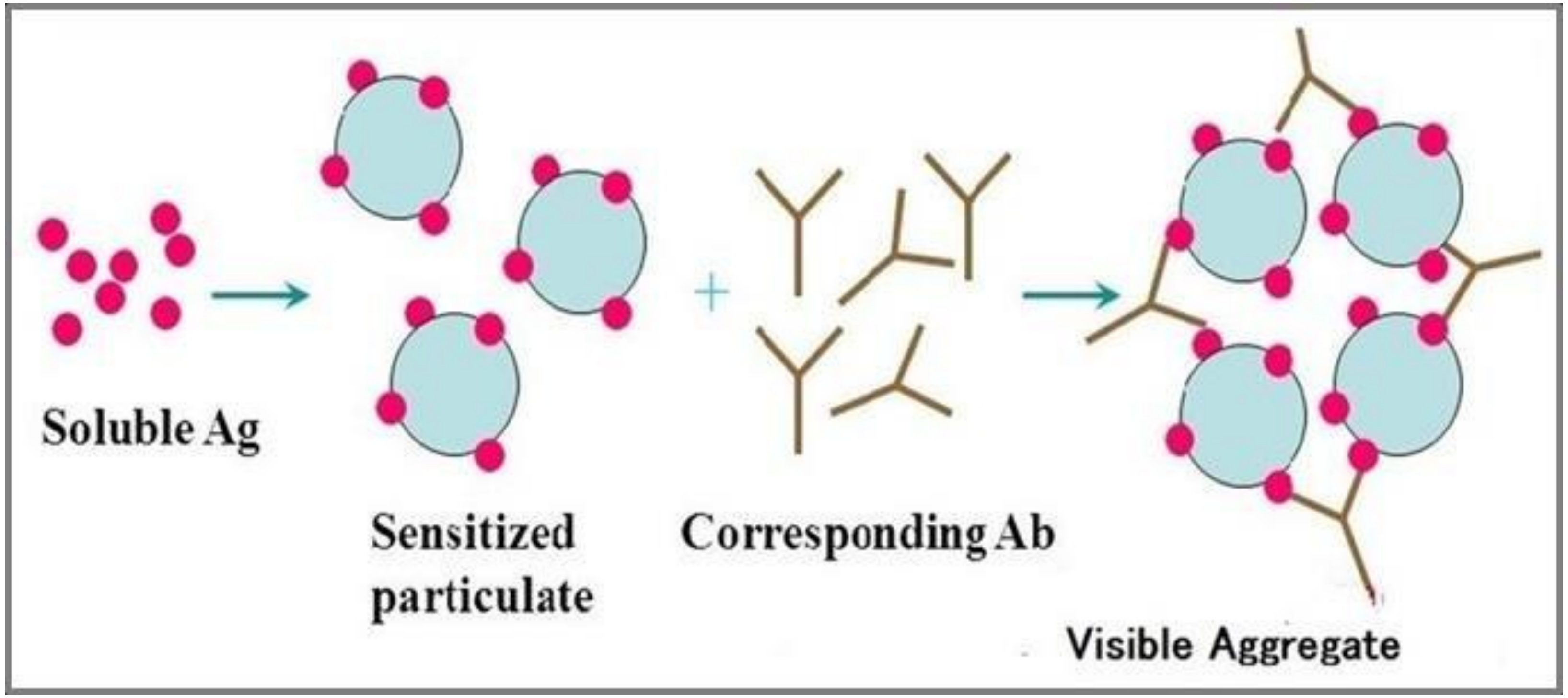
HEMAGGLUTINATION-INHIBITION (HI) ASSAY

- This reaction is used to identify some viruses (influenza, measles, tick-borne encephalitis, etc.).
- To determine the type of viruses in the examined material, serum containing antibodies against certain viruses is added to it.
- If there is a corresponding virus in the material, due to the effect of their antibodies, they lose their ability to agglutinate erythrocytes and the titer of the reaction decreases significantly.



PASSİVE HEMAGGLUTİNATİON REACTİON (PHAR)

The erythrocytes with adsorbed antigens come into contact with the corresponding antibodies in the blood serum, which causes the erythrocytes to stick together and settle to the bottom of the test tube or well in the form of sediment.



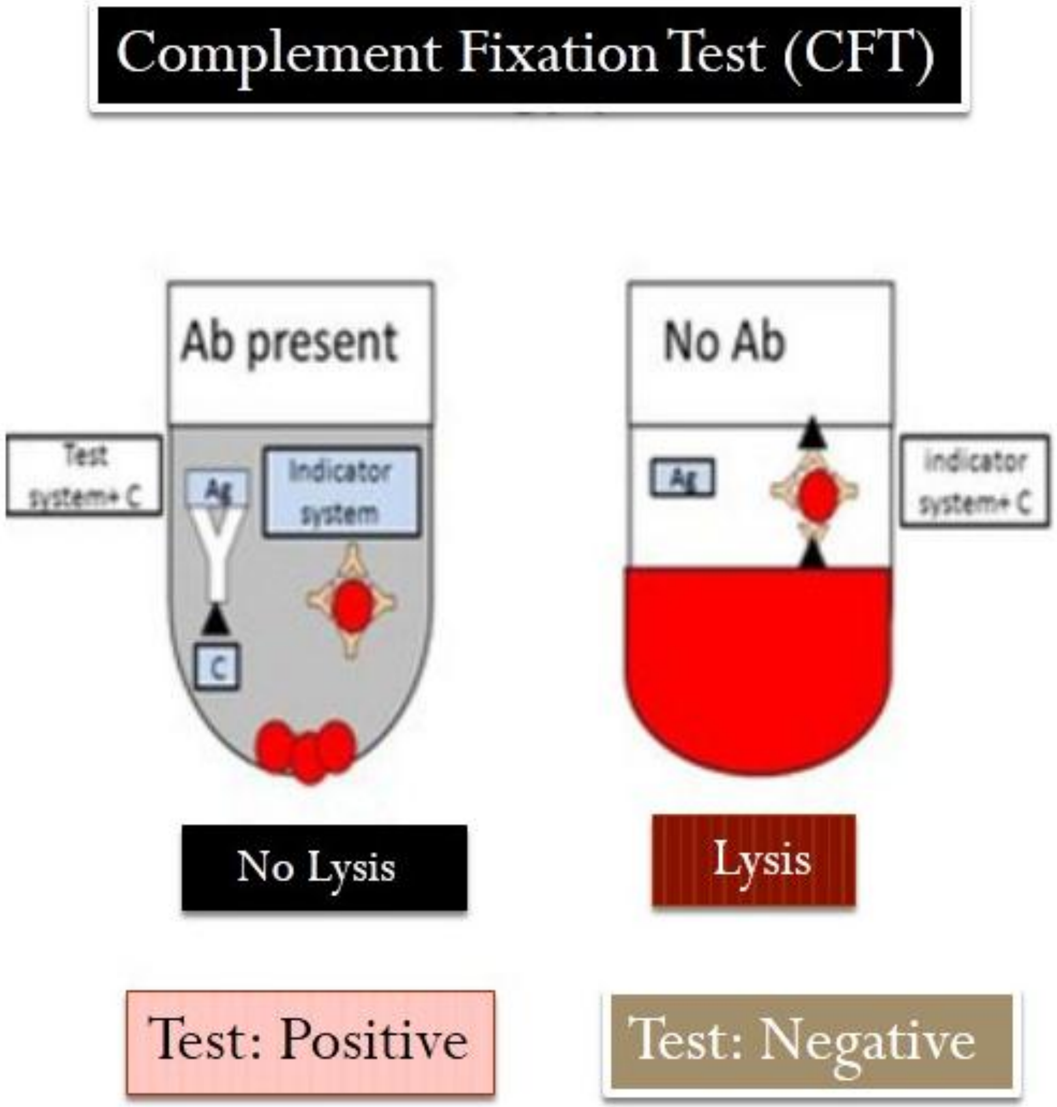
COMPLEMENT FIXATION TEST (CFT)

Complement fixation test (CFT) - when antigens and antibodies are compatible with each other, they form an immune complex, complement binds to it and a complement-antigen-antibody complex is formed. If the antigen-antibody complex is not formed, then the complement remains free.

CFT consists of two stages:

Phase 1st - incubation of the mixture containing antigen + antibody + complement,

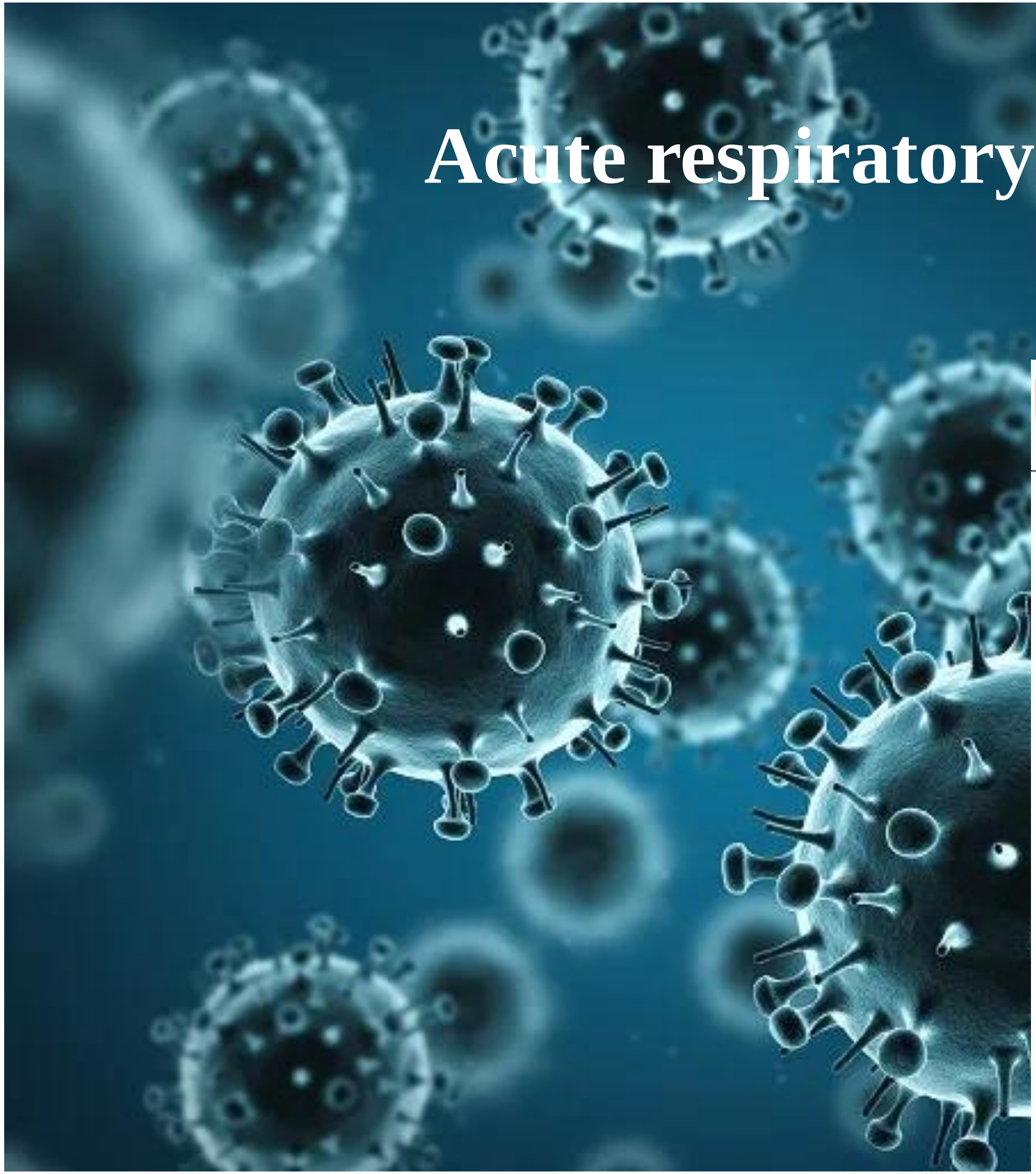
Phase 2nd (indicator) - detection of free complement in the mixture by adding a hemolytic system consisting of sheep erythrocytes and hemolytic serum. In the 1st phase of the reaction, when an antigen-antibody complex is formed, the combination of complement occurs, then in the 2nd phase, hemolysis of erythrocytes sensitized by antibodies will not occur (the reaction is **positive**). If the antigen and antibody do not match (no antigen or antibody in the test sample), the complement remains free and binds to the erythrocyte-anti-erythrocyte antibody complex in phase 2, causing hemolysis (**negative** reaction).

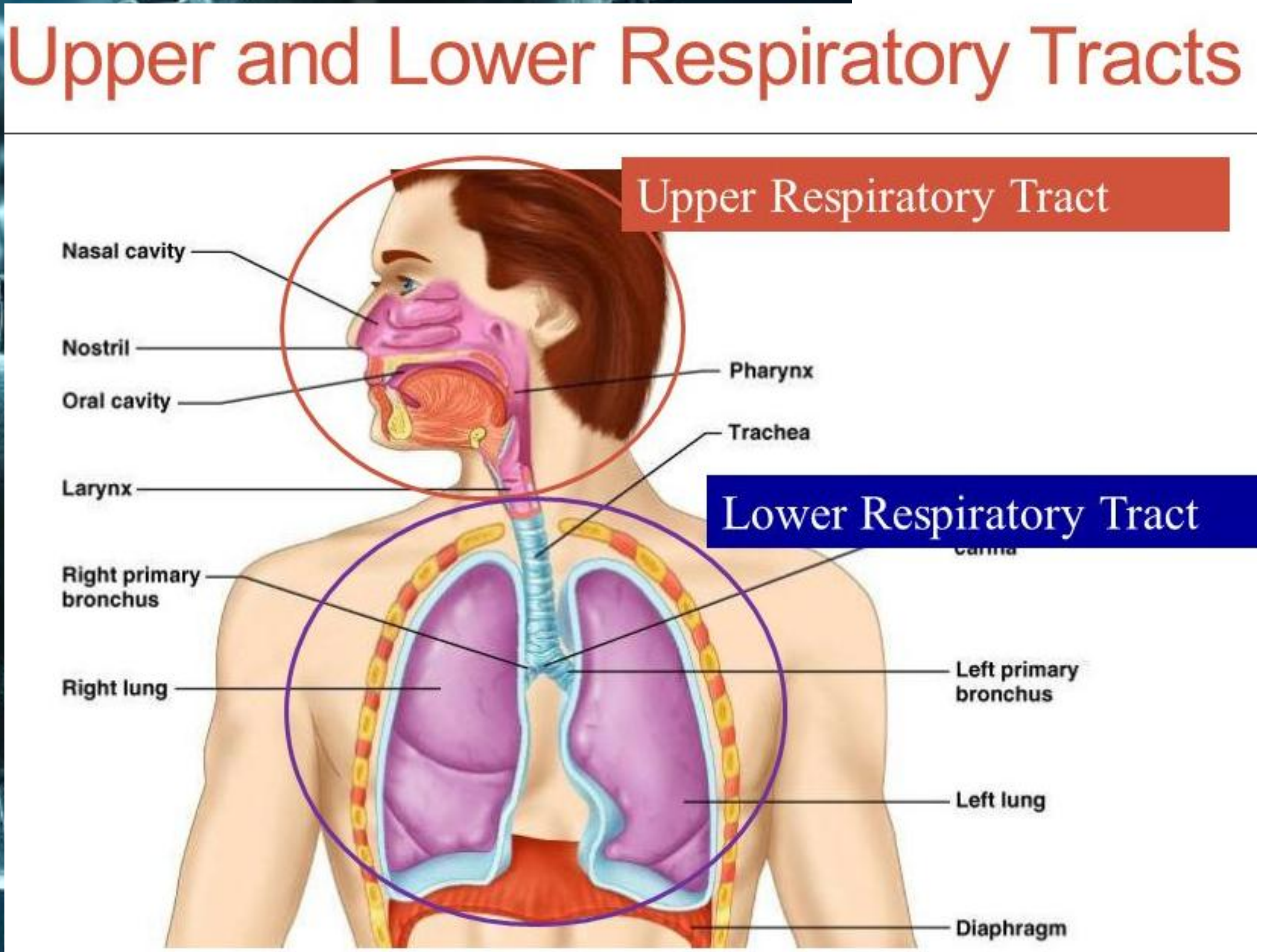


SEROLOGICAL METHOD

- Serological method - determination of antibodies in the blood of sick or recovered people. At this time, an increase in the titer of antibodies in the paired serum of the patient using viral diagnostics is considered as a positive result.
- Paired sera - two sera taken from the patient at the beginning of the disease and after 1-4 weeks.
- Serological reactions (PHAR, CFT, HIR, NR, ELISA, etc.) are performed with both sera to determine and compare antibody titers. The presence of IgM in the serum is determined for early diagnosis of the disease.

Acute respiratory viral infections

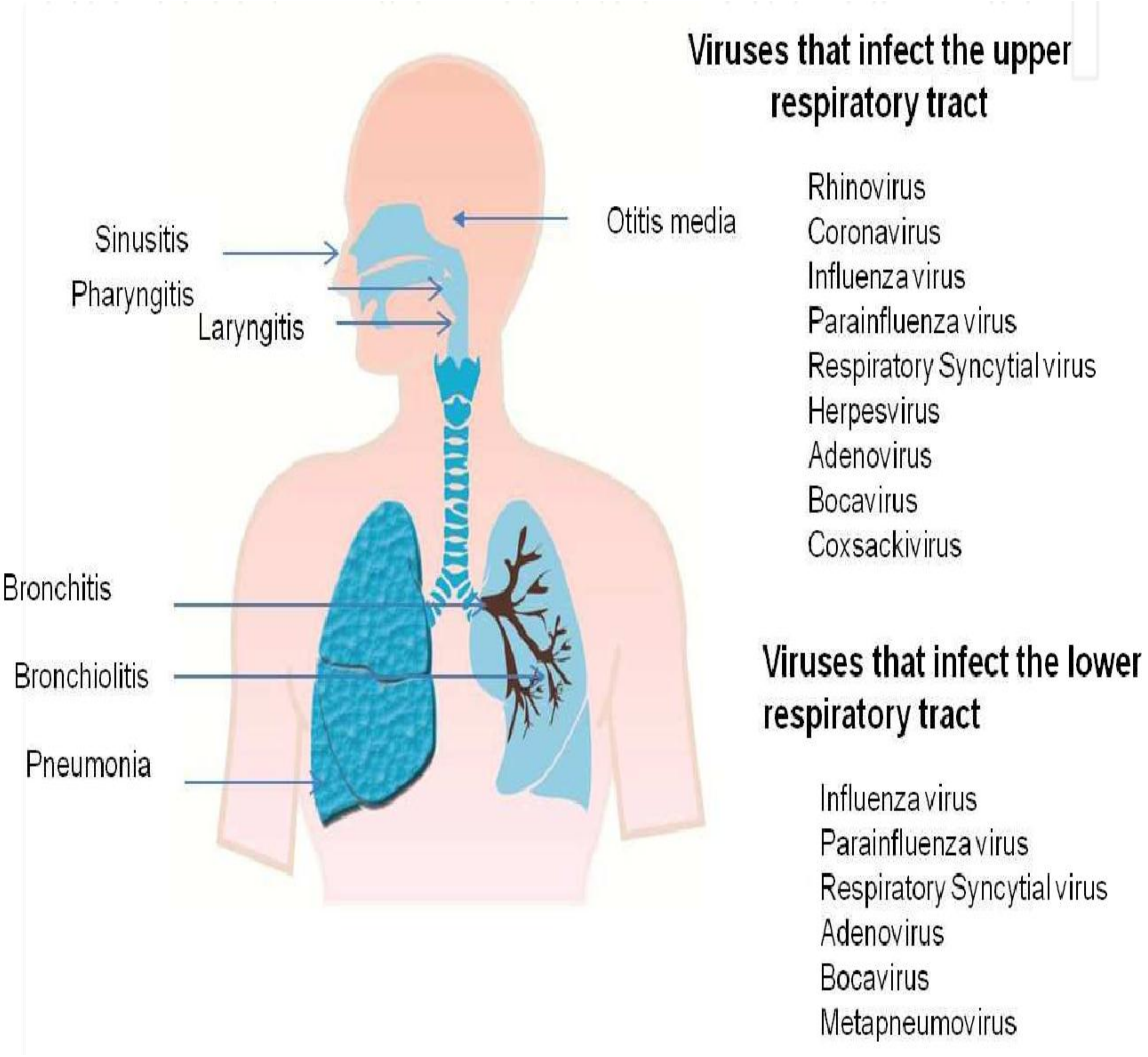




Oral and Respiratory Diseases

Disease	Etiologic Agent
Common cold	Rhinovirus[a] Coronavirus[a] Influenza viruses Parainfluenza viruses RSV Metapneumovirus Adenovirus Enteroviruses
Pharyngitis	Adenovirus[a] Coxsackievirus A[a] (herpangina, hand-foot-and-mouth disease) and other enteroviruses Epstein-Barr virus Herpes simplex virus
Croup, tonsillitis, laryngitis, and bronchitis (children <2 years)	Parainfluenza virus 1[a] Parainfluenza virus 2 Influenza virus Adenovirus Epstein-Barr virus
Bronchiolitis	RSV[a] (infants) Parainfluenza virus 3[a] (infants and children) Parainfluenza viruses 1 and 2 Metapneumovirus
Pneumonia	RSV[a] (infants) Parainfluenza virus[a] (infants) Influenza virus[a] Metapneumovirus Adenovirus Varicella-zoster virus (primary infection of adults or immunocompromised hosts) Cytomegalovirus (infection of immunocompromised host) Measles

[a]Most common causal agents.
RSV, Respiratory syncytial virus.



Viruses that initiate infection via respiratory tract

Site of infection	Family	Viruses
Local respiratory infection	Orthomyxoviridae	Influenza A and B viruses
	Paramyxoviridae	Parainfluenza viruses (4 types), respiratory-syncytial virus RSV (3 types)
	Picornaviridae	Rinoviruses (113 types)
	Reoviridae	Reoviruses (3 types)
	Coronaviridae	Types 1-4
	Adenoviridae	Types 1-7, 14, 21
Generalized diseases, usually with initial respiratory symptoms	Herpesviridae	Varicella virus, Epstain-Barr virus (EBV), cytomegalovirus
	Paramyxoviridae	Mumps and measles viruses
	Togaviridae	Rubella virus
	Picornaviridae	Some enteroviruses
	Bunyaviridae	Hantaviruses
	Arenaviridae	Lassa fever virus

Myxo = affinity to mucin

Myxoviruses

Orthomyxo viruses

-Smaller
-Segmented RNA genome
-Liable to Agic variation

Influenza viruses

Paramyxo viruses

-Larger
-Single piece of RNA
- Not liable to Agic variation

- Parainfluenza
- Mumps vairus
- Measles virus
- Respiratory syncytial virus

Orthomyxovirus (Influenza) Family

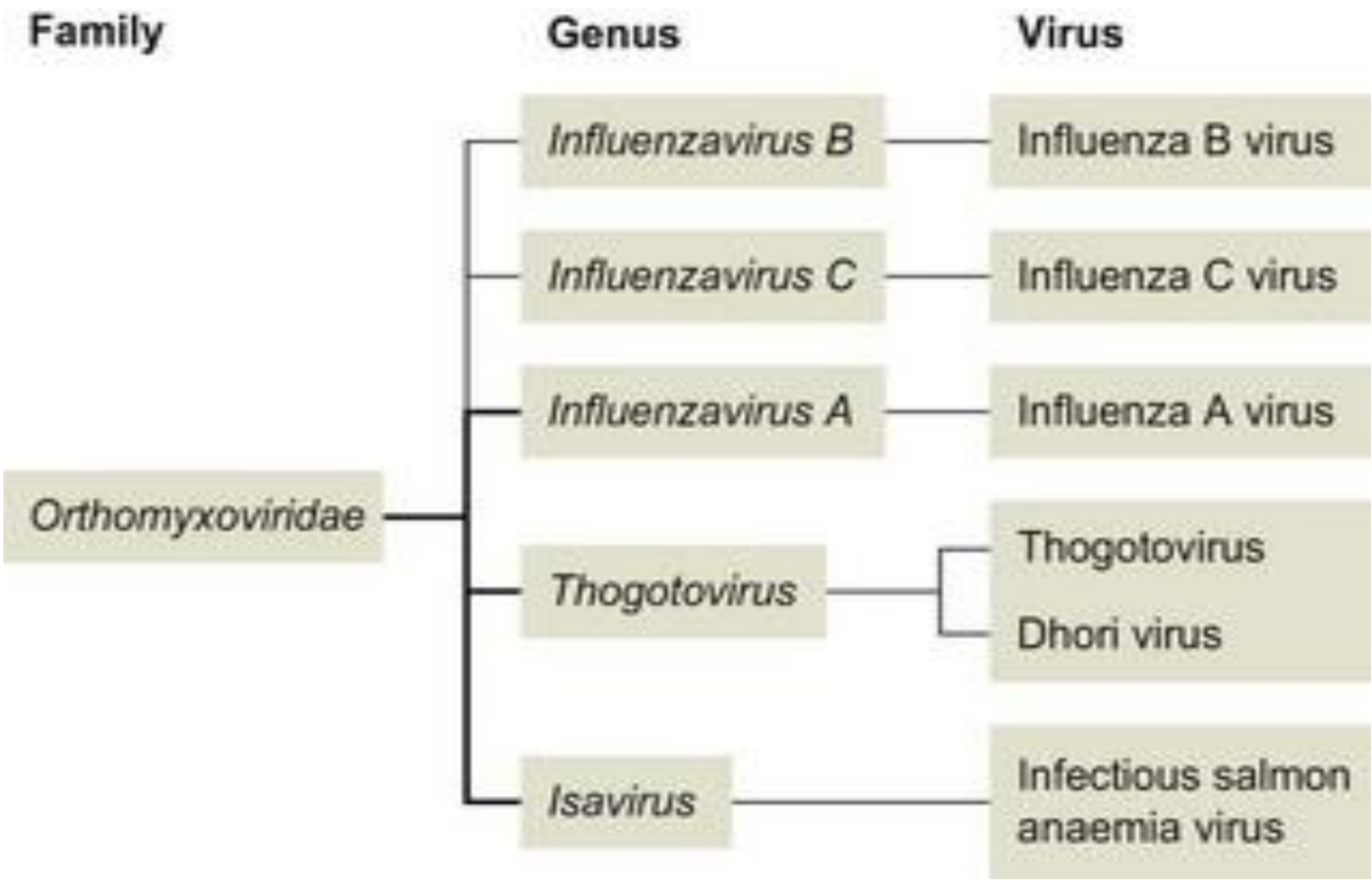
The name myxovirus was originally applied to influenza viruses. It meant virus with an affinity for mucins. Now there are 2 main groups – the orthomyxoviruses and the paramyxoviruses

Differences between orthomyxoviruses and paramyxoviruses

Feature	Orthomyxoviruses	Paramyxoviruses
Viruses and diseases	Influenza A,B,C	Mumps, measles, respiratory syncytial, parainfluenza
Genome	Single-stranded RNA in 8 pieces, MW $2\text{-}4 \times 10^6$	Single-stranded RNA in single piece, MW $5\text{-}8 \times 10^6$
Inner ribonucleo-protein helix	9-nm diameter	18-nm diameter

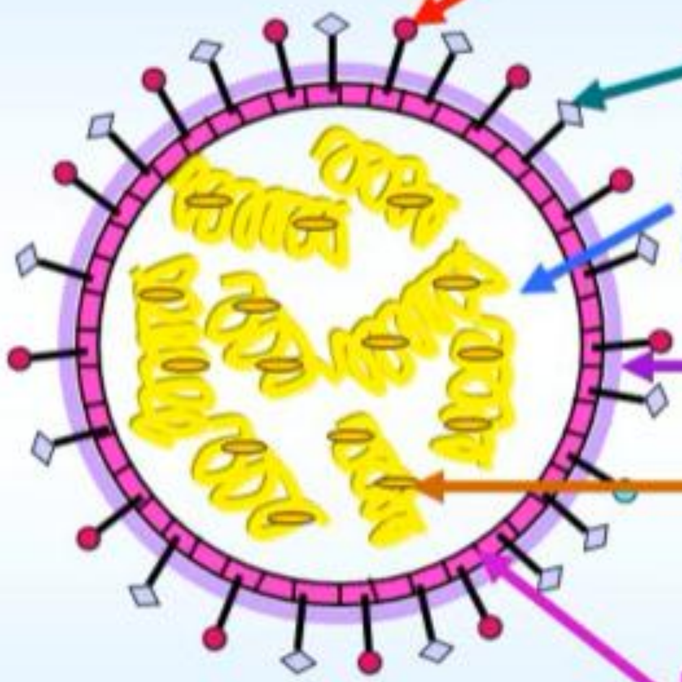
Ortomyxoviridae - Taxonomy

- Kingdom: Orthornavirae
- Phylum: Negarnaviricota
- Class: Insthoviricetes
- Order: Articulavirales
- Family: Orthomyxoviridae
- Genus:
- Alphainfluenzavirus
- Betainfluenzavirus
- Gammainfluenzavirus
- Deltainfluenzavirus
- Isavirus
- Quaranjavirus
- Thogotovirus



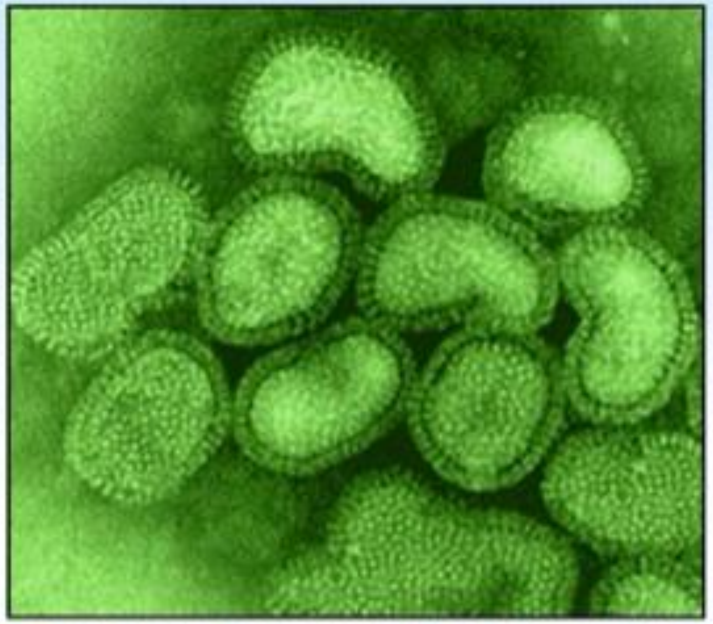
- **Influenzavirus D** - primarily affect cattle and are not known to infect or cause illness in people. (Medical Microbiology, 9th Edition, 2020, Patrick Murray, Ken Rosenthal, Michael Pfaller)

ORTHOMYXOVIRUSES



type A, B, C : **NP**, **M1** protein
sub-types: **HA** or **NA** protein

Influenza virus A

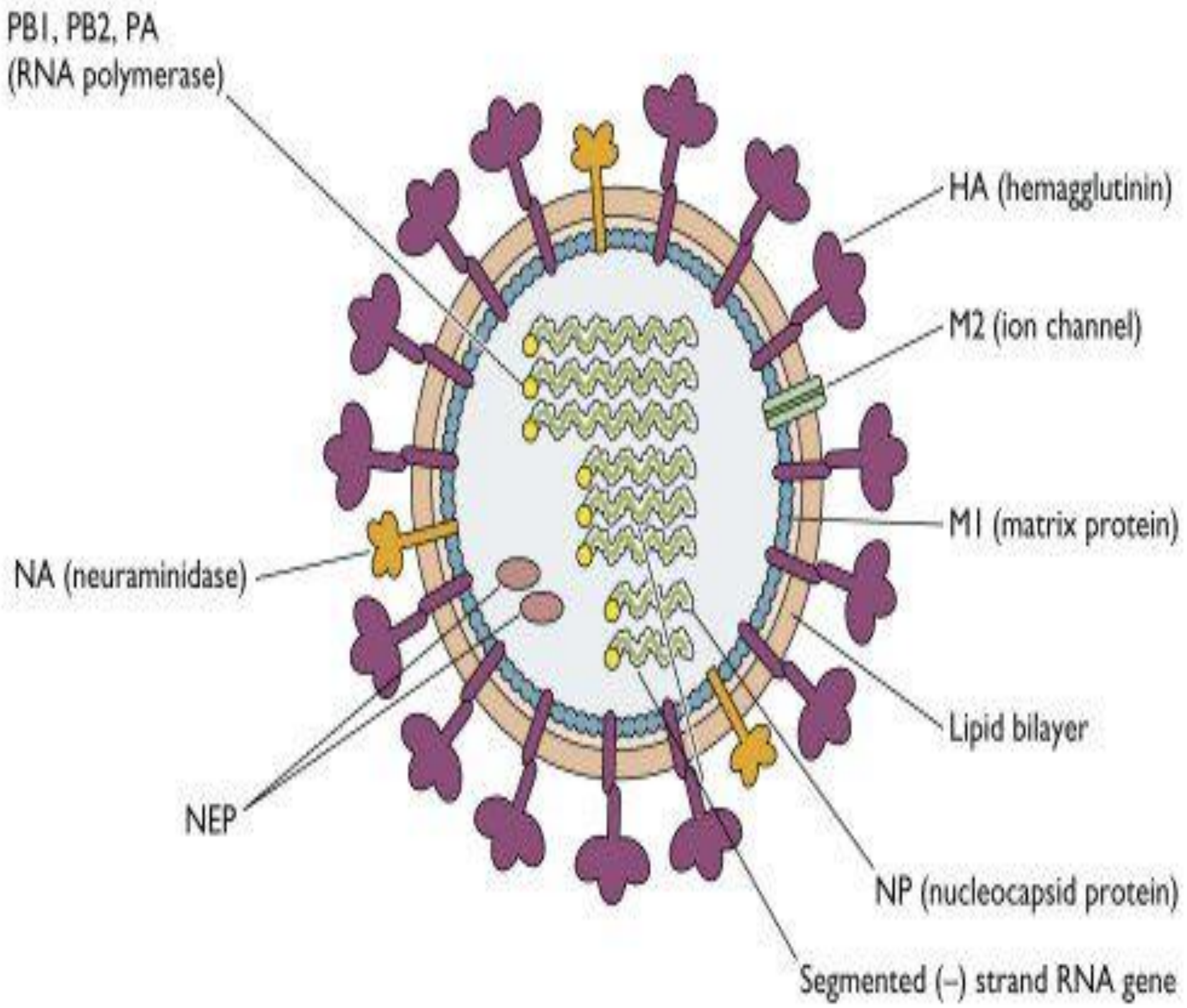


Orthomyxoviruses: medium-sized, enveloped, (-) sense that vary in shape from spherical to helical. Their genome is segmented into eight pieces

Figure 10-3d Microbiology 4/e

INFLUENZA VIRUS (STRUCTURE)

- The virion is **polymorphic**, mostly **spherical**, but sometimes rod-shaped. Influenza viruses, which vary in size in a wide range, are approximately **100 nm** in diameter.
- A nucleocapsid with **helical symmetry** is located in the center of the complex virion. In addition to ribonucleoprotein, nucleocapsid also includes three proteins of enzyme nature **(P1, P2 and P3).**
- The genome consists of **single-stranded segmented negative-RNA strand.** Influenza A and B viruses have **8** segments, and C virus has **7** segments.
- Nucleocapsid is surrounded by **matrix (M1)** and membrane or **ion channel (M2)** proteins.



INFLUENZA VIRUS (STRUCTURE)

The virion is surrounded by a lipoprotein membrane from the outside.
There are glycoprotein spikes on its surface. These spikes are composed of two complex glycoproteins: **hemagglutinin (H) and neuraminidase (N).**
Influenza type C virus does not have **neuraminidase.**

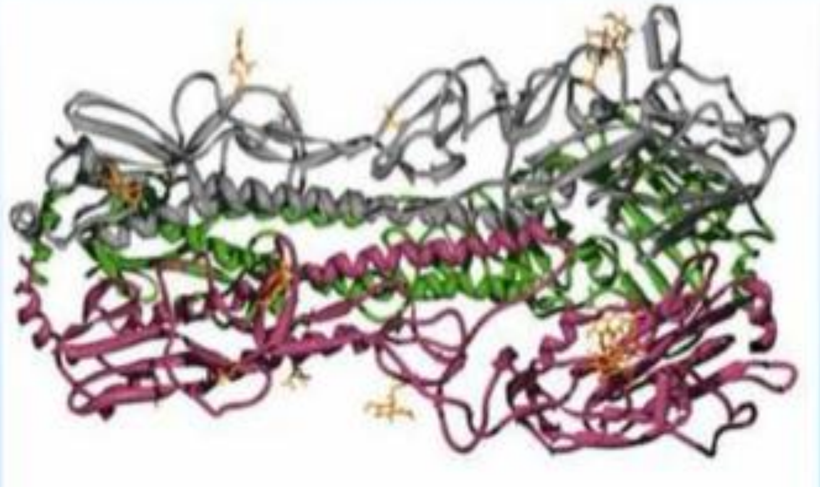
Haemagglutinin (HA)

Encoded by **RNA** segment # 4

Can agglutinate red blood cells - hence the nomenclature

Cleavage by host-cell protease is required (resulting in **HA1** and **HA2**) for infection to occur

Hemagglutinin glycoprotein is the viral attachment protein and fusion protein, and it elicits neutralizing, protective antibody responses



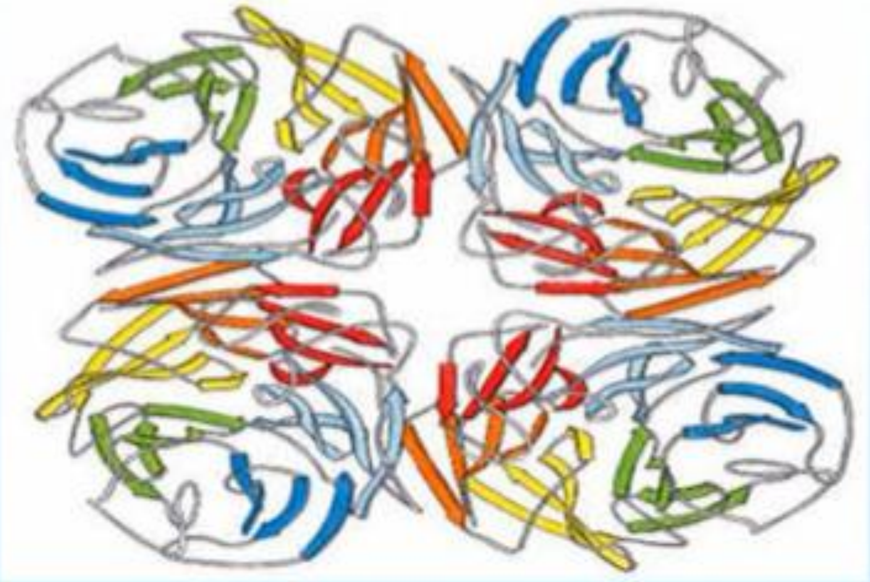
Neuraminadase (NA)

Encoded by **RNA** segment # 6

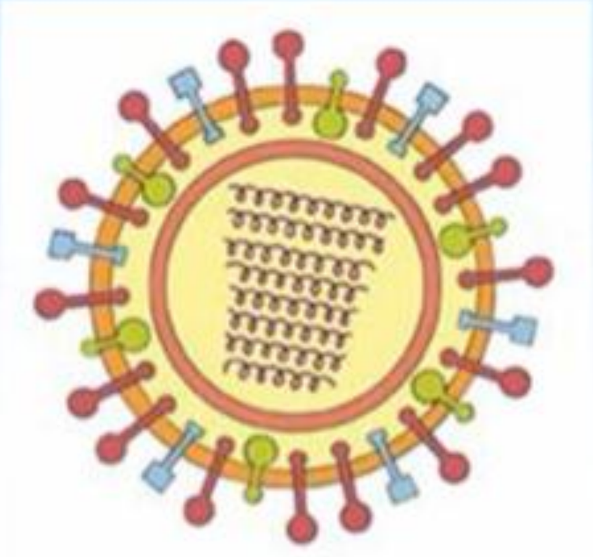
Removes neuraminic (sialic) acid from cell and permits dissemination of viruses

Important in releasing mature virus from cells

Stimulates production of protective antibodies



Orthomyxoviruses. Nomenclature



Human influenza virus

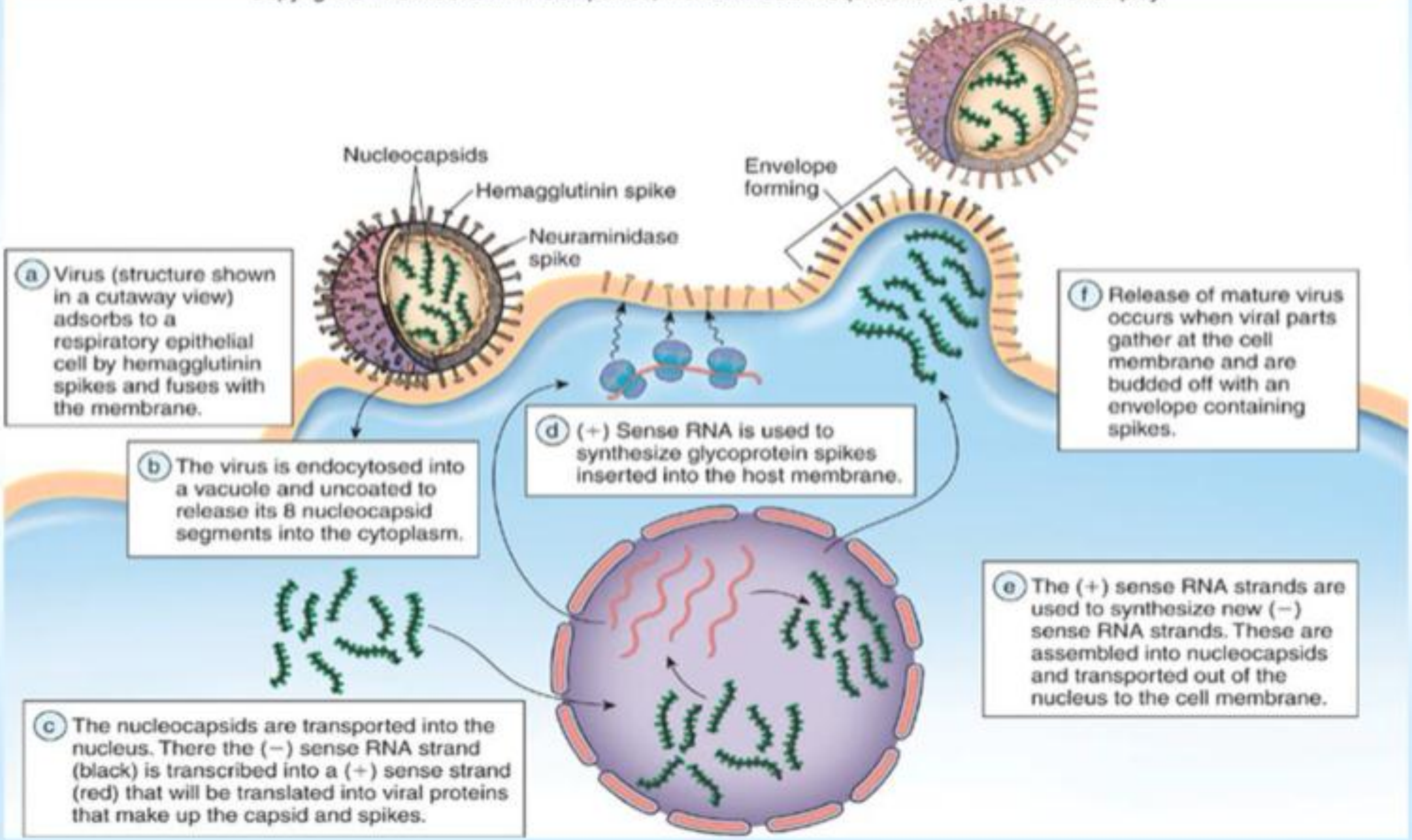
Influenza A/Bangkok/1/79(H3N2)
Influenza A/Singapore/1/57(H2N2)
Influenza B/Ann Arbor/1/86

Influenza type — Year of isolation — Hemagglutinin subtype

A/Sydney/5/97 (H3N2)

Geographic source — Isolate number — Neuraminidase subtype

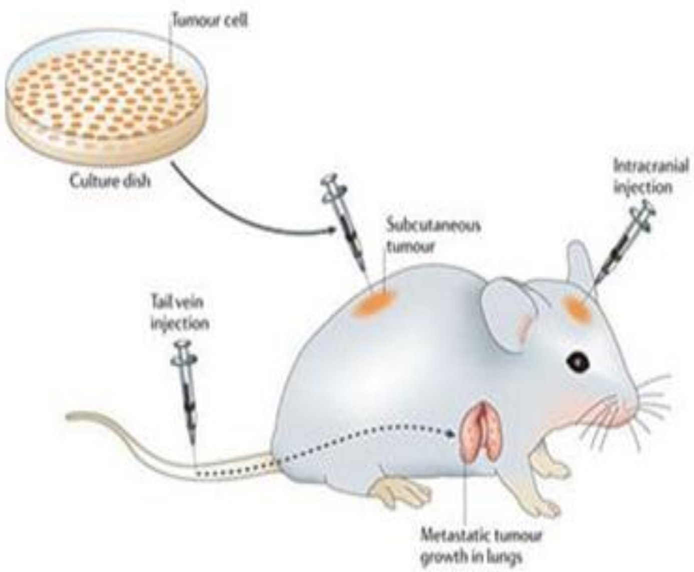
Influenza virus reproduction

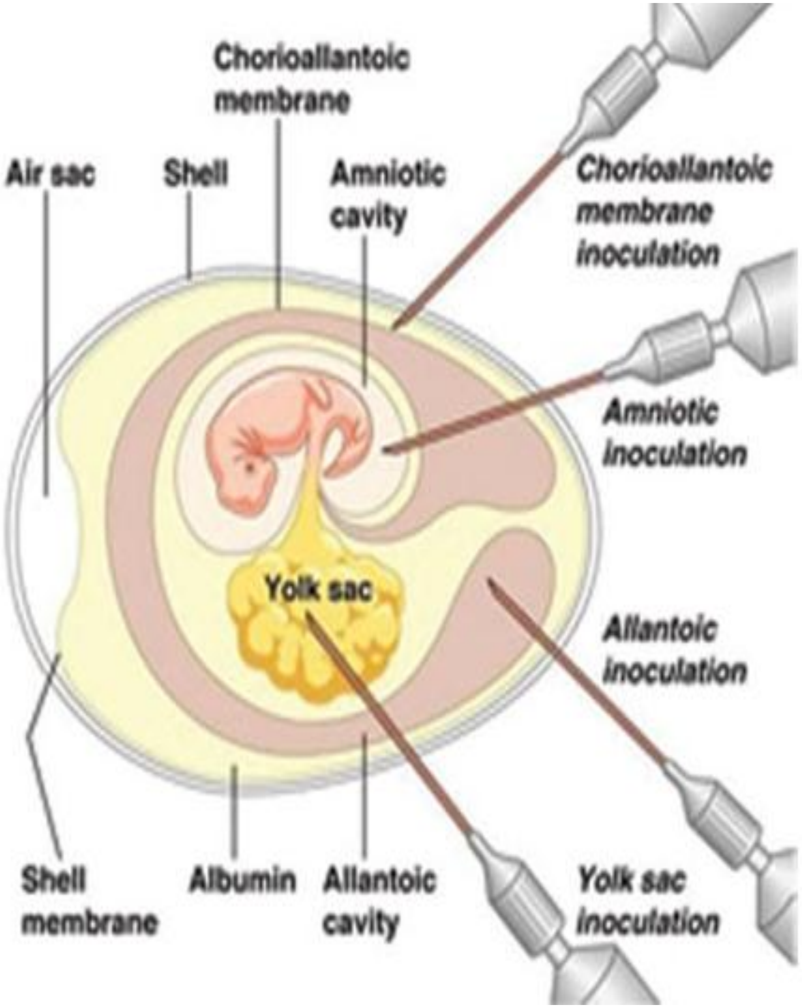


INFLUENZA VIRUS (REPRODUCTİON)

INFLUENZA VİRUS (CULTİVATİON)

- Chicken embryos are the optimal laboratory model for the cultivation of most strains of influenza viruses.
- Viruses can also be cultivated in **cell cultures** (primary cultures of monkey and dog kidney cells) and in **laboratory animals.**





Antigen

Influenza viruses are divided into 3 groups determined by the **ribonucleoprotein (RNP)** antigen and **M antigen**

- Soluble antigens: include **ribonucleoprotein** and **M protein** which are much stable in antigenicity.
- Surface antigens: include **HA** and **NA** which are much variable in antigenicity.

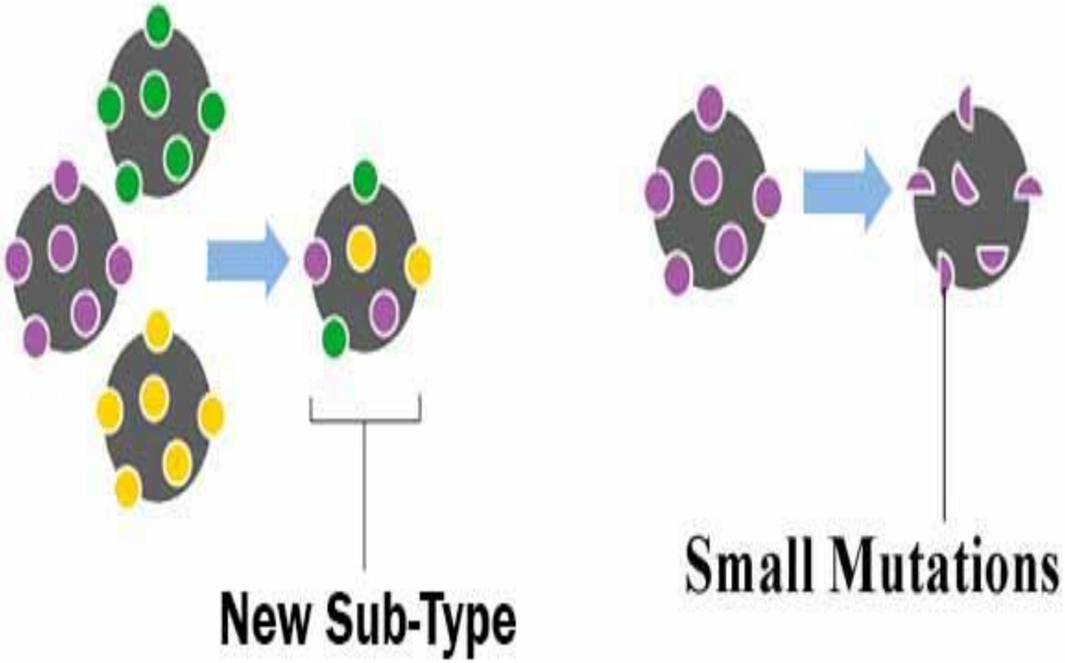
Antigenic Shift

- Major change in genom
- Gene reassortment
- Happen accidently
- Only in A viruses
- Result in new subtype

Antigenic Drift

- Minor change in genom
- Point mutation
- Happen gradually
- Both in A and B viruses
- Result in new strain

Differences Between
Antigenic shift & Antigenic drift

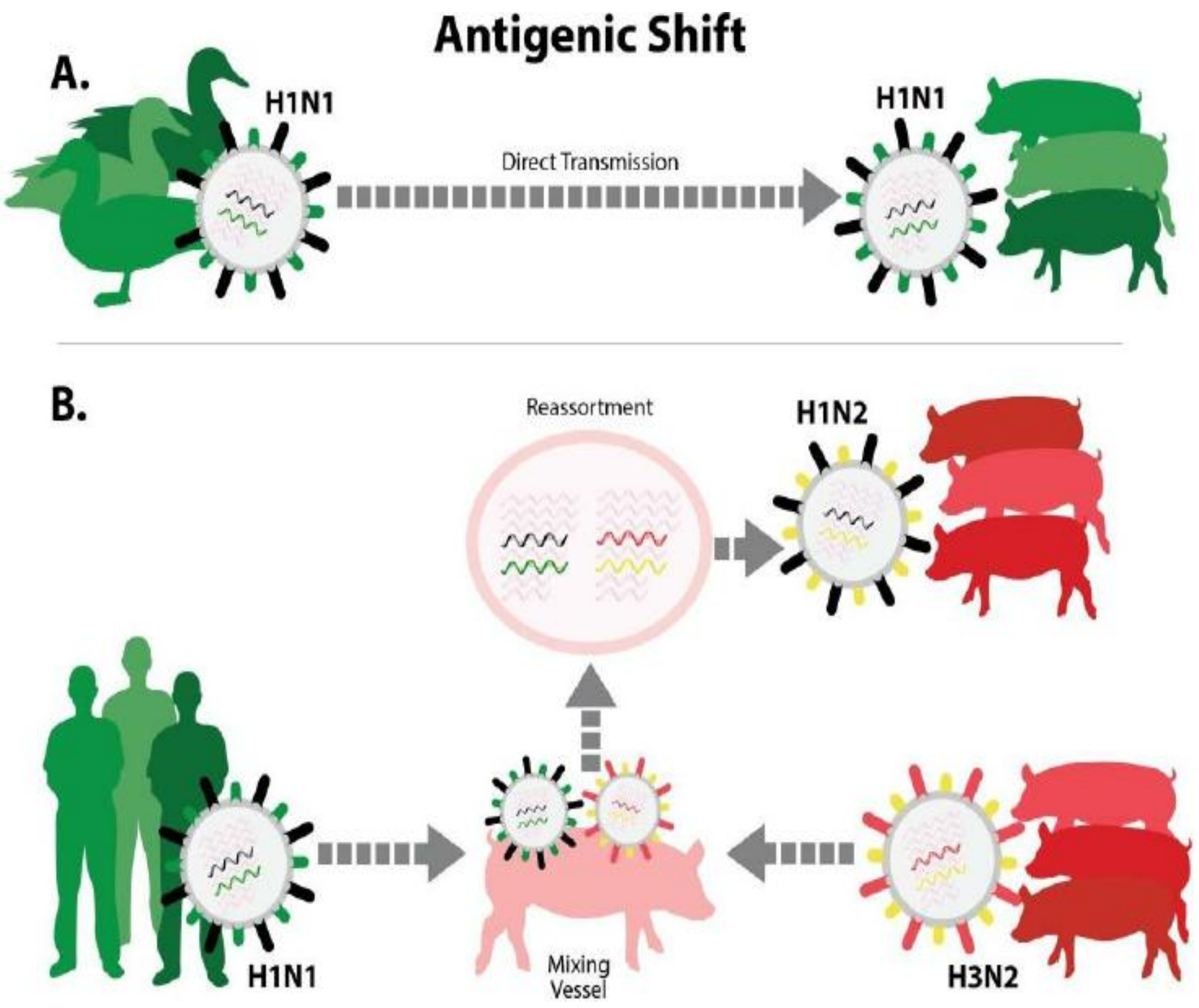


ANTİGENIC SHIFT

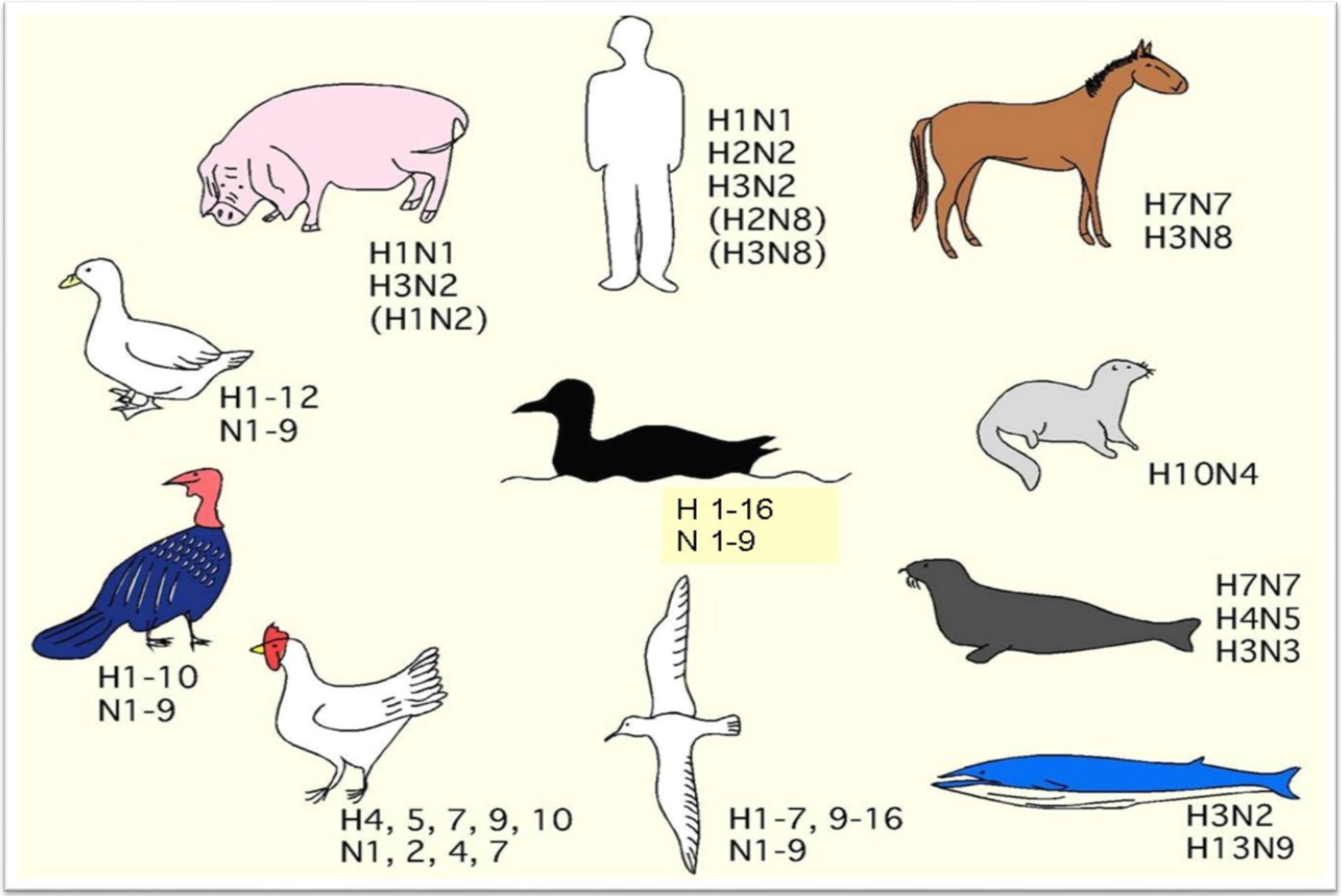
There are two ways that an influenza virus with new antigenic properties may enter the pig population.

(A) Virus that was previously adapted to another animal host, such as avian species, enters pigs and adapts to circulate efficiently in swine. The diagram portrays the inter-species transmission of an avian H1N1 virus, which became established in European swine populations;

(B) Virus previously adapted to another host, such as birds or humans, co-infects a pig along with a common swine-adapted strain. This can lead to gene reassortment, producing a new "reassortant" virus that contains an HA and/or NA antigenically different from those that previously circulated in swine. The diagram portrays reassortment between human seasonal H1N1 and swine H3N2 viruses. In both (A) and (B), the swine population lacks antibodies to important surface proteins of the new virus.

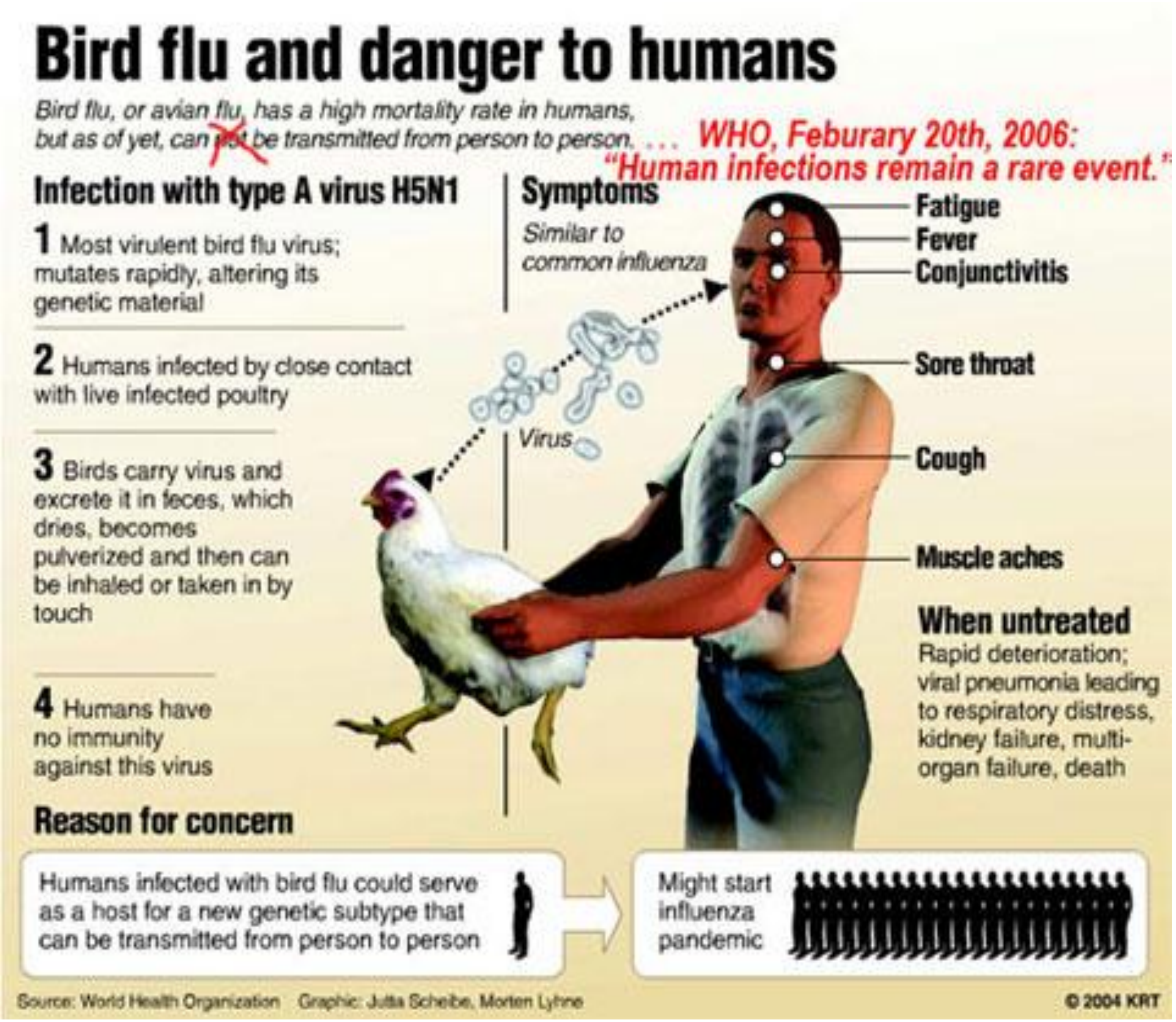


Occurrence of influenza virus subtypes in different organisms



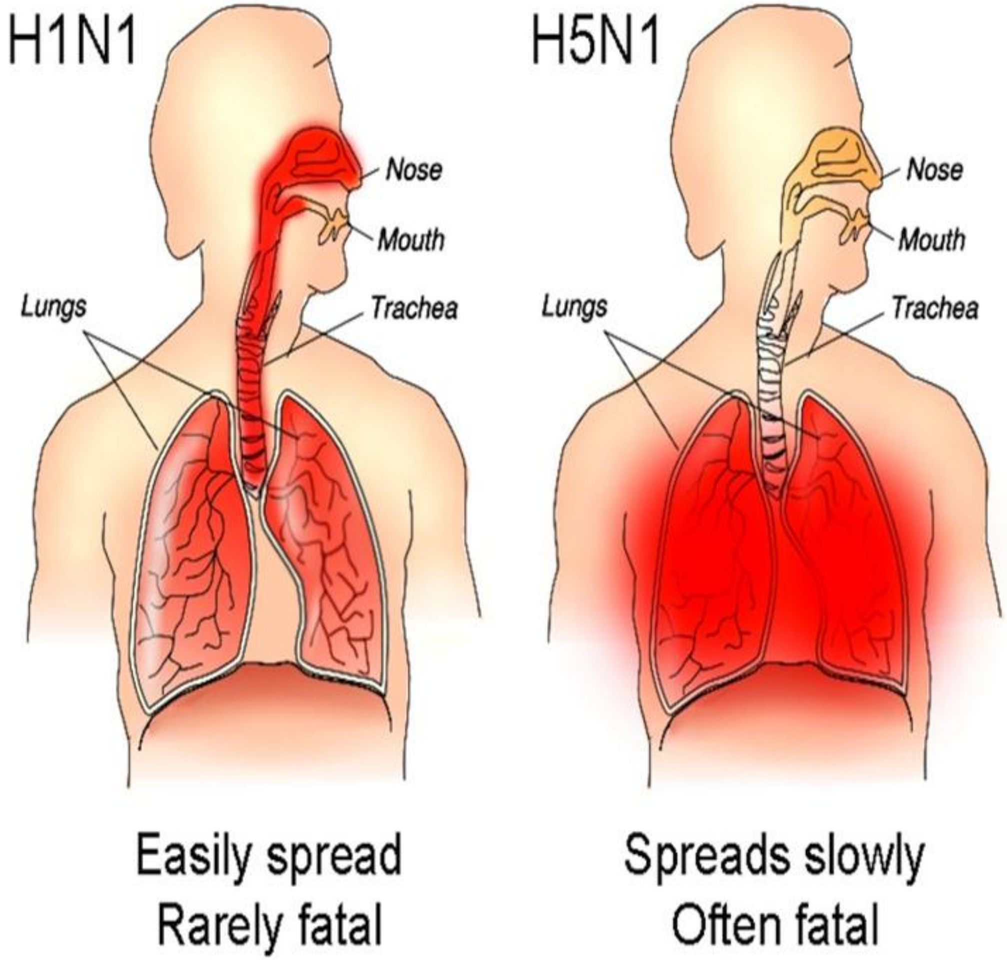
BIRD FLU (H5N1)

- In 1997, the first case of bird flu virus (H5N1 subtype of influenza A virus) was registered in Hong Kong. The source of infection was domestic birds. Avian influenza causes diseases of varying severity in some birds, from asymptomatic infections to lethal infections.
- Infection in ducks is usually asymptomatic. Influenza viruses in their bodies multiply in the intestinal epithelium, fall into the water in high concentrations with feces, and remain viable there for weeks. In this way, influenza viruses infect poultry and pigs.
- So far, pandemic influenza virus strains have emerged as a result of genetic sorting of avian and human influenza viruses. It is assumed that the genetic sorting of avian and human influenza viruses occurs in the body of pigs, since the body of pigs has receptors against both avian and human viruses.



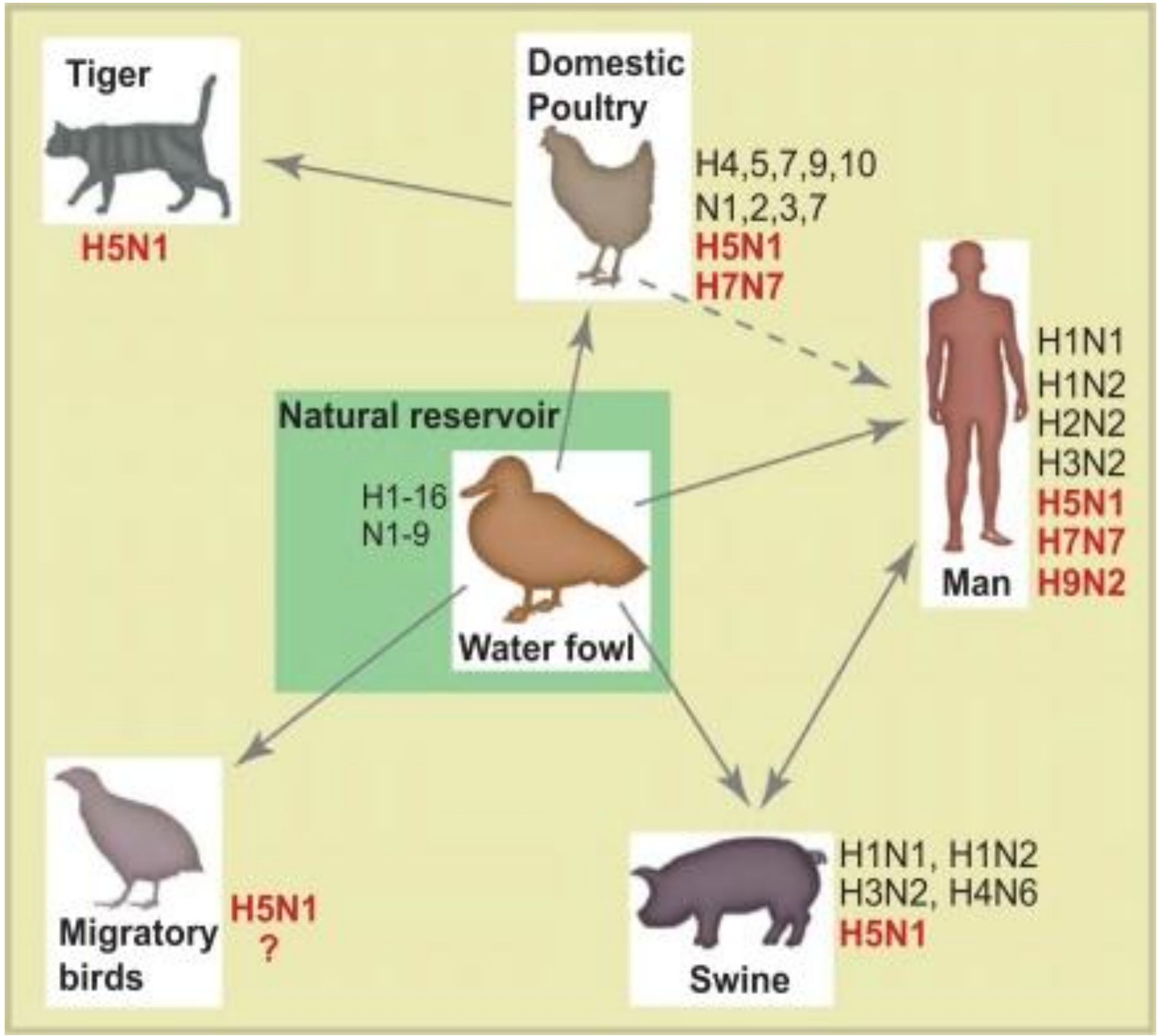
SWINE FLU (H1N1)

♦ In 2009, in California - the first case of H1N1 subtype of influenza A virus (swine flu virus) was registered;
♦ the source of infection was pigs;
♦ swine flu - which quickly spread across most of the world's continents and caused many deaths - has become a pandemic;
♦ due to its antigenic structure - this virus, which is not different from the *«Spanish»* virus, differs in its high pneumotropism and causes high mortality rates mainly in elderly or weakened persons;
♦ unlike common flu viruses - "swine flu" virus is highly pathogenic and kills white mice quickly.

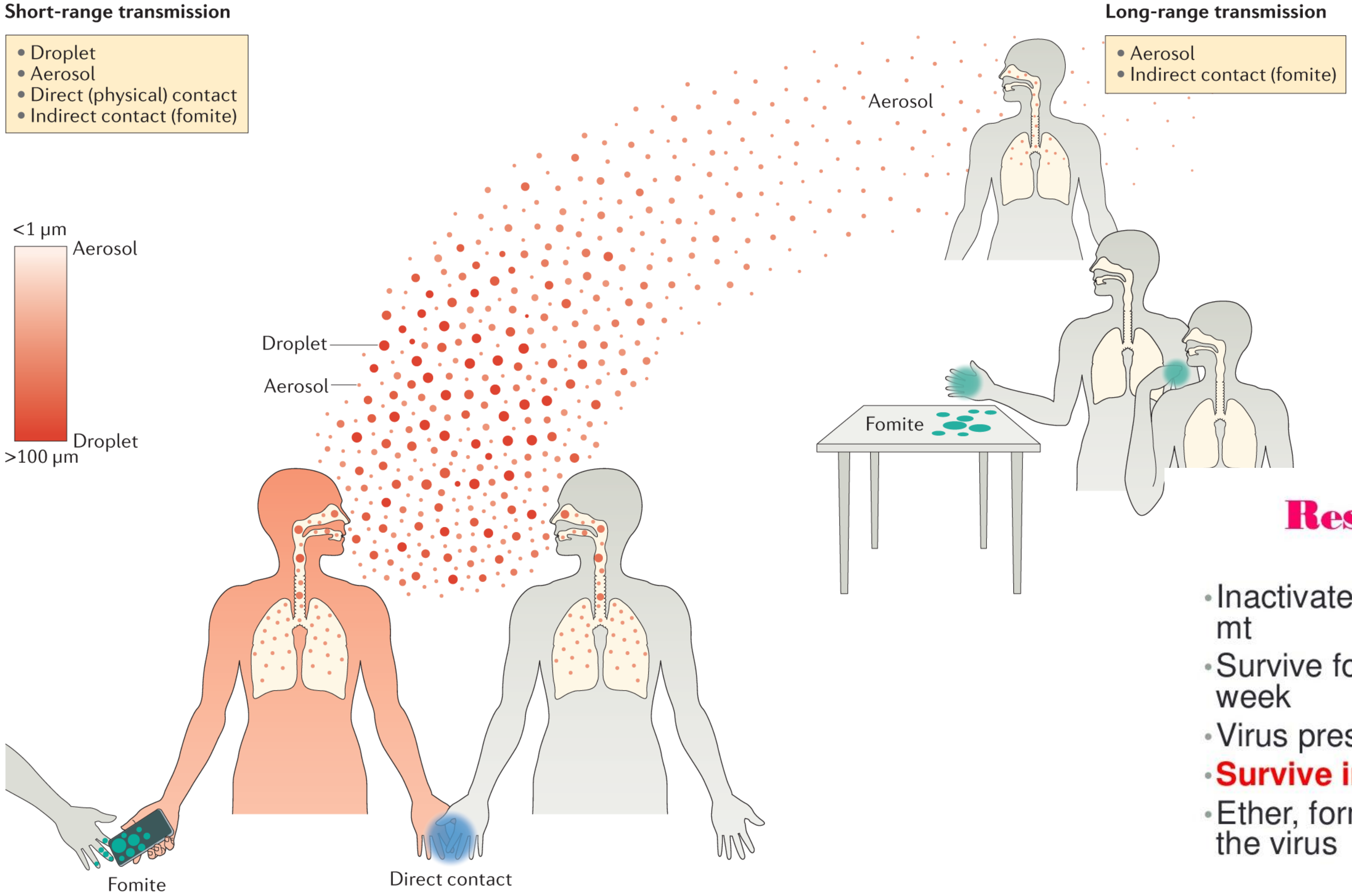


SOURCE OF INFECTION AND MODE OF TRANSMISSION:

- The source of infection is **sick people,** sometimes **birds and animals**. People are very susceptible to the flu virus. Infection occurs mainly through **air droplets** (when coughing, sneezing, talking).
- Type A influenza virus periodically causes pandemics. Southeast Asia (China) is the epicenter of the emergence of new pandemic strains of influenza A viruses. Here, high compact population, close contact with domestic animals and birds create conditions for the recombination of human and animal viruses.
- Since the end of 2005, the "bird flu" caused by the H5N1 subtype of influenza A virus, and the "swine flu" caused by the H1N1 subtype have started in the world since 2009.



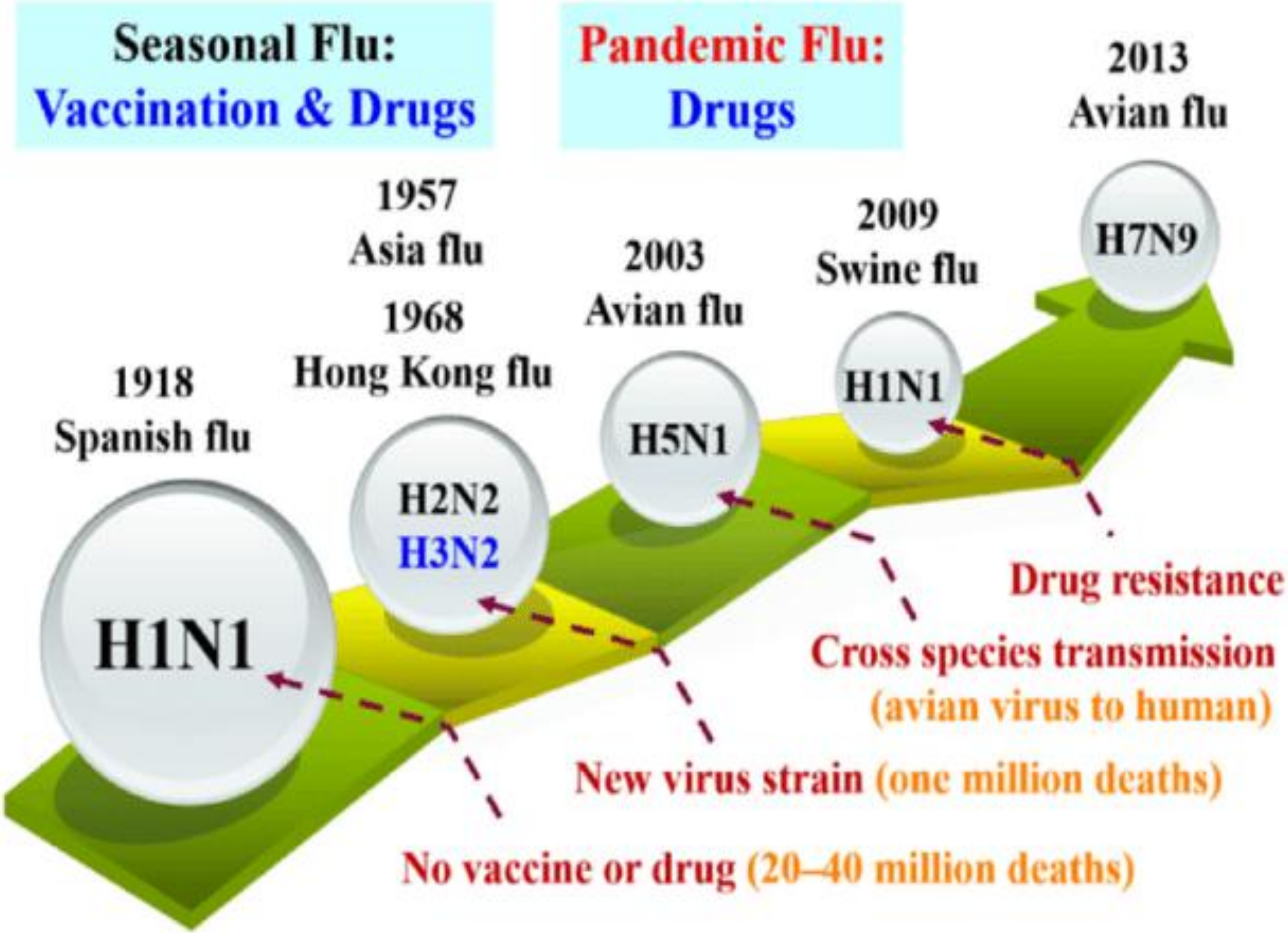
TRANSMISSION



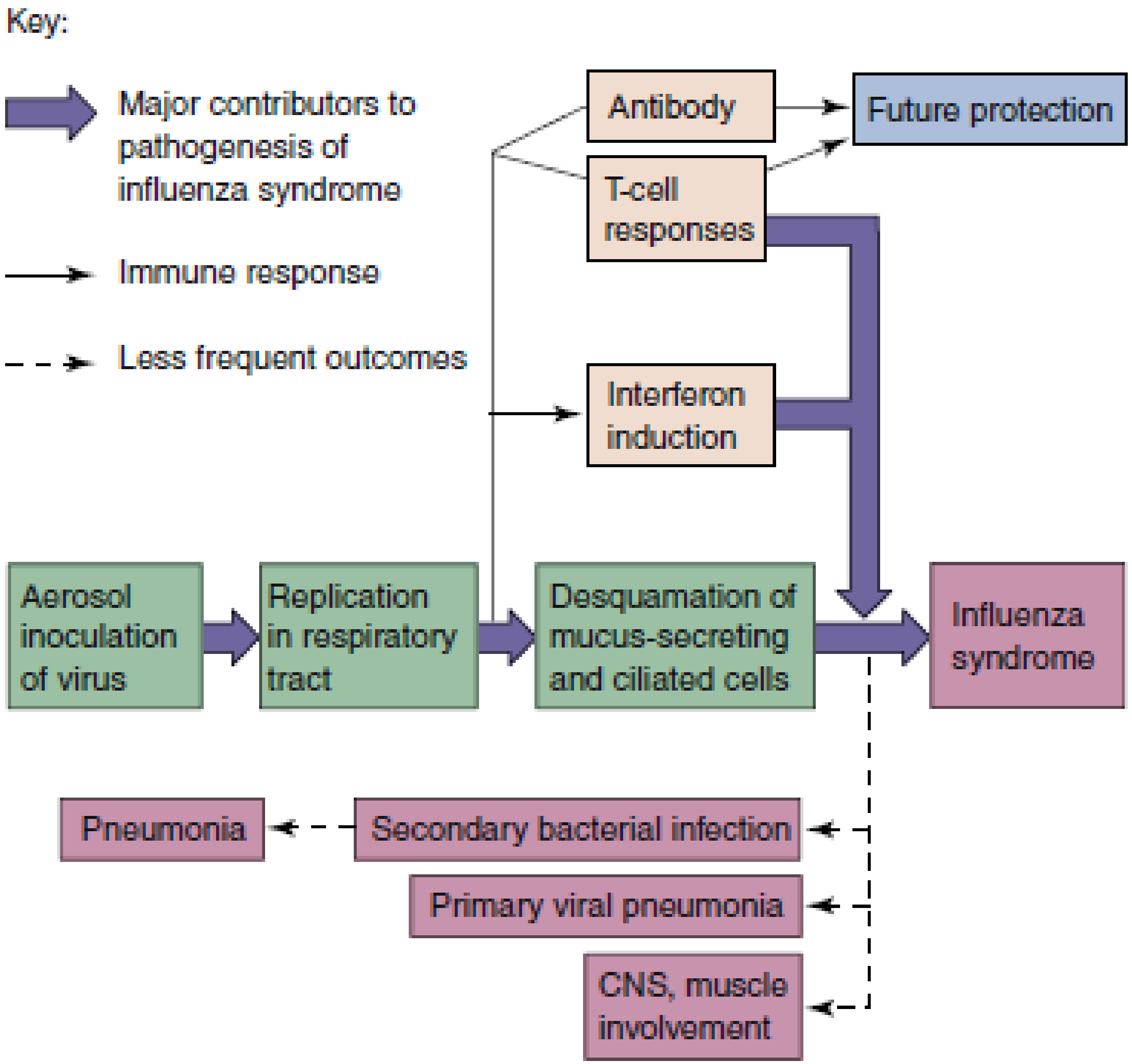
Resistance of Virus

- Inactivated by heating at 50^0c for 30 mt
- Survive for 1 week at 0 – 4^0c for 1 week
- Virus preserved at – 70^0c
- **Survive in the blankets for 2 weeks**
- Ether, formaldehyde, Phenol destroy the virus

EPİDEMİOLOGY



Pathogenesis



The primary reproduction of the influenza virus that has entered the body occurs in the **epithelial cells** of the **upper respiratory tract** (sometimes lung alveoli). As a result of the destruction and desquamation of the surface epithelium, inflammation and edema develop here, but the basal membrane of the epithelium is not damaged.

Some subtypes of influenza viruses can immediately enter the alveoli and cause the development of primary acute pneumonia (atypical pneumonia). This is one of the main causes of death in high-risk patients

The virus is rarely transmitted to the blood.

Damage to the epithelium of the respiratory tract creates conditions for the development of secondary bacterial infections, especially caused by staphylococci, streptococci (pneumococcus) and hemophilic bacteria.

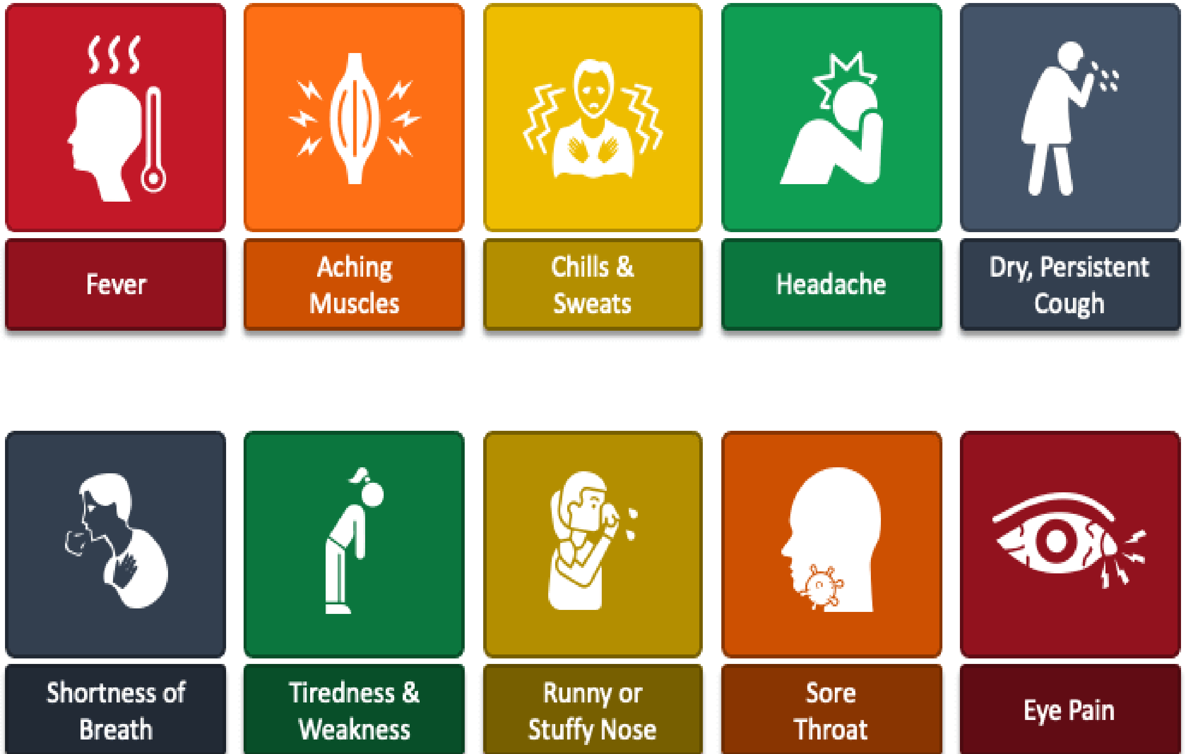
During the flu, transient secondary immunodeficiency develops, which creates conditions for the development of secondary bacterial infections.

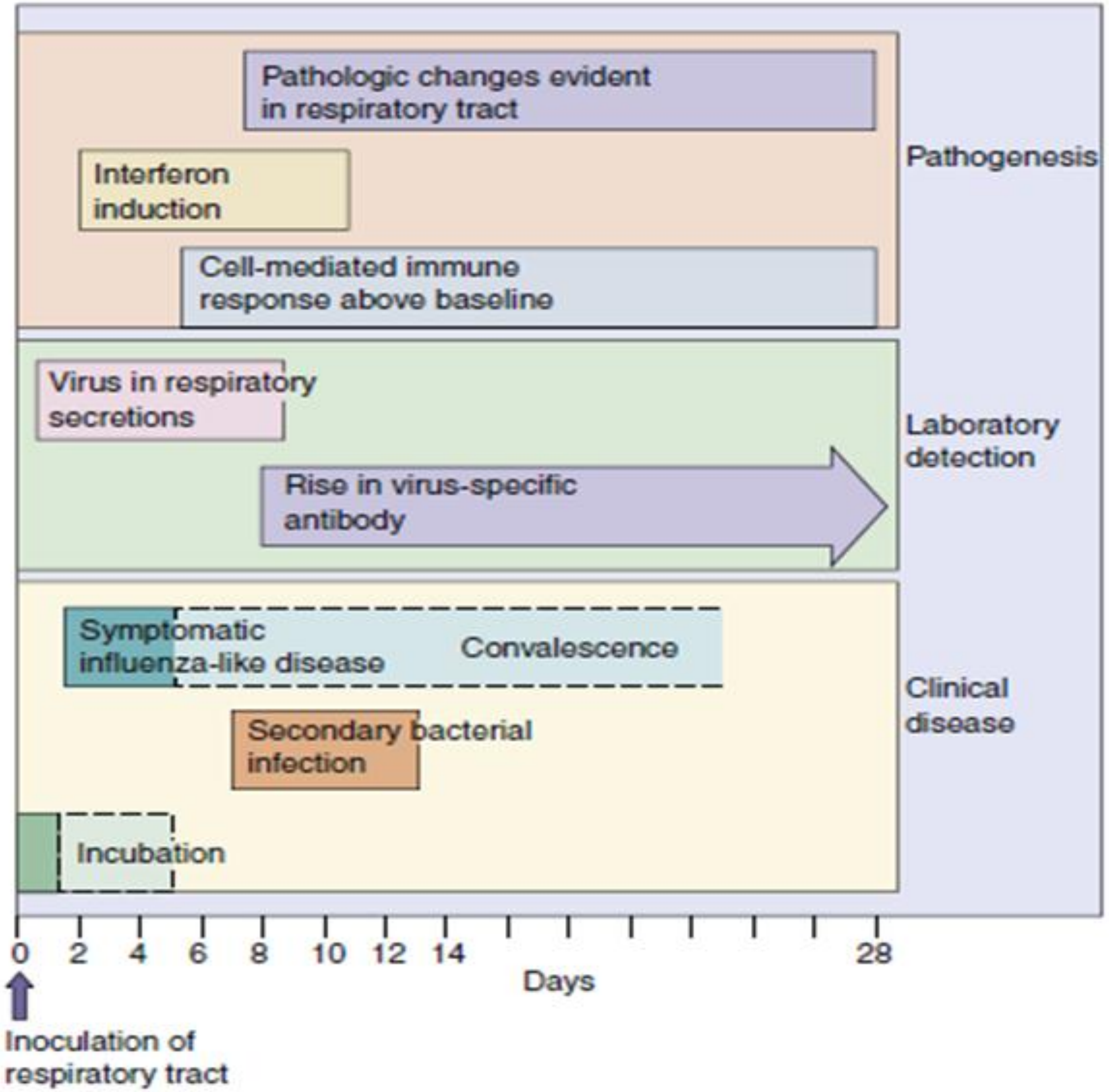
Secondary bacterial **pneumonias** are also one of the main causes of death.

Clinical manifestations of influenza:

•The **latent period** of influenza can last from **1 to 4 days**.

•**Uncomplicated flu.** The disease begins acutely, usually with high fever, headaches, joint and muscle pain felt throughout the body, and weakness. Catarrh of the upper respiratory tract - cough, back pain, rhinitis and rhinorrhea develop.



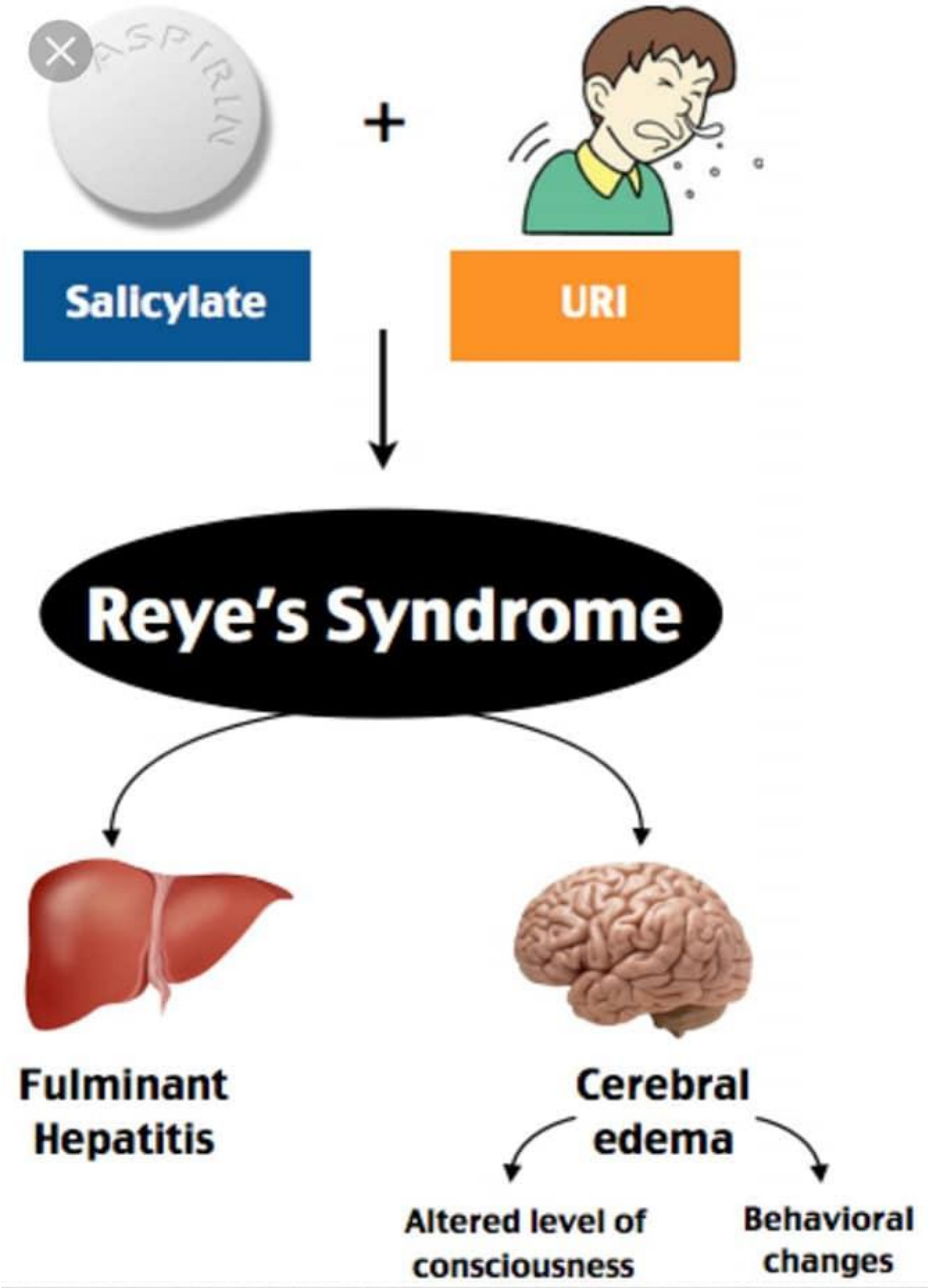


Time course of influenza A virus infection. The classic "flu syndrome" occurs early. Later, pneumonia may result from bacterial pathogenesis, viral pathogenesis, or immunopathogenesis.

Complications of the flu

•**Pneumonia**, the most serious complication of influenza in the elderly and debilitated with chronic diseases, as well as in pregnant women, is one of the main causes of death during influenza. Viral pneumonia, secondary bacterial pneumonias, as well as mixed viral-bacterial pneumonias are possible during influenza. Bacterial pneumonias are most commonly caused by *S.aureus*, *S.pneumoniae and H.influenzae*.

•**Reye's syndrome** is an acute encephalopathy that occurs in children and teenager aged 2-16 years and is rarely observed. Mortality is approximately 10-40%. The higher occurrence of Reye's syndrome after administration of salicylates in these infections suggests a possible relationship between salicylates (**aspirin**, etc.) and this syndrome.



Immunity in Influenza

Immunity to an influenza virus is type-specific and lasts for many years. Recurrent cases of influenza are caused primarily by antigenically different strains.

- Antibody to HA - >protective
- Antibody to NA - > decrease severeity
- Serum antibody - > years
- Secretory antibody - > months

Laboratory Diagnosis of Influenza Virus Infection

Test	Detects
Cell culture in primary monkey kidney or Madin-Darby canine kidney cells	Presence of virus; limited cytopathologic effects
Hemadsorption to infected cells	Presence of hemagglutinin protein on cell surface
Hemagglutination	Presence of virus in secretions
Hemagglutination inhibition	Type and strain of influenza virus or specificity of antibody
Antibody inhibition of hemadsorption	Identification of influenza type and strain
Immunofluorescence, ELISA	Influenza virus and antigens in respiratory secretions or tissue culture
Serology: hemagglutination inhibition, hemadsorption inhibition, ELISA, immunofluorescence, complement fixation	Seroepidemiology
Genomics: rapid viral RNA detection assays, RT-PCR, multiplex RT-PCR, sequence analysis	Detection and identification of influenza type and strain

ELISA, Enzyme-linked immunosorbent assay; *RT-PCR*, reverse transcriptase-polymerase chain reaction.

Microbiological diagnosis of influenza

Examination material - during the first three days of the disease, it is possible to obtain viruses as a result of the examination of the materials taken from the nose, or pharynx, as well as swabs from the sputum. Sometimes, nasal mucosal impression smear are studied.

Virological

Materials intended for virological examination should be stored at +4^{0}C until examination. Freezing reduces the possibility of detecting the influenza virus, if the examination is to be carried out later than 5 days, the material is stored frozen at -70^{0}C. Chicken embryos are mostly used for cultivation. In cell cultures, the virus can be indicated by the hemadsorption test 3-5 days after inoculation, and in the culture fluid after 5-7 days by the hemagglutination (**HAR**) reaction. The subtype of the acquired virus is determined by the hemagglutination inhibition reaction (**HIR**), and the type is determined by **CFT**.

Express diagnostics

Virus antigens can be detected in the examined material by **IFR** (direct and indirect variant), but it has poor sensitivity compared to the virological method.

It is possible to determine the viral genome in the material by **PCR**. In 2006, Real-time reverse transcriptase PCR was proposed to detect the "bird flu" virus (A/H5N1).

Serological

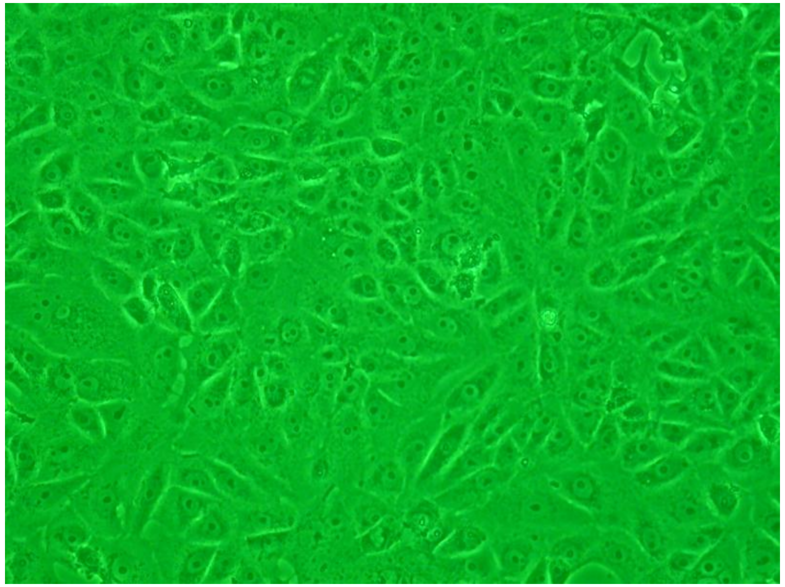
Preliminary serodiagnosis of influenza is carried out by **HIR** and **ELISA**. Since the blood serum of healthy people can contain antibodies against influenza viruses, paired blood sera of the patient (taken at an interval of 10-14 days during the acute period of the disease and during the convalescence period) are studied.

A four-fold increase in the titer of antibodies in the blood serum confirms the diagnosis. The serological method is often used for retrospective diagnosis.

Microbiological diagnosis of influenza

Examination material - during the first three days of the disease, it is possible to obtain viruses as a result of the examination of the materials taken from the nose, or pharynx, as well as swabs from the sputum. Sometimes, nasal mucosal impression smear are studied.

Virological



Express diagnostics

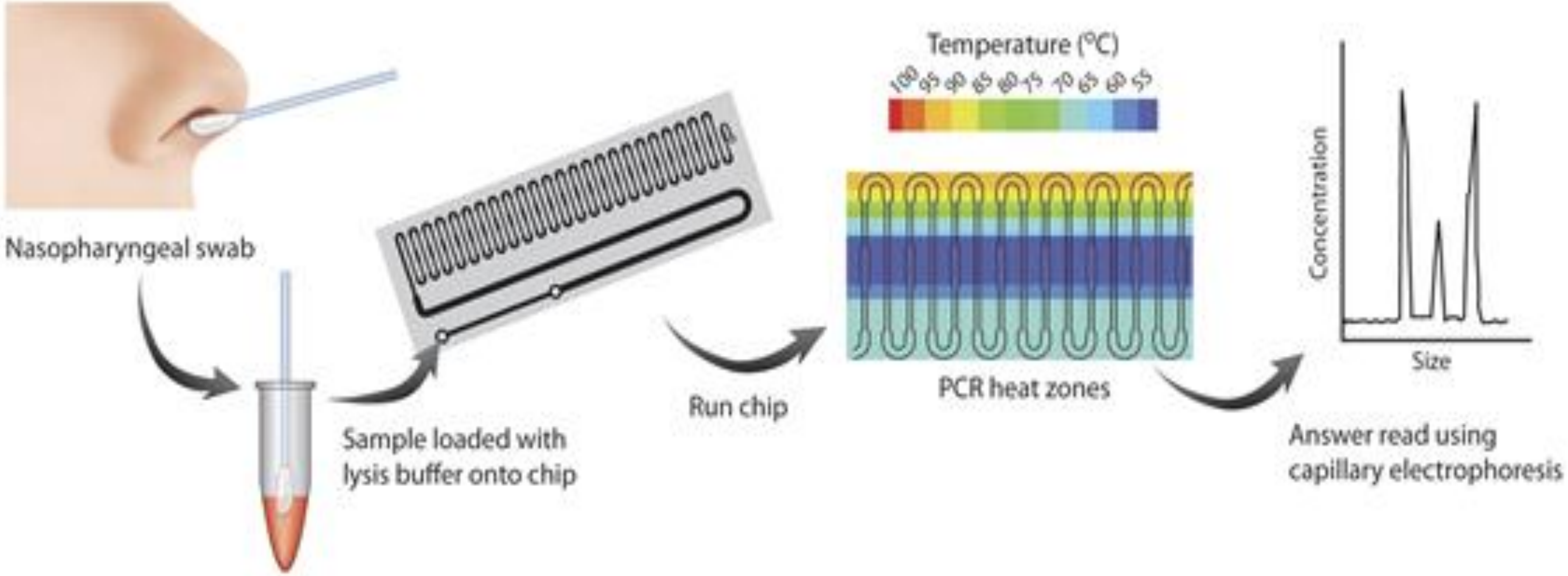


TABLE 61-6 Laboratory diagnosis of influenza virus infection

Method	Test	Detection
Direct antigen detection	IF, ELISA	Influenza virus antigen in respiratory secretions
Virus isolation		
Cell culture	Primary monkey kidney cell, Madin-Darby canine kidney cell, and hemadsorption to infected cells	Limited cytopathic effects Presence of HA protein on cell surface
Chick embryo	Allantoic and amniotic cavity	Fluid is tested for hemagglutination of virus
Serology	Hemagglutination inhibition, Hemadsorption inhibition, ELISA, CFT, and IF	Demonstration of a rise in serum antibody titer
Molecular diagnosis	RT-PCR	Viral nucleic acid in the nasopharyngeal cells

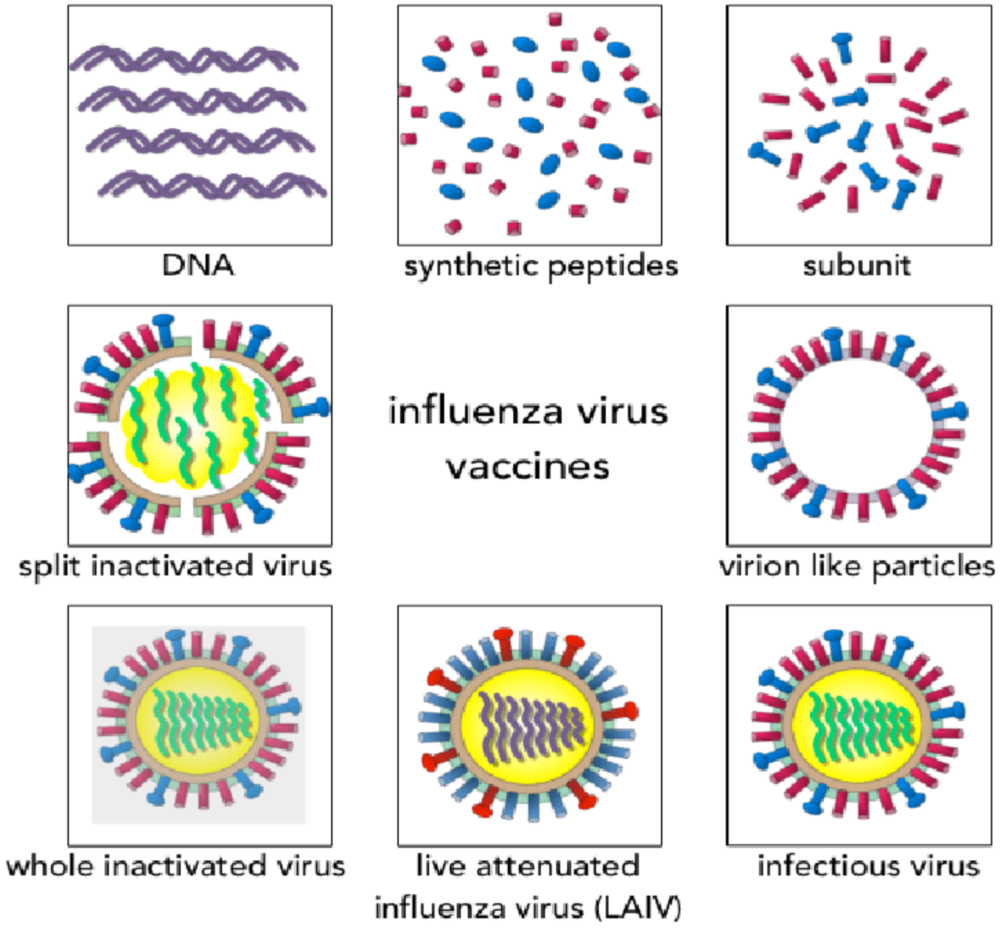
ELISA, enzyme-linked immunosorbent assay; CFT, complement fixation test; IF, immunofluorescence; RT-PCR, reverse transcriptase-polymerase chain reaction.

TREATMENT

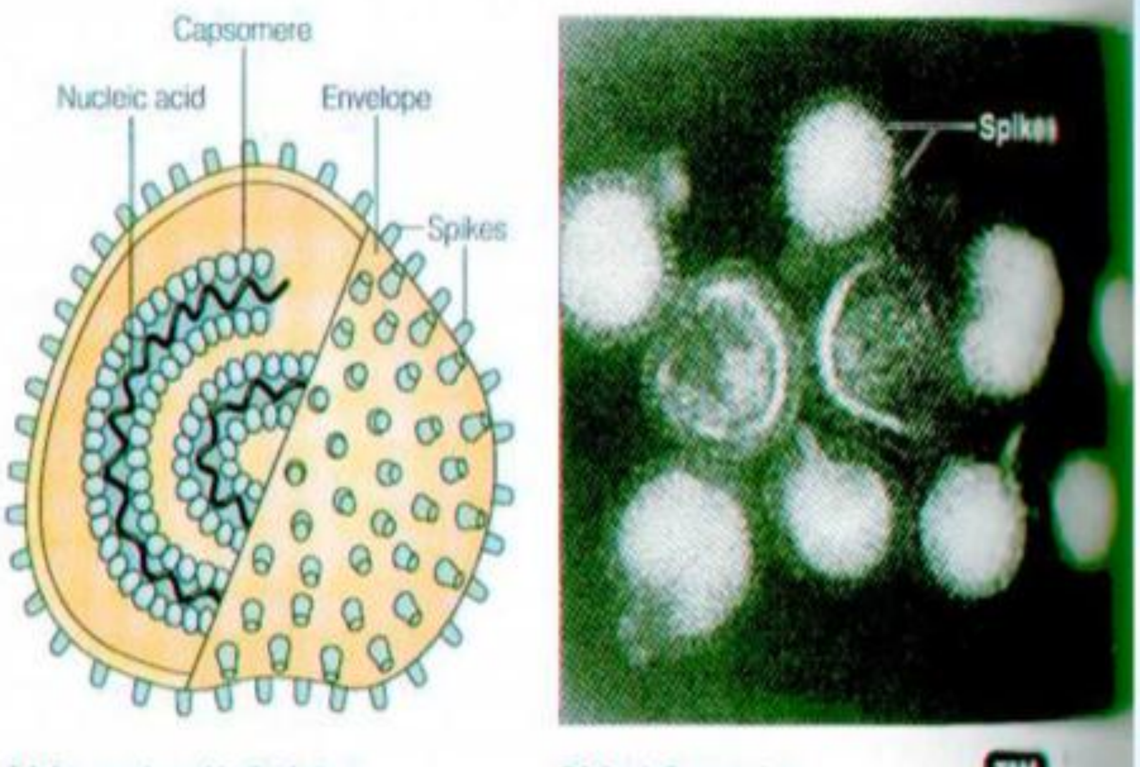
- **RIMANTADINE** (blocks the M2 ion channel) (M2)
  - type A only, needs to be given early
- **AMANTADINE** (blocks the M2 ion channel) (M2)
  - type A only, needs to be given early
- **ZANAMIVIR** (neuraminidase inhibitors) (NA)
  - types A and B, needs to be given early
- **OSELTAMIVIR** (neuraminidase inhibitors) (NA)
  - types A and B, needs to be given early

VACCINES

- Rimantadine can be used for emergency **chemical prevention** during flu epidemics.
- Various vaccines are used for **specific prevention**. Inactivated (killed) and live vaccines are available.
- **Whole virion vaccines** consist of inactivated viruses
- **Subvirion vaccines** - consist of virus particles that have been broken down by detergents.
- Vaccines made from **surface antigens** include purified H and N glycoproteins.
- **Live (live, attenuated) vaccines**. Thermolabile mutants of the influenza virus have recently been obtained that can growth at 25^{0}C but cannot growth at body temperature (37^{0}C). Such viruses can replicate in the nasopharynx, where the temperature is relatively low (33^{0}C), but cannot replicate in the lower respiratory tract. Live vaccines made from such viruses are administered as an intranasal spray.



Influenzavirus B

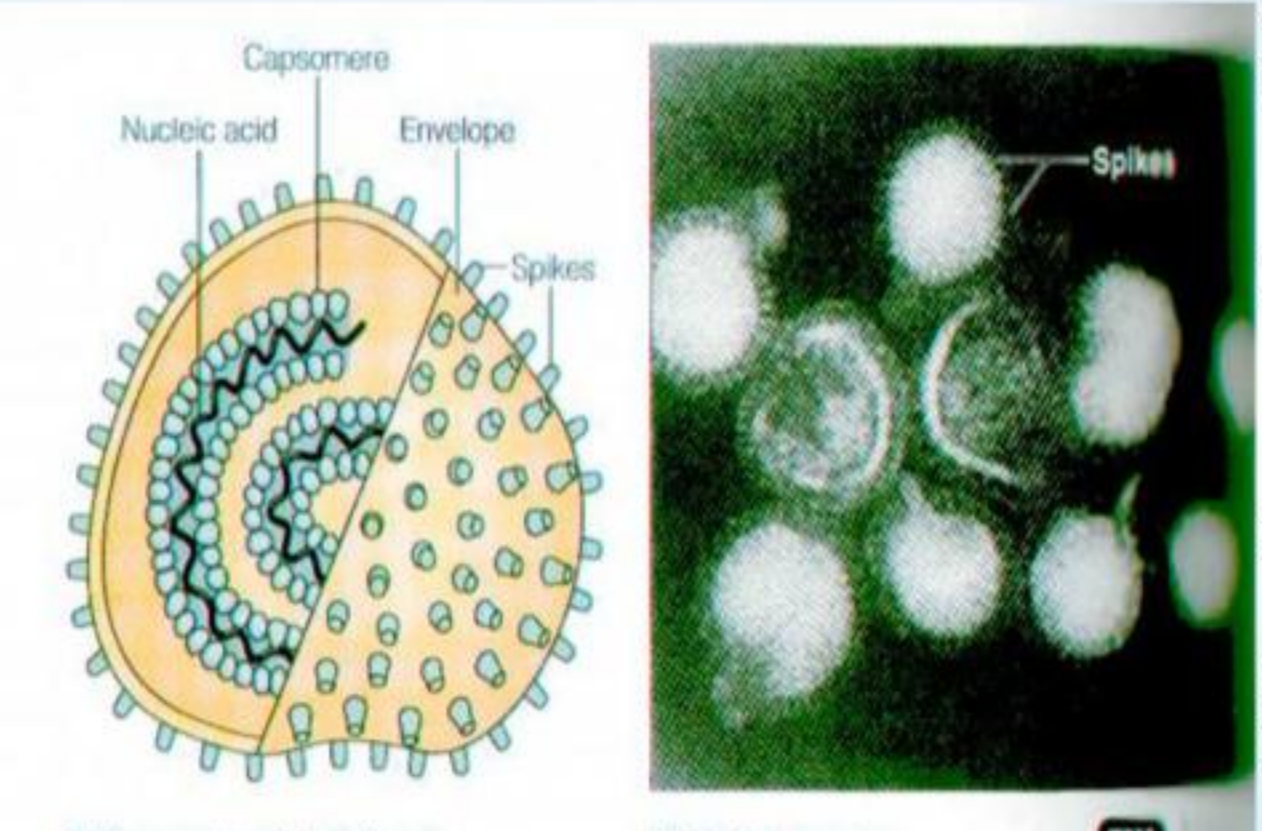


(a) An enveloped helical virus
(b) An *Influenzavirus*

- **Virions enveloped**
- **About 500 spikes**
- **Nucleocapsid enclosed within lipoprotein membrane**
- **Virions contain 8 segments of linear negative-sense single stranded RNA**
- **Total genome length is 13588 nt**
- **The largest segment 2341 nt**

- Infect much man and birds.
- Cause human disease but generally not as severe as A types.
- Believed to be epidemiologically important - reassortment with type A leads to epidemics.

Influenzavirus C



(a) An enveloped helical virus (b) An *Influenzavirus*

- Virions enveloped
- Many spikes
- Nucleocapsid enclosed within lipoprotein membrane
- Virions contain 7 segments of linear negative-sense single stranded RNA
- Total genome length is 12900 nt
- Glycoprotein
- -hemagglutinin esterase fusion (HEF)
- esterase -> receptor destroying enzyme

Family Paramyxoviridae

Subfamily Paramyxovirinae:

Genus	Human Pathogen
Morbillivirus	Measles virus
Paramyxovirus	Parainfluenza viruses 1-4
	Mumps virus
Pneumovirus	Respiratory syncytial virus
	Metapneumovirus

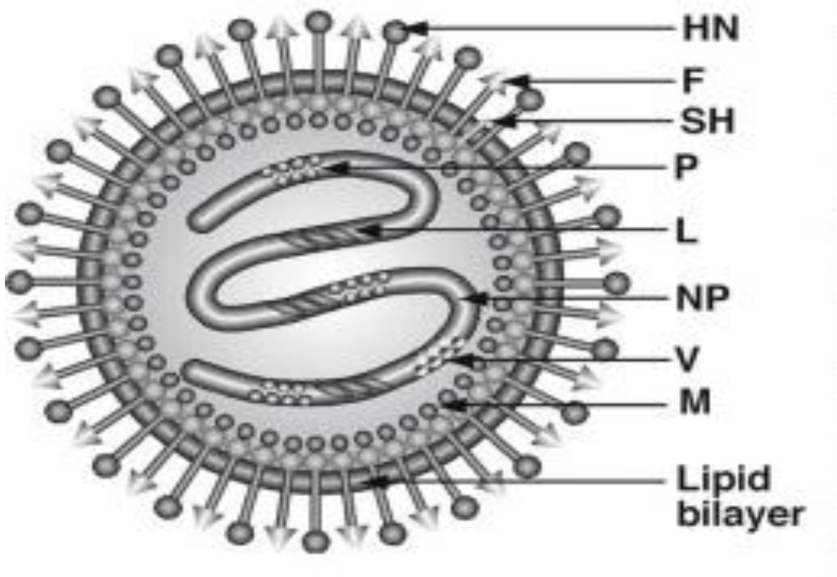
Major Viral-Encoded Proteins of Paramyxoviruses

Gene and Proteins[a,b]	Virion Location	Protein Function
N: nucleoprotein	Major internal protein	Protection of viral RNA
P: phosphoprotein and C and V proteins	Association with nucleoprotein	Part of transcription complex; C and V are antagonists of innate responses
M: matrix	Inside virion envelope	Assembly of virions
F: fusion protein	Transmembranous envelope glycoprotein	Protein promotes fusion of cells, hemolysis, and viral entry
G: glycoprotein (HN, H, G)[c]	Transmembranous envelope glycoprotein	Viral attachment protein
L: polymerase (large)	Association with nucleoprotein	Polymerase

[a]In order on the genome.
[b]Pneumoviruses also encode an SH and M2 protein.
[c]Glycoproteins differ for the different paramyxoviruses: HN, hemagglutinin-neuraminidase; H, hemagglutinin; G, glycoprotein.

Paramyxoviruses:

- **Structure**. The virions of *Paramyxoviruses* are **enveloped**, **polymorphic**, 150 nm and larger (sometimes 700 nm).
- The genome of the virus consists of **linear single-stranded RNA**, which combines with a number of proteins to form a nucleocapsid with **helical symmetry.**
- The lipid virion membrane has two types of transmembrane glycoprotein spikes: one of them consists of three glycoproteins **(HN, or H, or G)** with hemagglutinin and/or neuraminidase activity.
- The activity of these glycoproteins, which ensures the virus's connection with the host cell, allows different types of the family to be differentiated**. It is found in HN - parainfluenza and mumps, H - measles virus, G - respiratory syncytial virus.**
- Another glycoprotein consists of **fusion protein F-protein** (in English, fusion), connects cell membranes and has hemolytic activity.

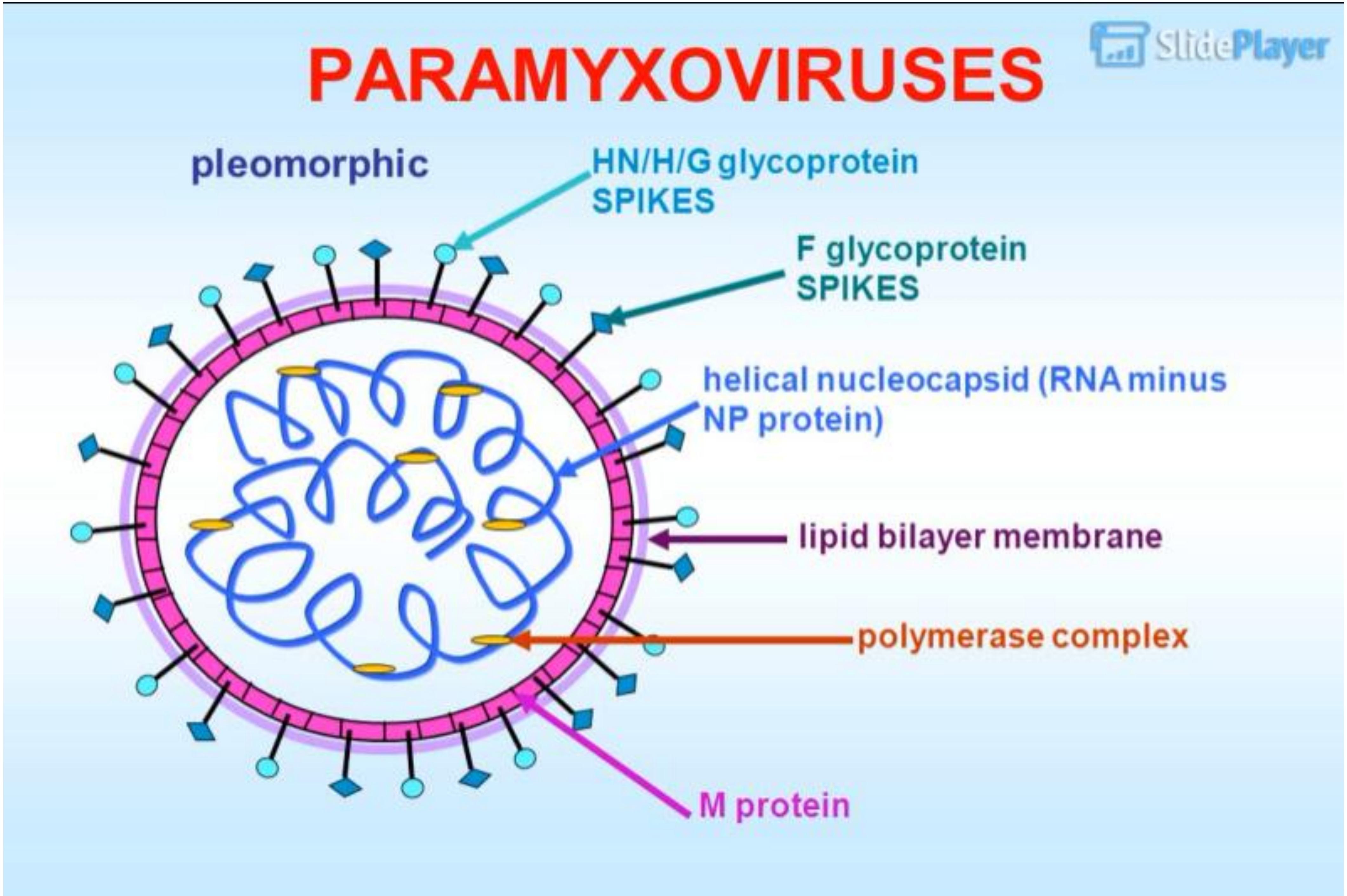


PARAMYXOVIRUS FAMILY
properties of attachment protein

Major Viral-Encoded Proteins of Paramyxoviruses

GENUS	GLYCOPROTEINS	TYPICAL MEMBERS
Paramyxovirus genus	HN, F	HPIV1, HPIV3
Rubulavirus Genus	HN, F	HPIV2, HPIV4 mumps virus
Morbillivirus genus	H, F	measles virus
Pneumovirus genus	G, F	respiratory syncytial virus

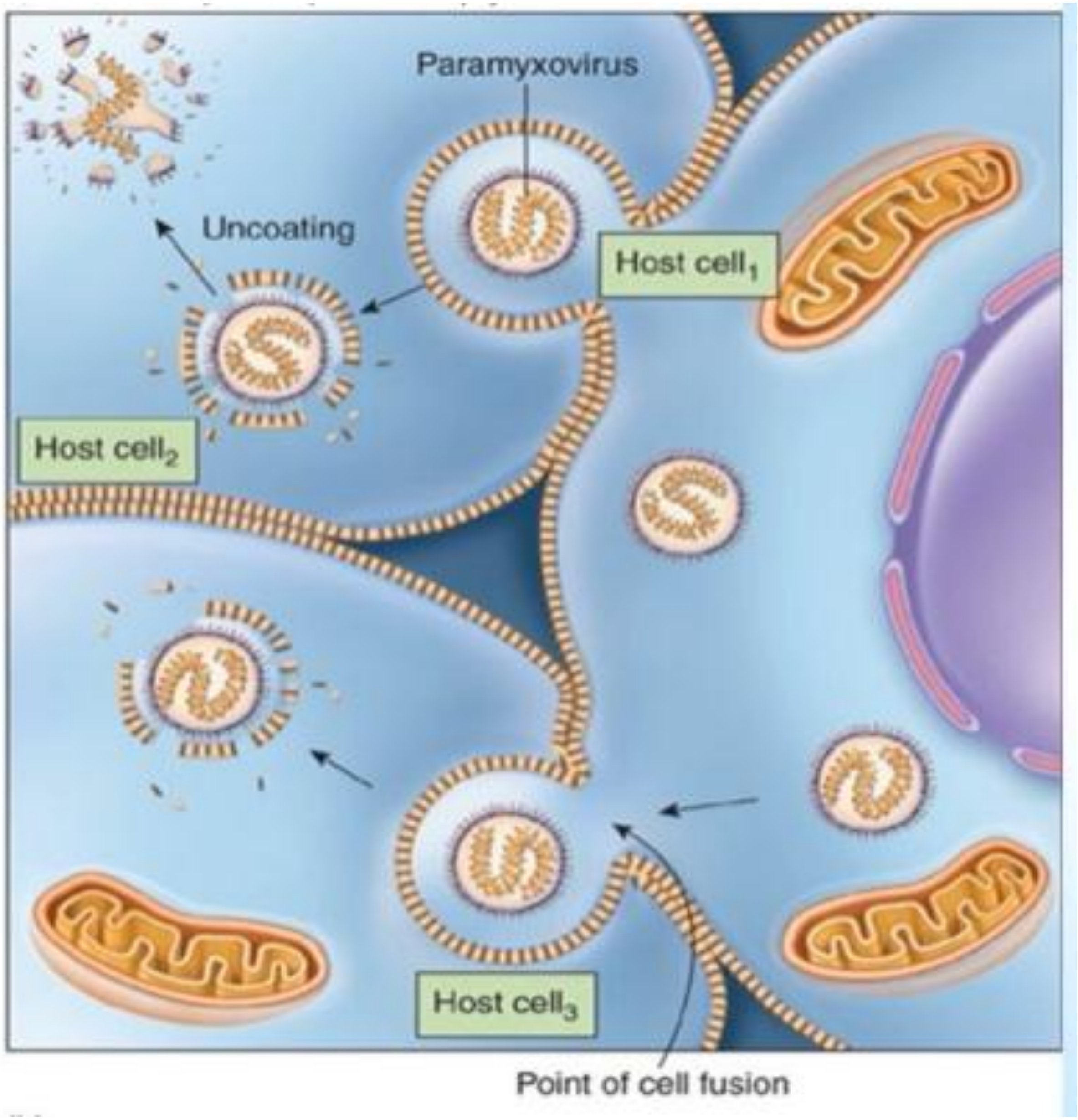
Major Viral-Encoded Proteins of Paramyxoviruses



PARAMYXOVIRUSES
pleomorphic
HN/H/G glycoprotein SPIKES
F glycoprotein SPIKES
helical nucleocapsid (RNA minus NP protein)
lipid bilayer membrane
polymerase complex
M protein

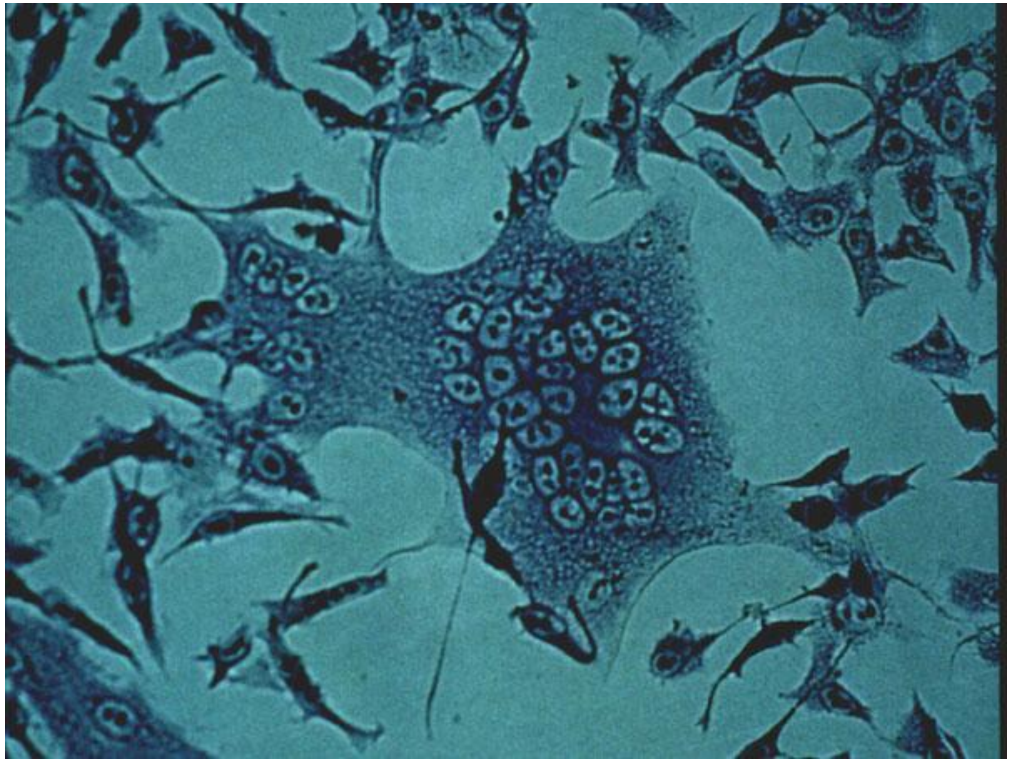
Reproduction of Paramyxoviruses:

•***Paramyxoviruses*** are adsorbed to sialic acid receptors on the surface of the host cell through **HN-, H- or G-proteins** in the membrane.
•The virion enters the cell directly without endosome formation.
•Genome transcription, replication and protein synthesis take place in the cytoplasm of the host cell.
•The virion exits the cell by budding.
•F_0-glycoproteins formed as a result of proteolytic cleavage of F-glycoproteins in the cell membrane under the influence of appropriate proteases of the host cell form **syncyts** by connecting the membranes of neighboring cells.



Paramyxoviridae cultivation

- Cultivation of *Paramyxoviruses* are carried out in primary and continuous cell cultures.
- The **cytopathic effect** is manifested by the formation of **syncytium** (polykaryons) and acidophilic inclusions in the cytoplasm.

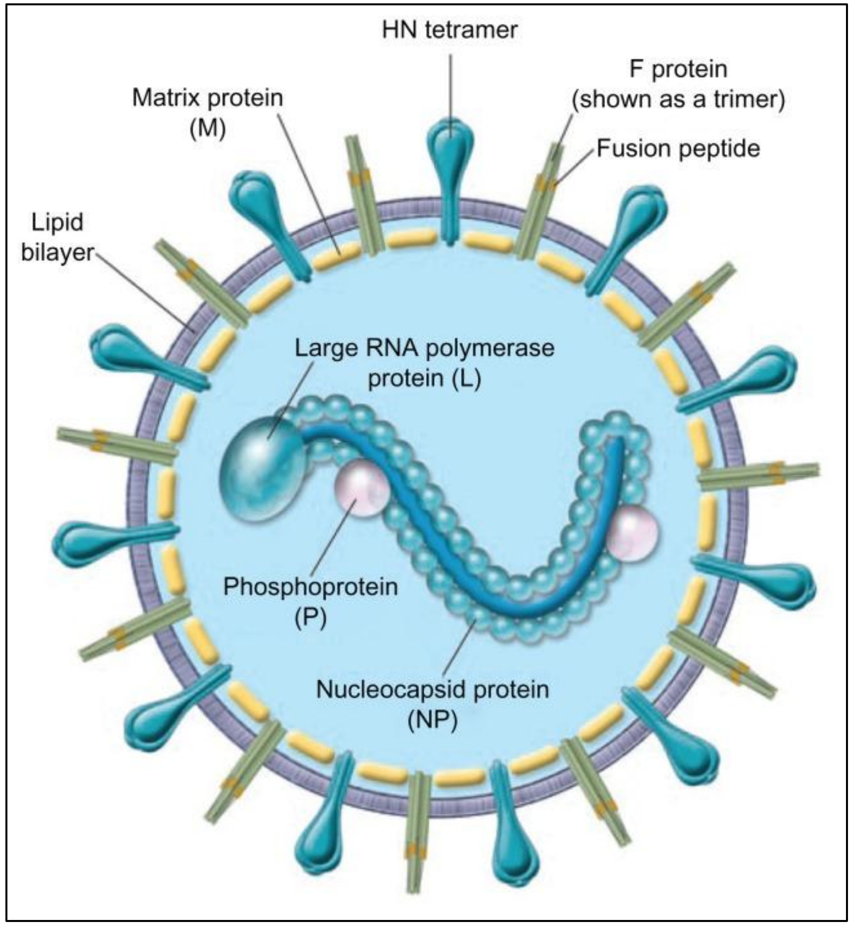


Resistance to environmental factors:

- *Paramyxoviruses* are among the most **persistent** viruses in the environment.
- They are **sensitive** to temperatures above 50^0C, detergents, disinfectants and other factors.
- They have higher resistance to low temperature.

Parainfluenza virus

- Human *Parainfluenza (HPIV)* virus serotypes 1 and 3 belong to the *Respirovirus* genus, and serotypes 2, 4a and 4b belong to the *Rubulavirus* genus.
- 4 main serotypes of *Parainfluenza* viruses are distinguished according to the antigens of glycoprotein spikes in the membrane - **HN-**, **NP-** and **F-proteins**.
- Viruses of serotypes 1, 2, 3 have common antigens with epidemic mumps viruses.

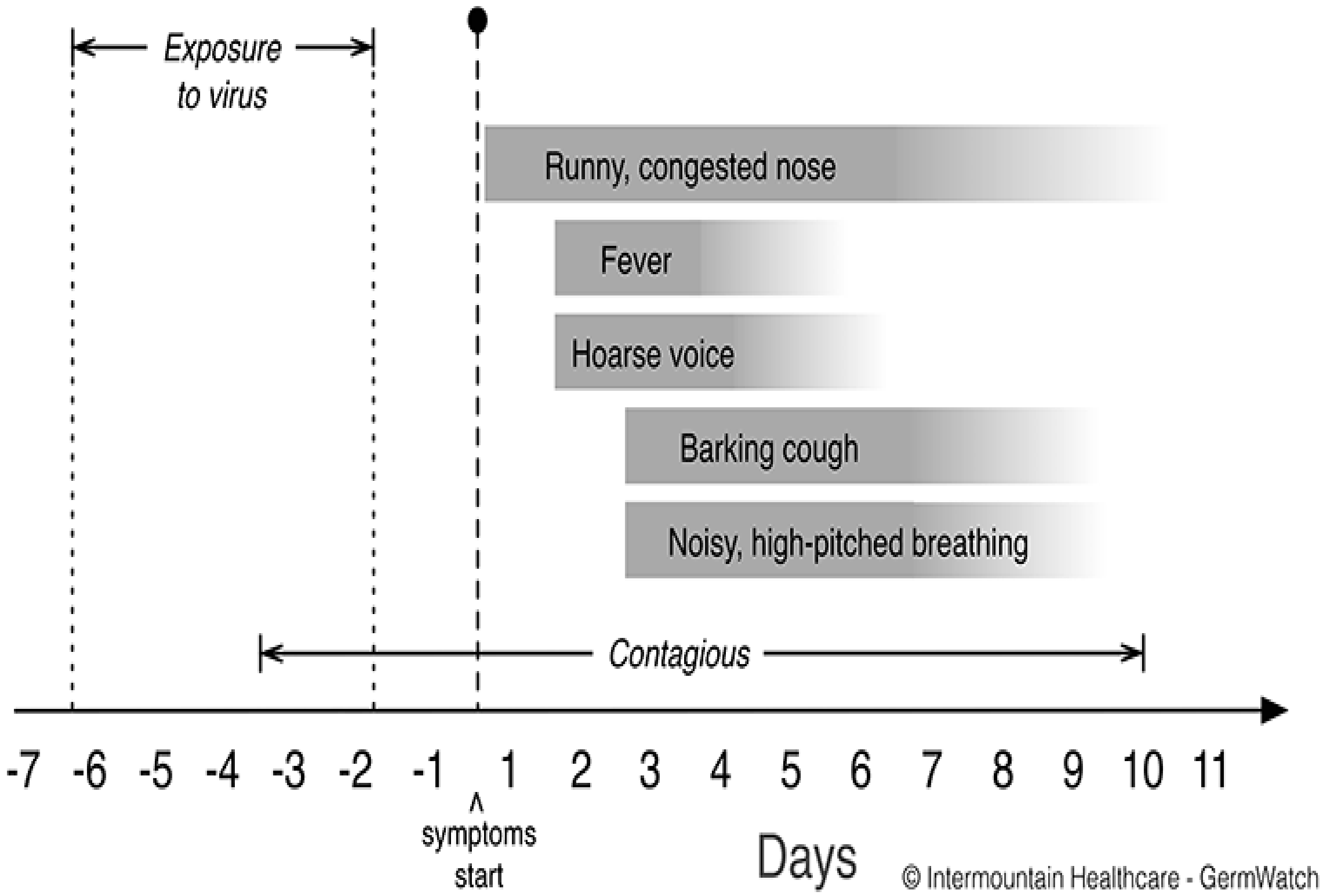


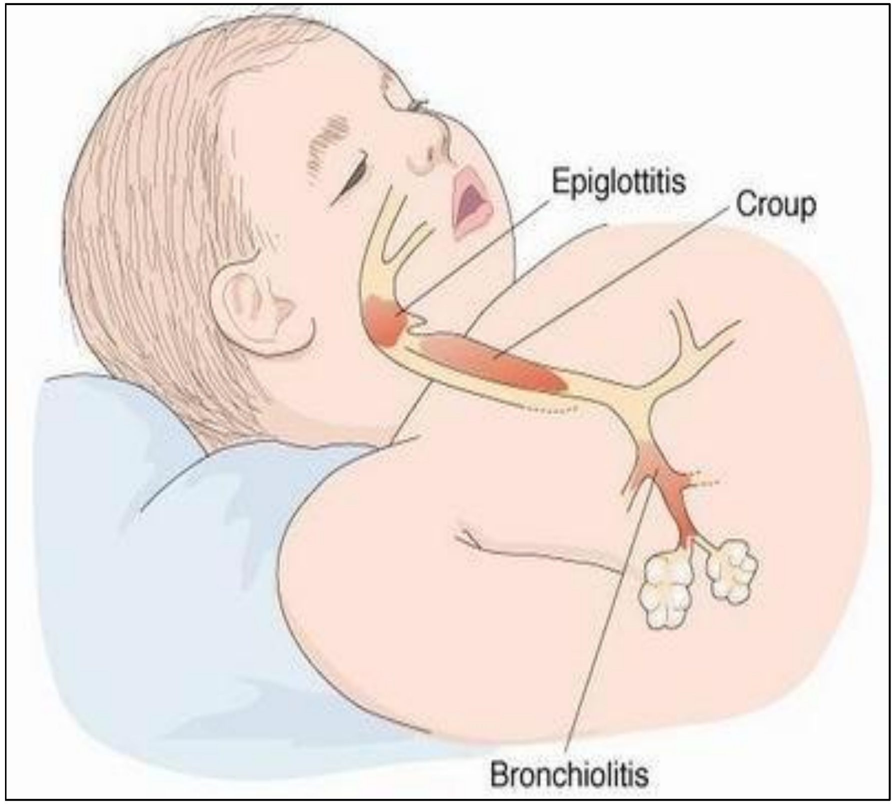
Clinical features of parainfluenza (PIV)

- The **source** of infection is **patient**. Infection occurs mainly through **airborne droplets.**
- Incubation period is 2 to 6 days.

- **Clinical symptoms:**
- Rhinitis, pharyngitis, cough, fever, croup (laryngotracheobronchitis), bronchiolitis, and pneumonia.
- **<u>Croup</u>** - the subglottic region becomes narrower and results in difficulty with breathing, a seal bark-like cough and hoarseness.
- **<u>There is clinical variation between the different PIV types.</u>**
- **PIV-1 and 2:** croup in children ages 2-6 years in autumn/early winter.
- **PIV-3:** bronchiolitis and pneumonia, and croup sporadically, without a particular seasonal occurrence.
- **PIV-4:** mild upper respiratory infections.

Parainfluenza Virus Infection Timeline





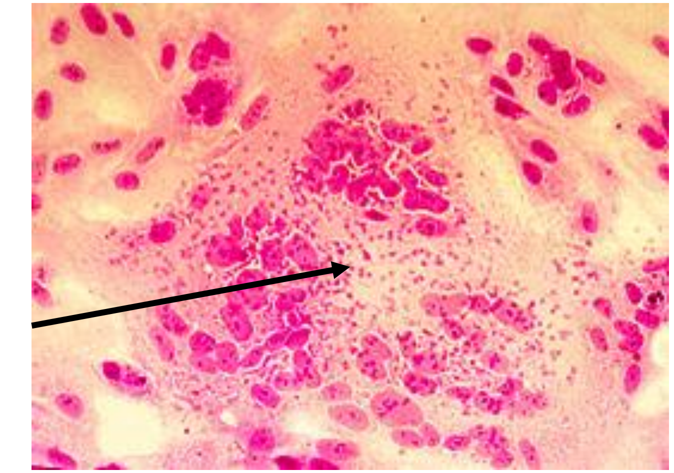
LABORATORY DIAGNOSIS OF PARAINFLUENZA

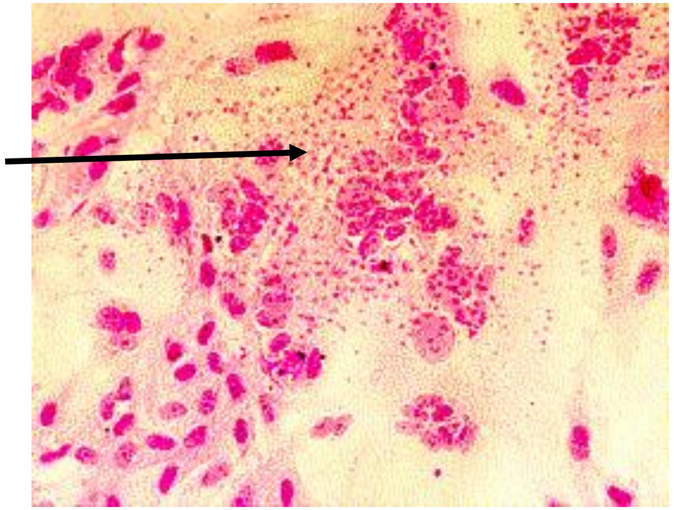
1. **Detection of antigen** from nasopharyngeal aspirates and throat swab **by IF and PCR**.

2. **The virus isolation** in cell culture.

Indication: Haemadsorption of erythrocytes on the surface of cells infected with virus.

Identification: **HadsI**, **HAI, NT, CFT.**

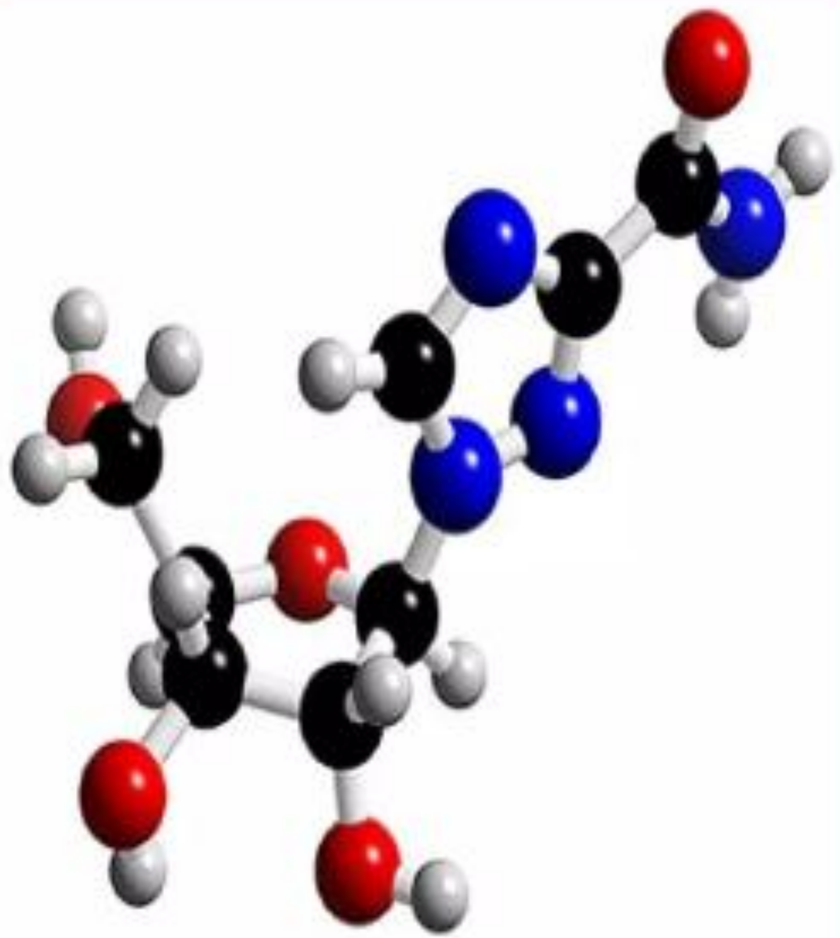




3. **Serology** – detection of rise in titer of IgG in paired sera:

NT, ELISA, CFT, **HAI.**

Treatment and Prophylaxis.

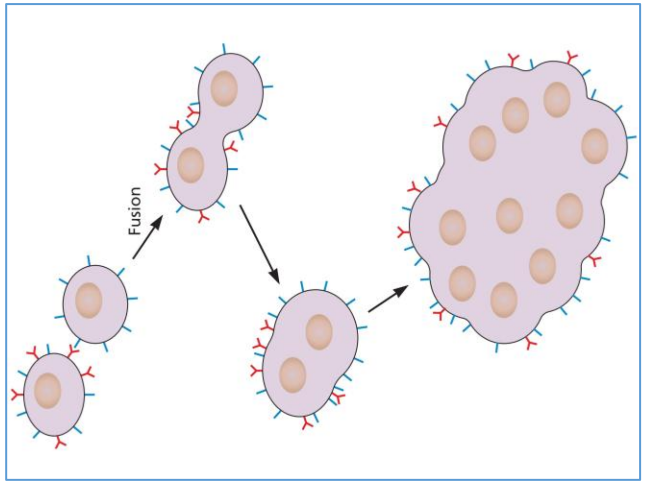


- Treatment with **Ribavirin**
- **No Vaccine to date.**

Respiratory syncytial virus (RS-virus)

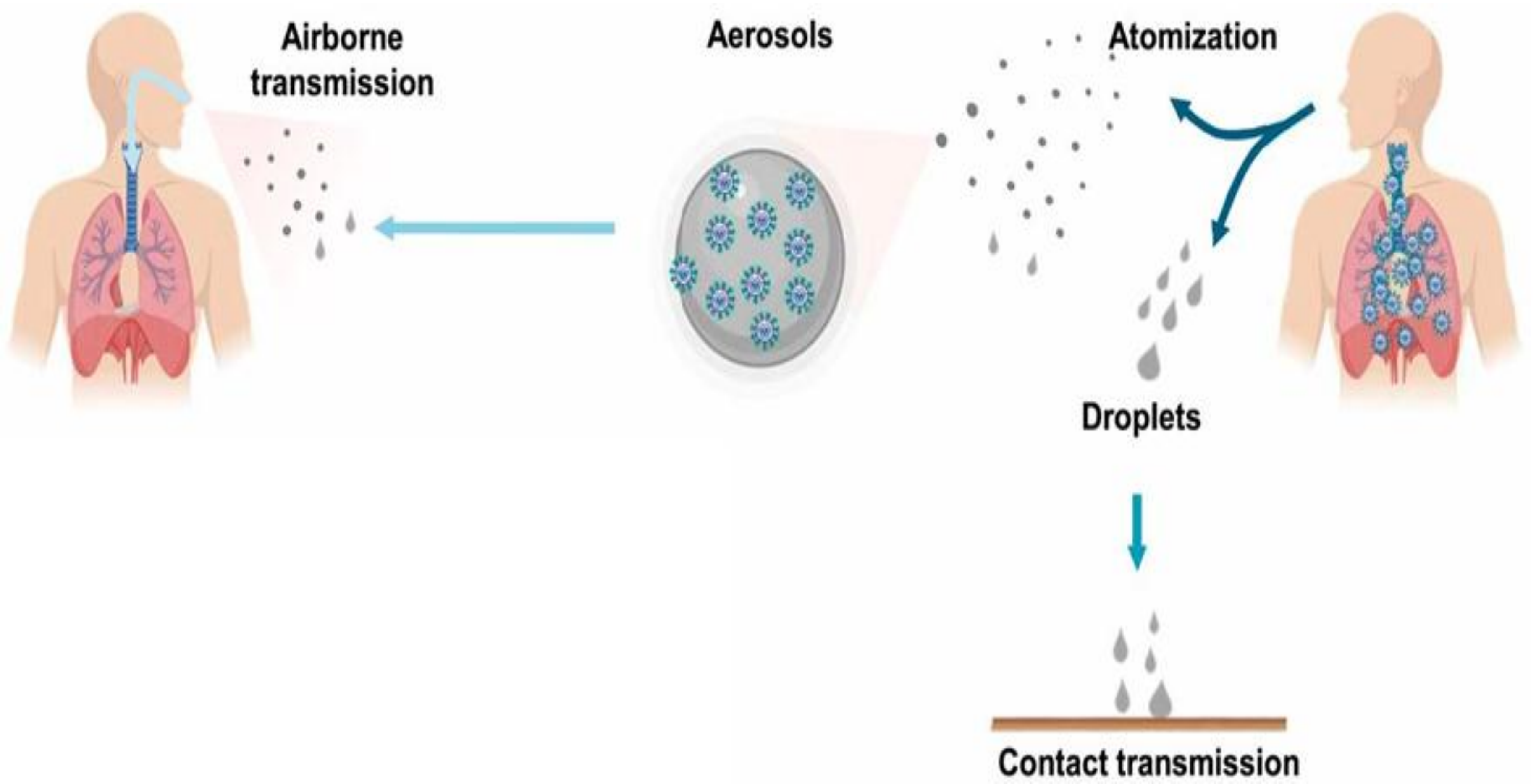
- RS-virus belongs to the ***Pneumovirus*** genus of the ***Paramyxoviridae*** family.
- RS-virus is distinguished by its **polymorphism**: in addition to the usual spherical forms, thread-shaped forms are also found. Large glycoprotein spikes in the lipoprotein membrane have no hemagglutinating and neuraminidase activity, so they are called **G glycoproteins**. This glycoprotein binds the virus to host cell receptors.
- F glycoproteins characteristic of RS-virus connect the membranes of neighboring cells, resulting in the formation of syncytium.
- RS-virus got its name due to the nature of cytopathic effect in cell culture - formation of **symplast** and **syncytium**.

Сейчас не удается отобразить рисунок.



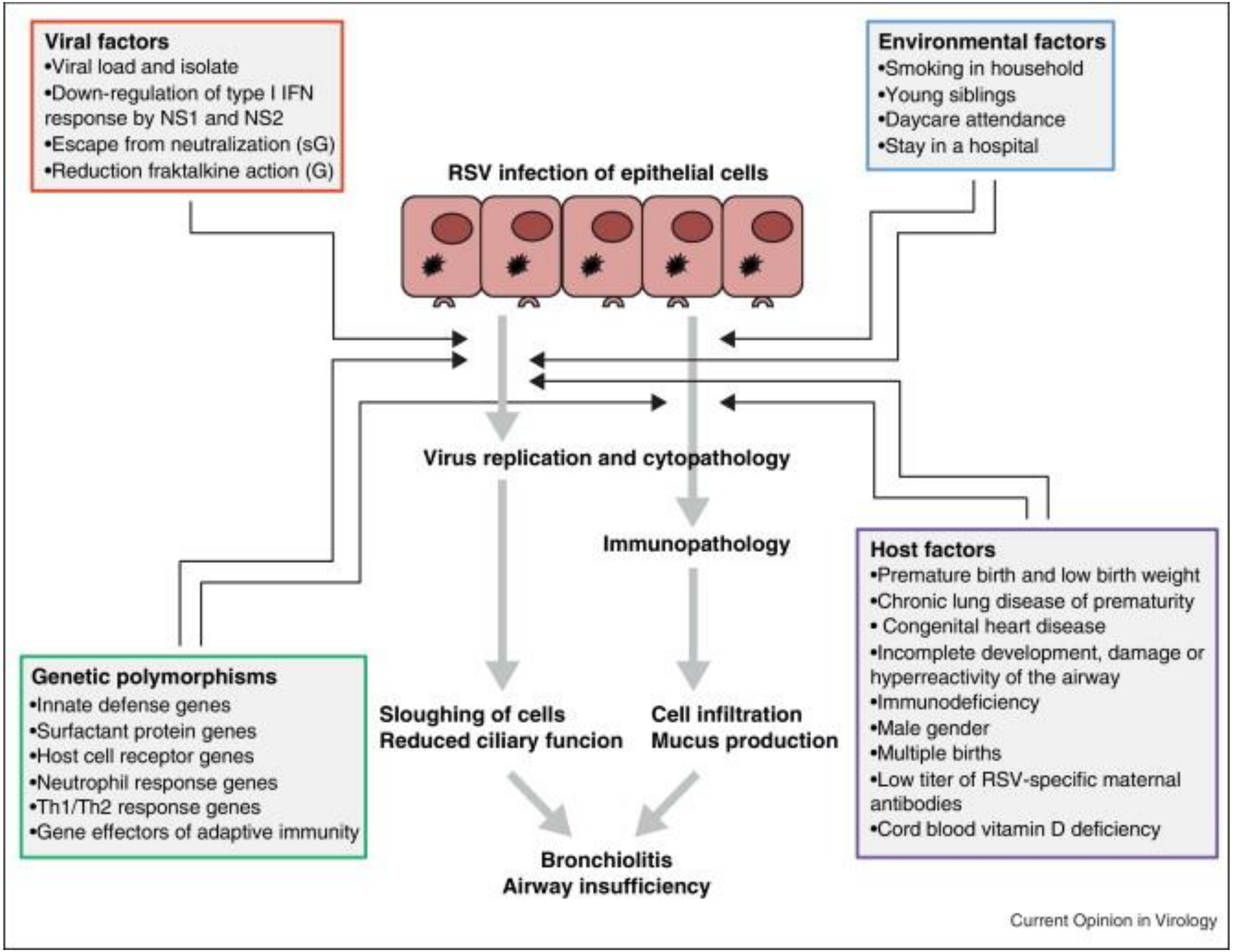
Mode of transmission of RS-virus infections:

- Infection occurs through **air-droplet**, as well as contact-household ways. The portal of the entry of the virus occurs in the mucous membranes of the upper respiratory tract - nasopharynx.



PATHOGENESIS

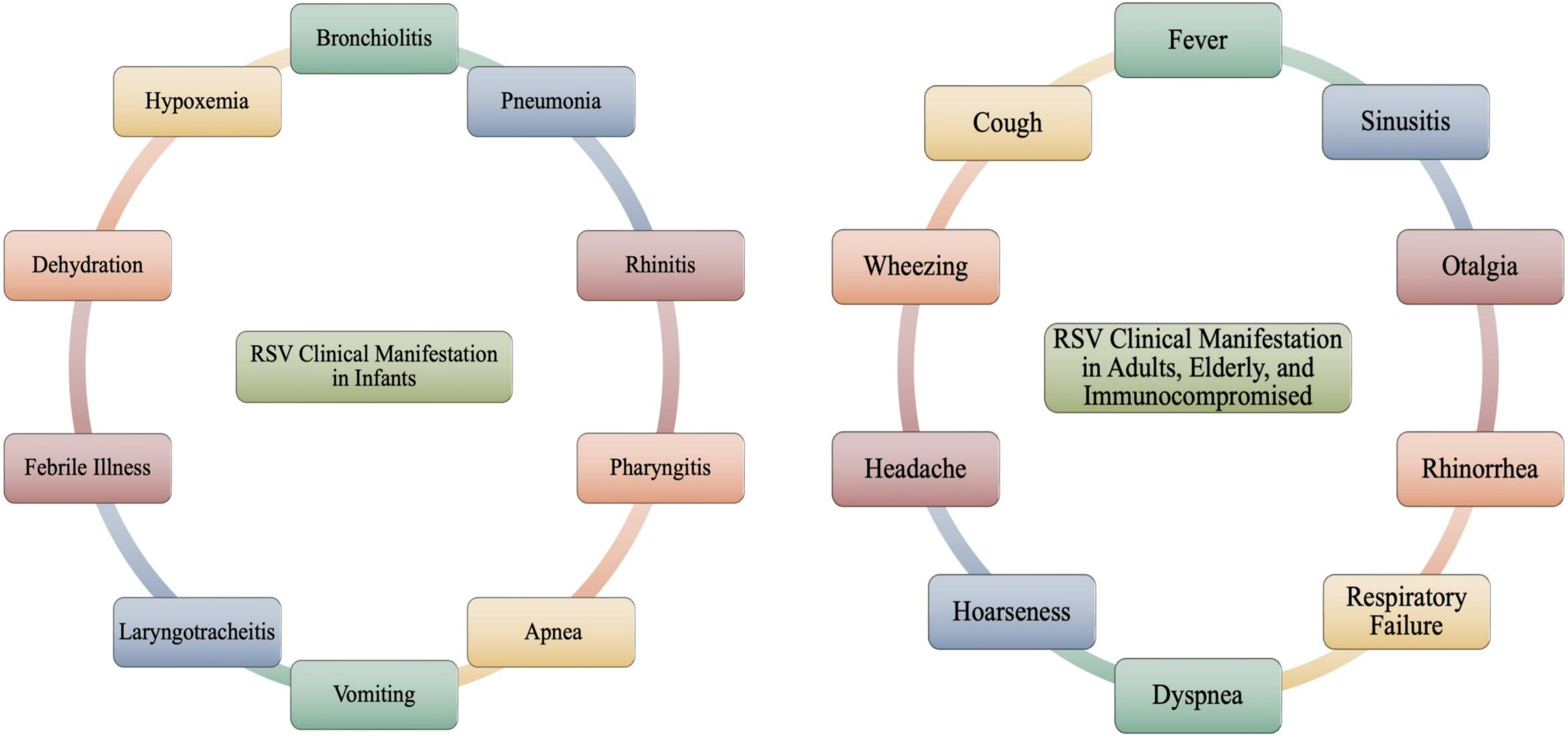
- The virus multiplies inside the epithelial cells, causing their destruction. The pathological process quickly spreads to the lower respiratory tract, causing bronchiolitis and pneumonia.
- Viremia is rarely observed.



Clinical manifestations of RS-virus infections:

- RS-virus is one of the most common causes of lower respiratory tract infections in infants and children. This virus is the leader among the microorganisms that cause **bronchiolitis** and **pneumonia** in children under one year of age.
- Clinical manifestations of RS-virus infections vary widely, from mild cold symptoms to pneumonia in infants and bronchiolitis in young children. After a latent period lasting 3-5 days, acute catarrhal symptoms of the upper respiratory tract first appear, followed by bronchiolitis and pneumonia. RS-virus is more dangerous for children up to 6 months old - they develop severe bronchiolitis and pneumonia.
- RS-virus infections can be complicated by inflammation of the middle ear. About half of **otitis** in infants are accompanied by RS-virus infections.

Clinical manifestations of RS-virus infections:



Microbiological diagnosis of RS-virus infections

Examination material - *nasopharyngeal lavage or nasopharyngeal swabs*

Virological

RS-virus can be obtained from nasal mucus of patients by virological method - by infecting **HeLa and Hep-2** cell cultures for this purpose. After 10 days of incubation in cell cultures, RS-virus induces a cytopathic effect by producing giant cells and **syncytium**.

Exspress diagnostics

It is possible to detect the RS-virus in materials taken from the nasopharynx with a swab by **IFR, ELISA** and **PCR**. Detection of the virus indicates current illness, as RS-virus is never found in healthy individuals.

Serological

A serological method based on the detection of specific antibodies in blood serum by **IFR, ELISA** and **NT** can be applied, but serological tests have little diagnostic value, they are mostly used in **epidemiological** studies.

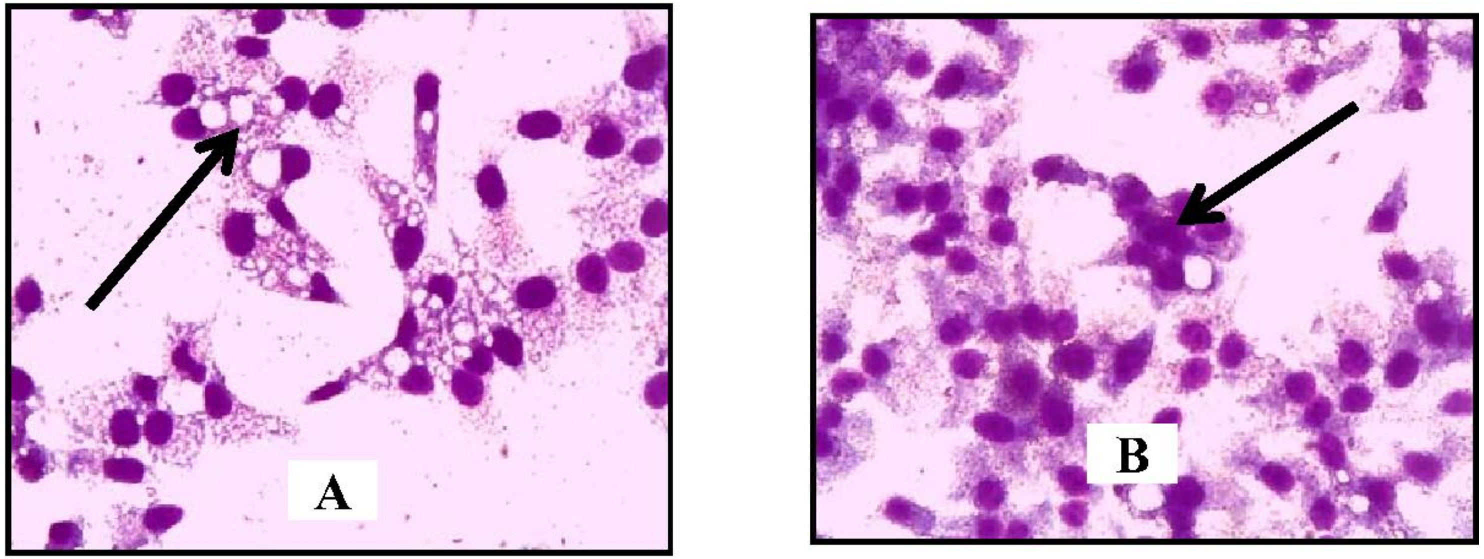


Figure 1: Cytopathic effects in HEp-2 cell line infected with the isolated HRSV, 5 days

TREATMENT

- A supportive management with tube feeding in cases of difficulty in suckling
- Use of oxygen if indicated.
- **Ribavirin** is a specific antiviral drug, proved to effective when given as a small particle aerosol although it is apparently not effective intravenous infusion.

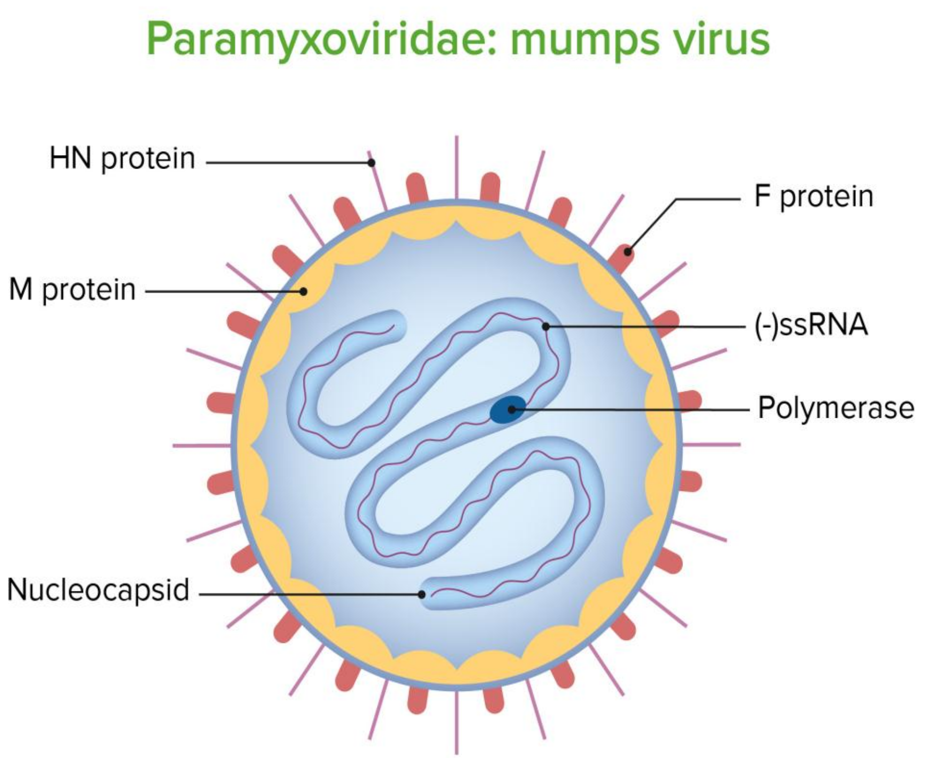
VACCINE - FAILURES

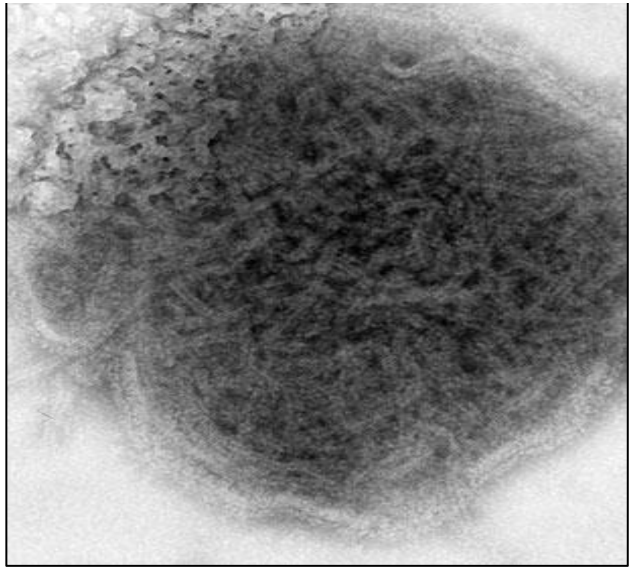
- **A formalin inactivated crude, whole virus vaccine was tried in 1960, but failed to produce immunity in the recipients**
- The difficulties in preparing safe vaccine for RSV lie with young and immunologically immature recipients.
- **Yet to date there is no safe vaccine available for universal use**

MUMPS

MUMPS VİRUS

- Mumps virus belongs to the ***Rubulavirus*** genus of the ***Paramyxoviridae*** family.
- The outer membrane of the virus contains **HN-** and **F**-glycoproteins. Therefore, the virus, having hemagglutinating activity, can be used in chicken, guinea pig, etc. causes erythrocytes to agglutinate. F-glycoproteins bind the membranes of host cells, that is, they have symplast formation and also hemolytic activity.
- Mumps virus can be **cultivated** in monkey kidney cell culture. The cytopathic effect is manifested by the formation of convoluted **giant cells**



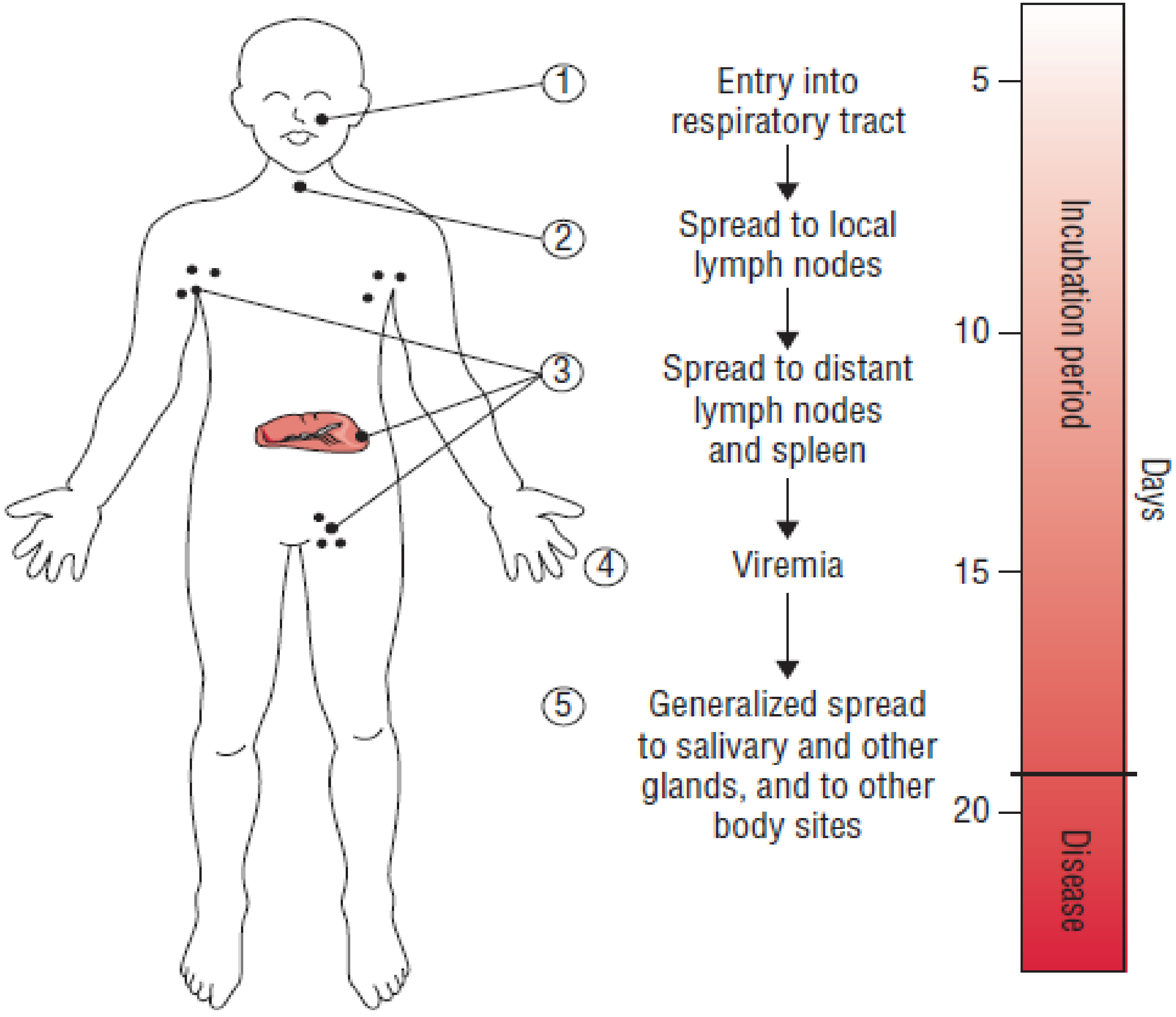


PATHOGENESİS

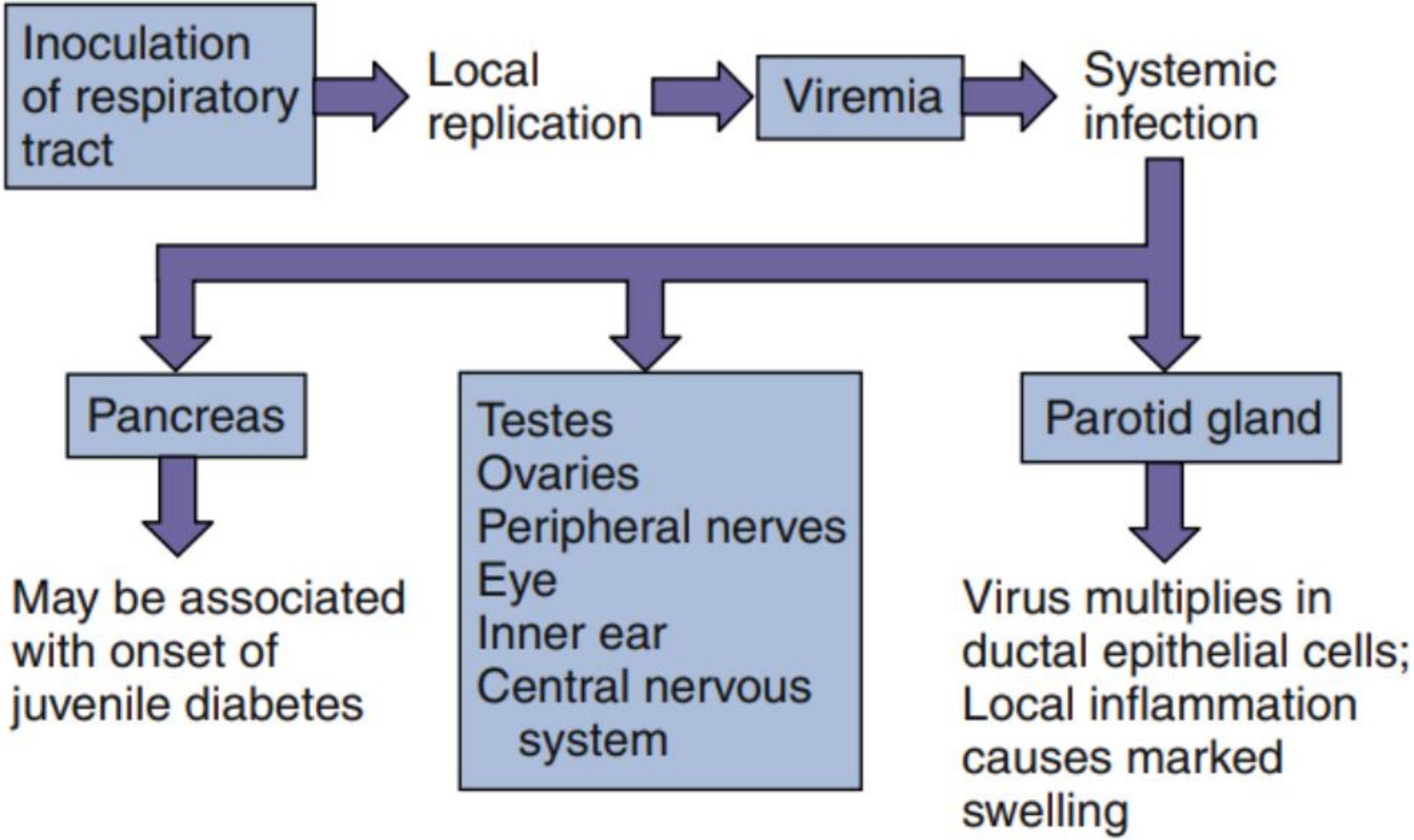
•Children aged 5-15 years are more vulnerable. The virus is excreted in the mouth until the 9th day of the disease, including the last three days of the incubation period. The disease is transmitted by **airborne droplets**, sometimes by **contact** with objects contaminated with saliva.

•Mumps is an **acute childhood infection** characterized by damage to the **parotid glands** and sometimes other organs. The virus multiplies in the epithelium of the mucous membranes of the upper respiratory tract, passes into the blood, spreads throughout the body, and enters the **salivary glands.**

•The virus infects testicles, ovaries, pancreas, thyroid gland, meninges and other organs and causes inflammation.



PATHOGENESİS



Mechanism of spread of mumps virus within the body.

Clinical manifestations of mumps

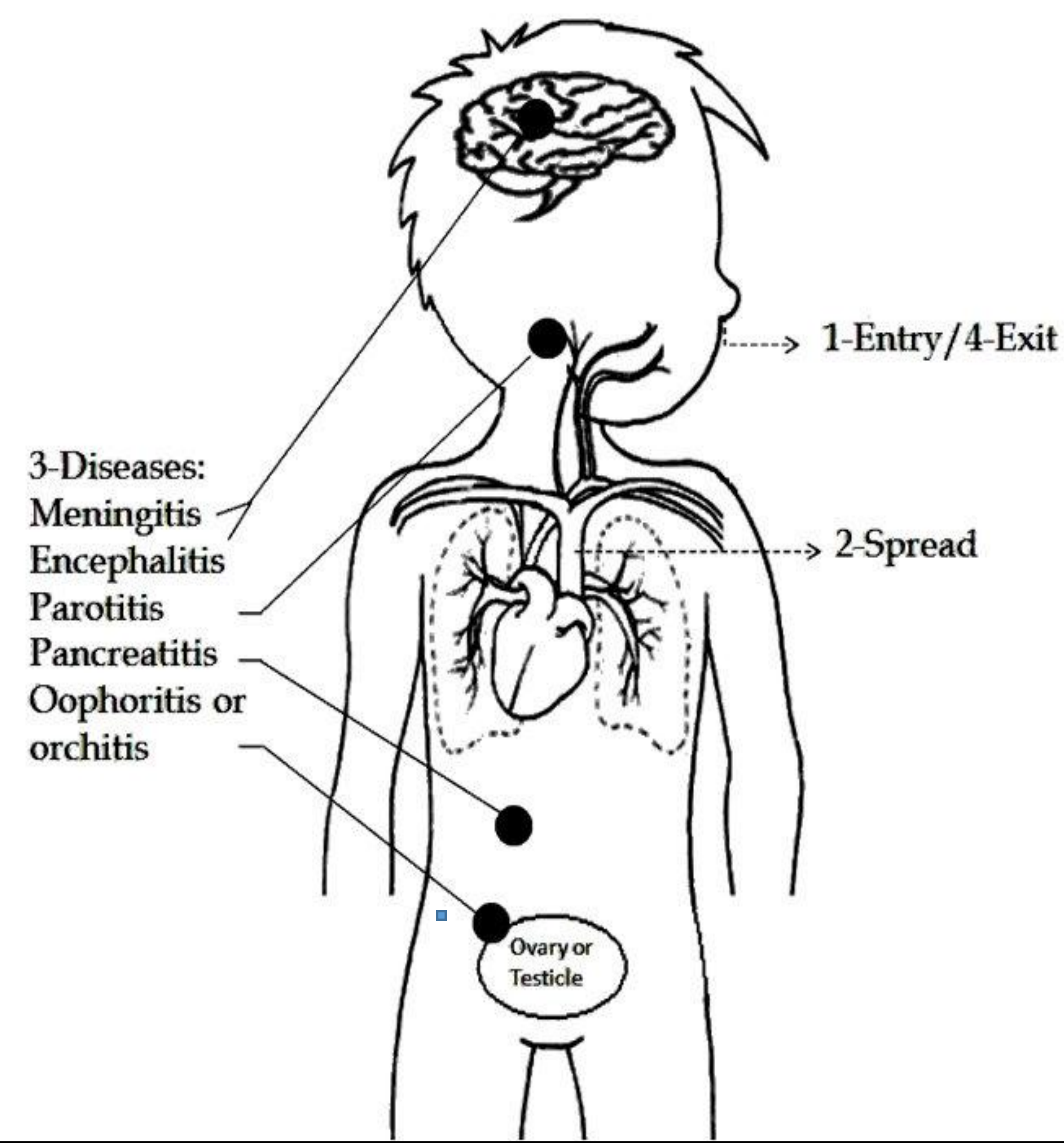
•The **latent period** lasts 2-4 weeks. The most characteristic symptom of the disease is the **swelling and pain of the parotid glands**. Swelling of one or both of the parotid glands gives the patient a characteristic appearance ***(Swine`s face).*** Other salivary glands can also be involved in the pathological process. In about one third of patients, parotitis has an asymptomatic course

•After puberty, mumps can be complicated by inflammation of the testicles **(orchitis)** in boys, and inflammation of the ovaries **(oophoritis)** in girls.

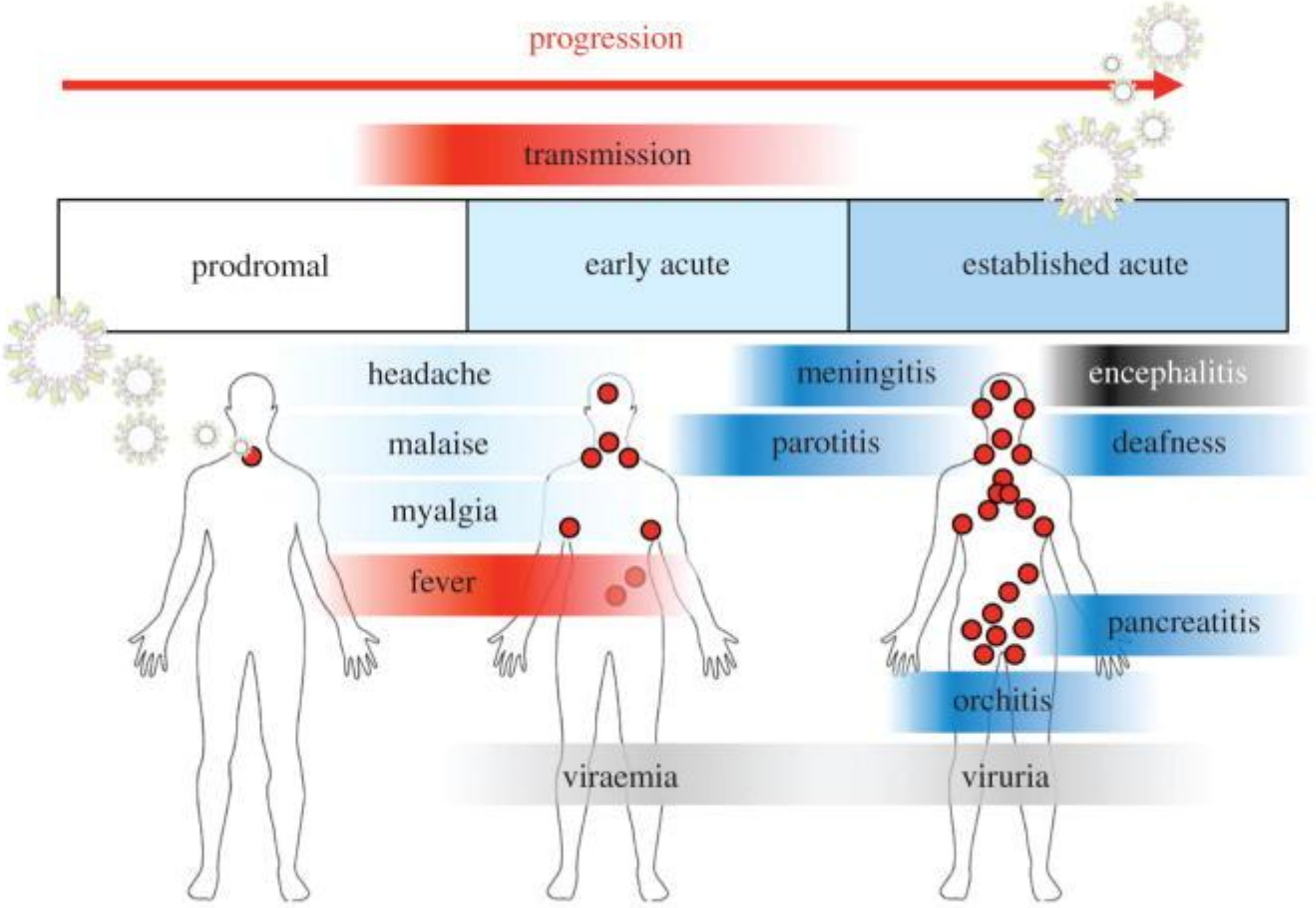
•**Aseptic meningitis and meningoencephalitis** are the most common complications of mumps.

•Mumps can be complicated by pancreatitis in about 4% of cases.

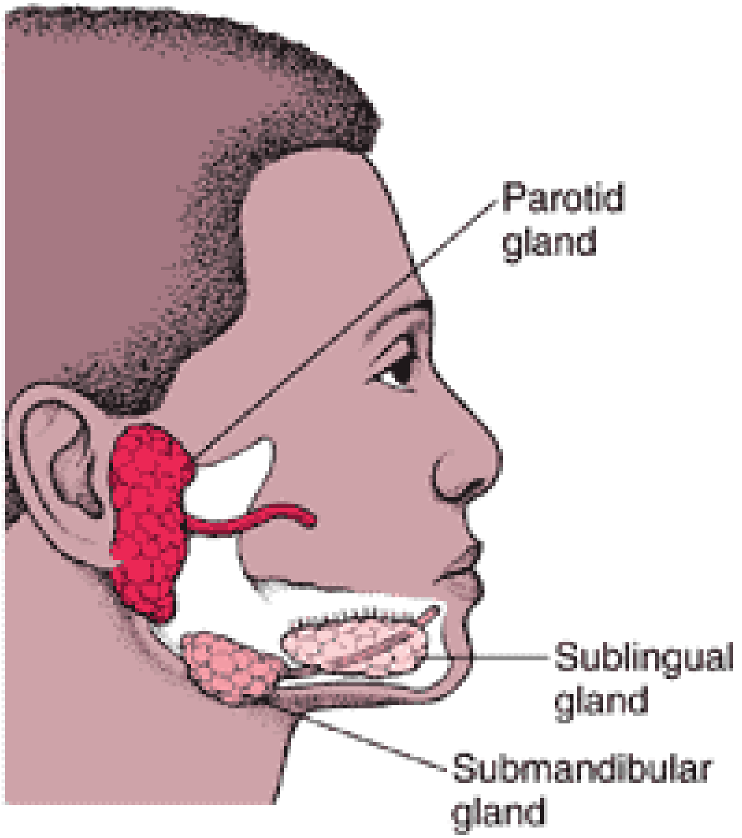
•**Lifelong immunity** is formed after the disease.

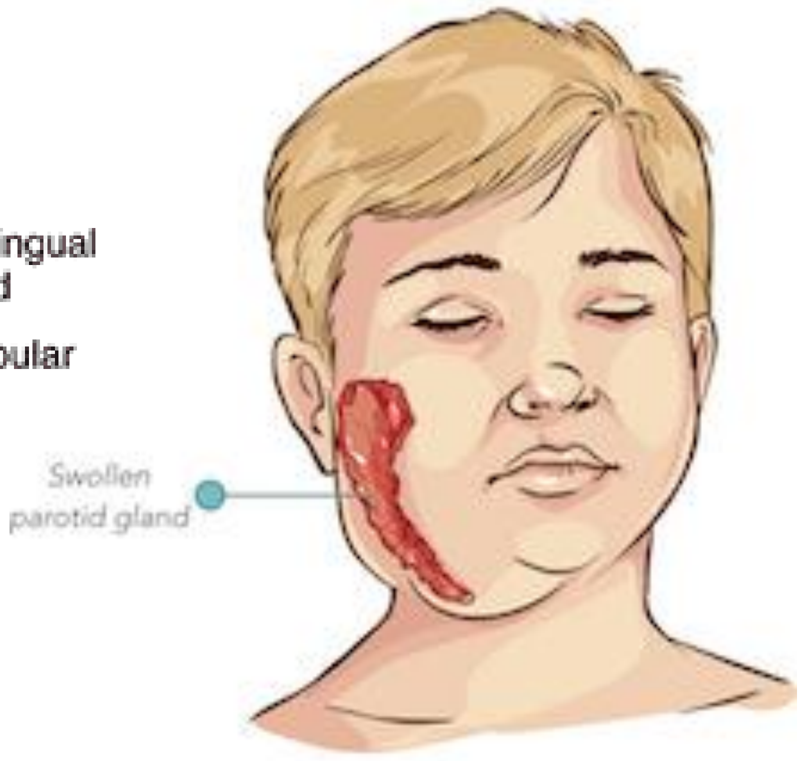


Clinical manifestations of mumps

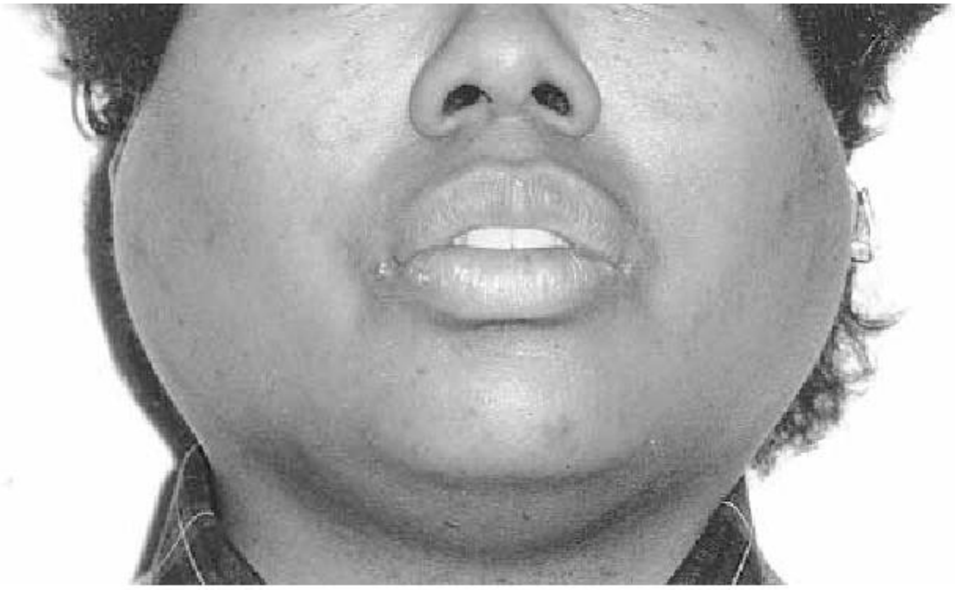


MUMPS





Characteristic mumps symptom



Swine`s face

Complications of mumps

- **Orchitis - 20-50 %**
- Meningitis and meningoencephalitis - 15 %
- Ovaritis - 5 %
- Pancreatitis - 2-5 %
- **Rare complications:** polyarthritis, diabetes, nephritis, thyroiditis, deafness, myocarditis.

Laboratory Diagnosis

- No Laboratory confirmation needed.
- Atypical infection needs laboratory Diagnosis.
- Virus isolated from

  Saliva

  Urine

  CSF.

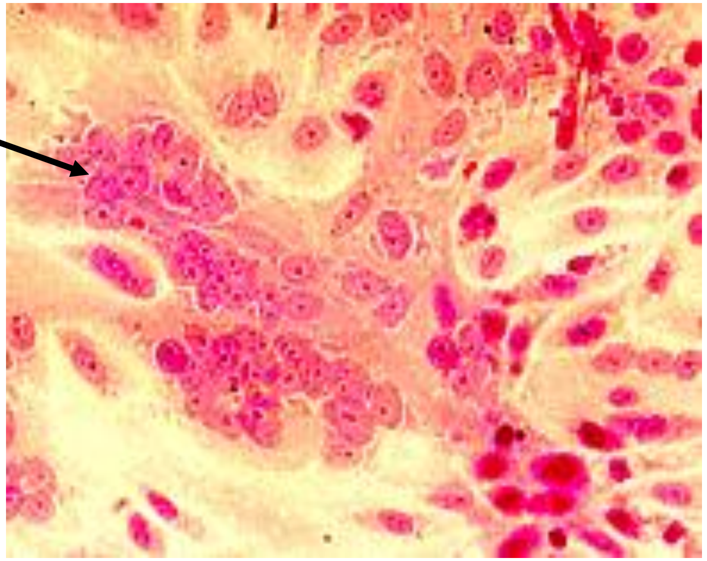
LABORATORY DIAGNOSIS OF MUMPS

1. **The virus isolation** from the saliva, liquor or urine in cell culture (or chicken eggs).

   CPE: giant multinucleated cells formation.

   Identification:

   HAI, NT, IF, CFT.



2. **Serology**: **HAI, NT, ELISA, CFT** (demonstrating IgM in the first serum and detecting IgG rise in paired sera).

3. **Molecular-genetic: PCR**

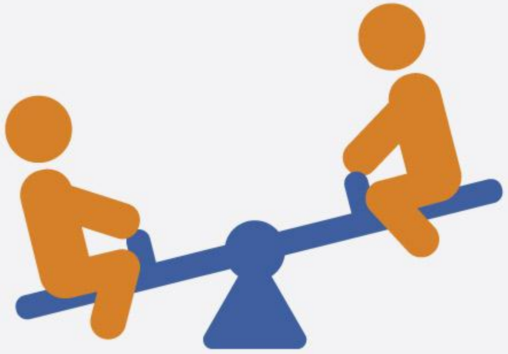
Treatment

- There is no medication to treat mumps so **self-care techniques are used** e.g. bed rest, painkillers, plenty of water, a compress for swollen glands and soft food
- **Good hygiene** and staying away from others is important to prevent the spread of mumps

***Specific immunoglobulin** can be used to ensure a mild course of the disease.*

MMR VACCINE

Protecting children and adults from measles, mumps and rubella



FIRST DOSE
12-15
months old

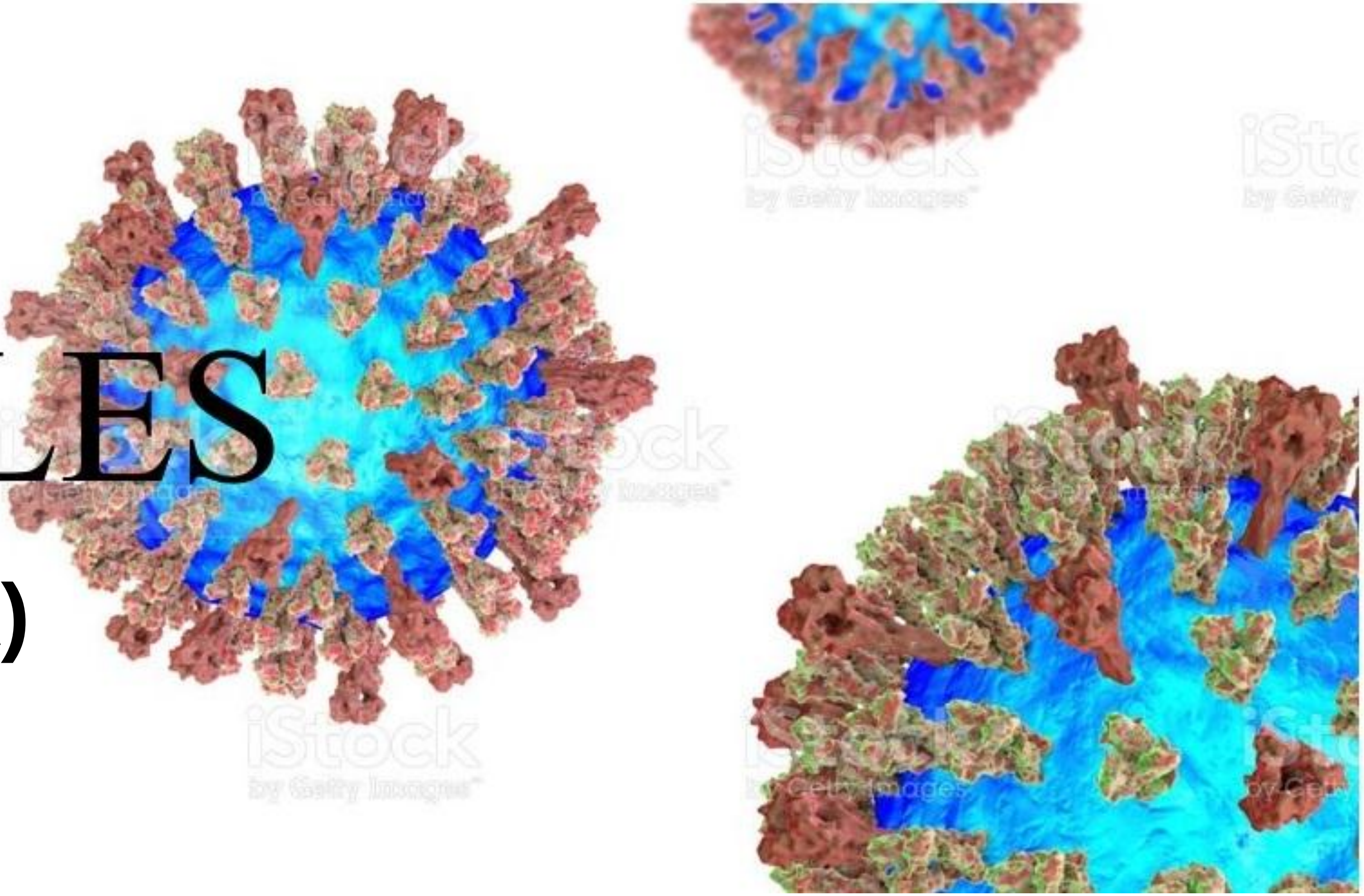
SECOND DOSE
4-6
years old

Percentage of children protected after 2 doses:

MEASLES **97%**
MUMPS **88%**
RUBELLA **97%**

MEASLES

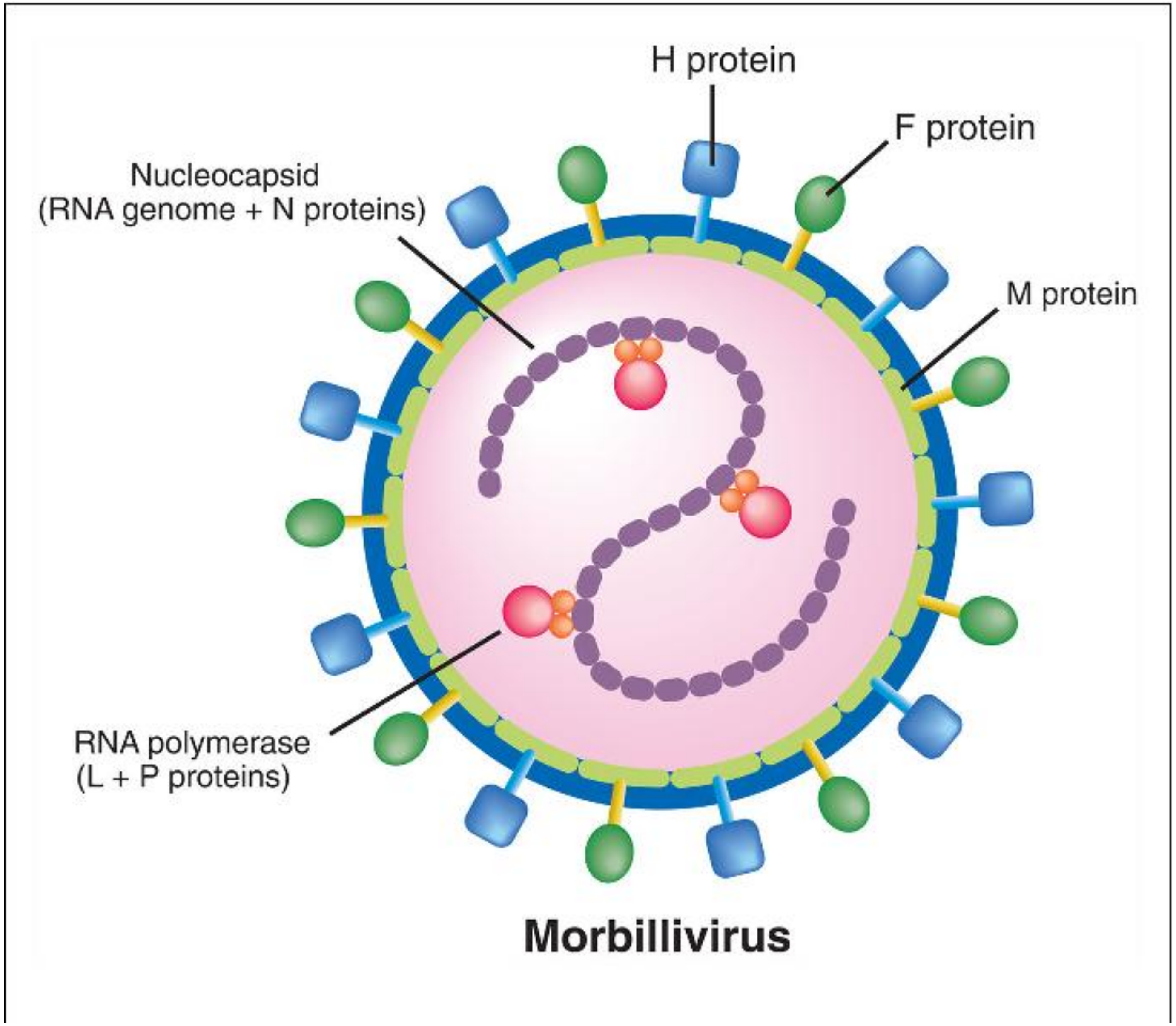
(RUBEOLA)



Measles	**Virus classification**	
	**Group:**	Group V ((-)ssRNA)
	Order:	*Mononegavirales*
	Family:	*Paramyxoviridae*
Measles virus	**Genus:**	*Morbillivirus*

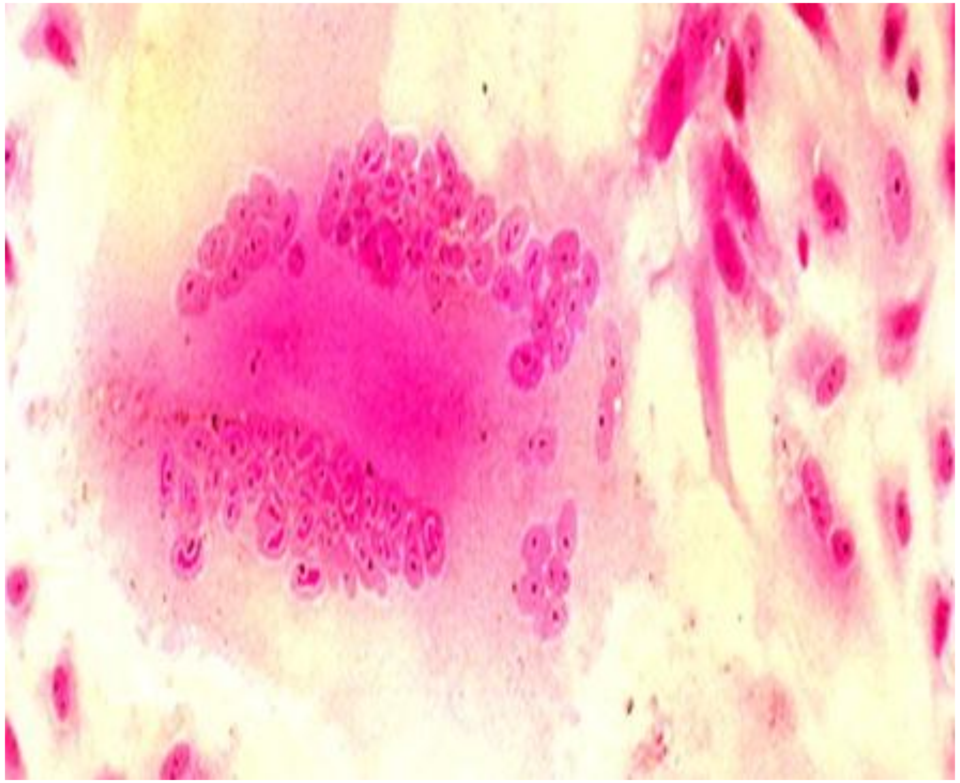
MEASLES (RUBEOLA) VİRUS

- **Enveloped**, pleomorphic spheres 100-300 nm diameter.
- Virions have an inner **helical** nucleocapsid that is a coiled helix of protein and RNA. Envelope has **hemagglutinin (H)** and **fusion (F)** glycoprotein spikes.
- Nucleic acid: **non-segmented, single-stranded, negative-sense RNA virus.**



MEASLES (RUBEOLA) VİRUS

- Measles virus is **cultured** in primary monkey and human kidney cell cultures. At this time, it causes a cytopathic effect with the formation of multinucleated giant cells - symplasts with intranuclear and cytoplasmic inclusions. Unlike other paramyxoviruses, it forms **intranuclear inclusions**.
- Measles virus is very **unstable** in the environment, it is inactivated after 3-4 hours at room temperature.



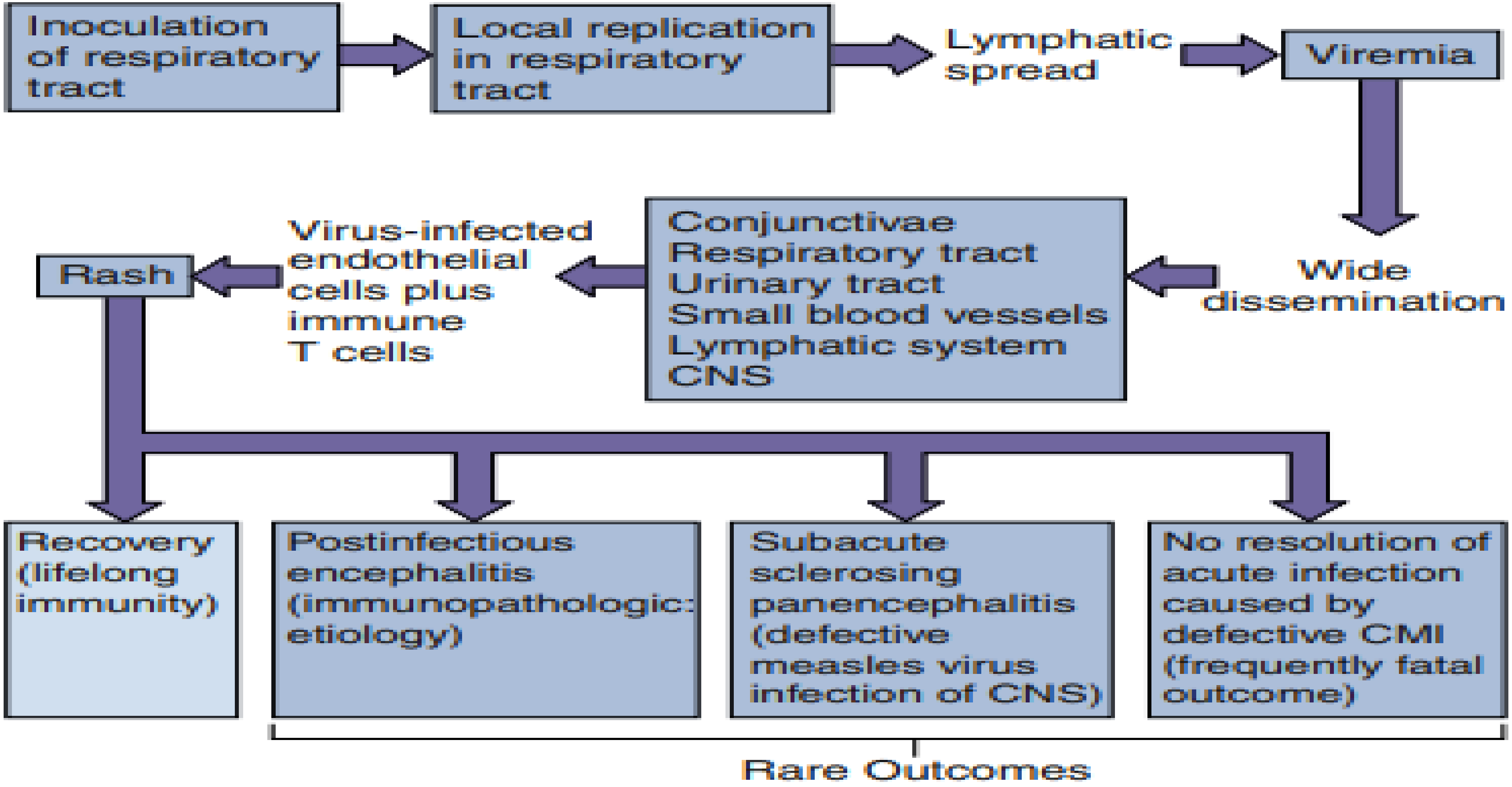
EPIDEMIOLOGY

- Infection sources
  - Patients of acute stage and viral carriers of atypical measles
- Transmission
  - Highly contagious, approximately 90% of susceptible contacts acquire the disease.
  - Respiratory secretions: maximal dissemination of virus occurs by droplet spray during the prodromal period (catarrhal stage).
  - Contagious from 5 days *before symptoms*, 5 days *after onset of rash*
  - Seasons: in the spring, peak in Feb-May

PATHOGENESIS AND PATHOLOGY

- Portal of entry
  - Respiratory tract and regional lymph nodes
  - Enters bloodstream (primary viraemia) ➔ monocyte – phagocyte system ➔ target organs (secondary viraemia)
- Target organs
  - The skin; the mucous membranes of the nasopharynx, bronchi, and intestinal tract; and in the conjunctivae, ect

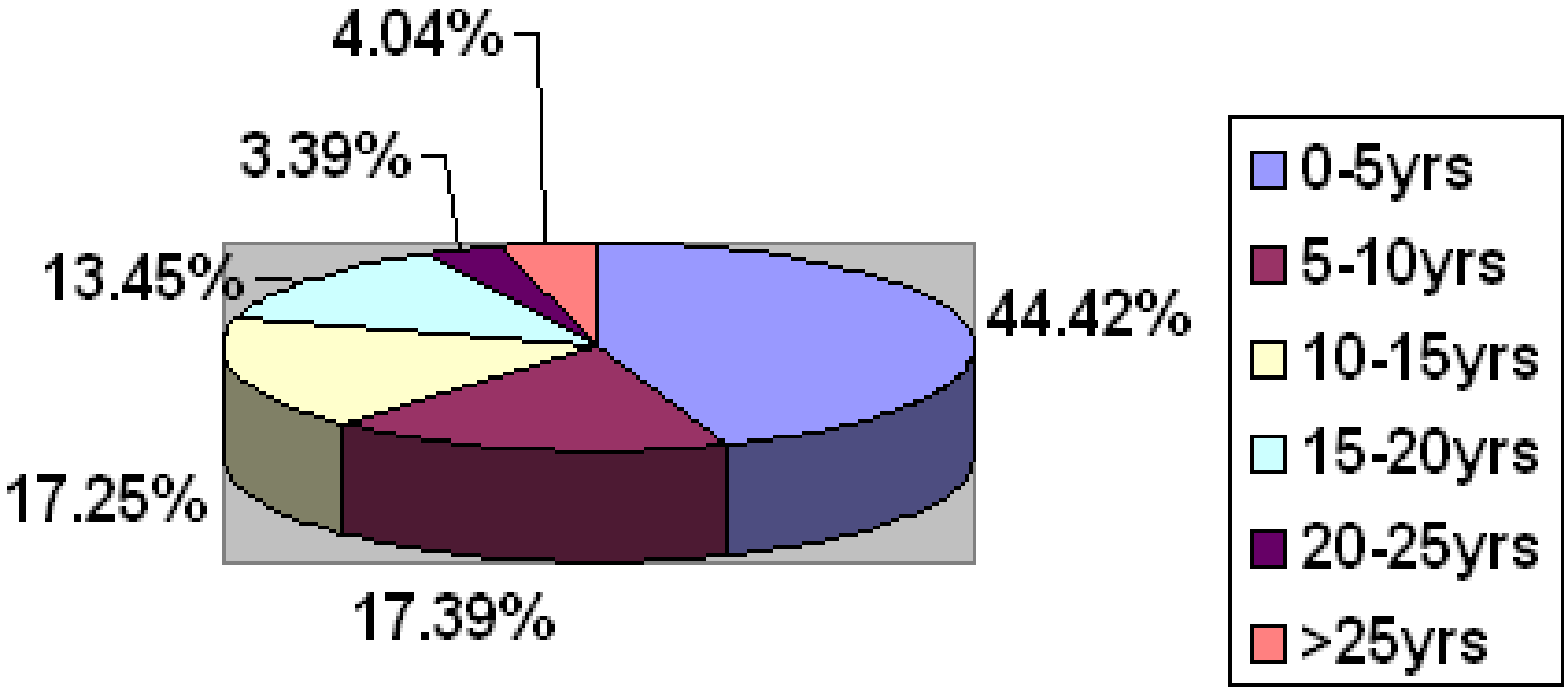
PATHOGENESIS



Mechanisms of spread of the measles virus within the body and the pathogenesis of measles. *CMI,* Cell-mediated immunity; *CNS,* central nervous system.

Age distribution of measles cases

- Measles is one of the most contagious of all human viruses, with about **40 million infections world wide each year, and one to two million deaths.**



CLINICAL MANIFESTATION

- **Measles** - a systemic infection, disseminated by viremia, with acute disease manifestations involving the lymphatic and respiratory systems, the skin, and sometimes the brain.

1. Incubation period (infection to symptoms) :

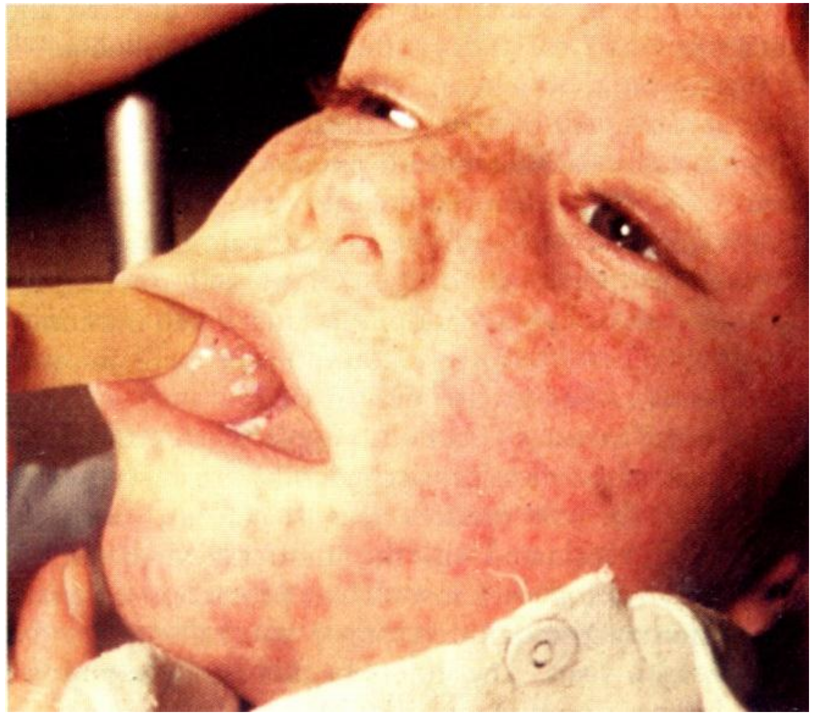
6-18days (average 10 days)

2. Prodromal period:

- 3-4 days
- Non-specific symptoms: fever, malaise, anorexia, headache
- Classical triad: cough, coryza, conjunctivitis (with photophobia, lacrimation)

Enanthem (Koplik spots):

- ***Pathognomonic for measles***
- 24-48 hr before rash appears
- 1mm, grayish white dots with slight, reddish areolae
- Buccal mucosa, opposite the lower 2nd molars
- increase within 1day and spread
- fade soon after rash onset



3. Rash period

3-4days

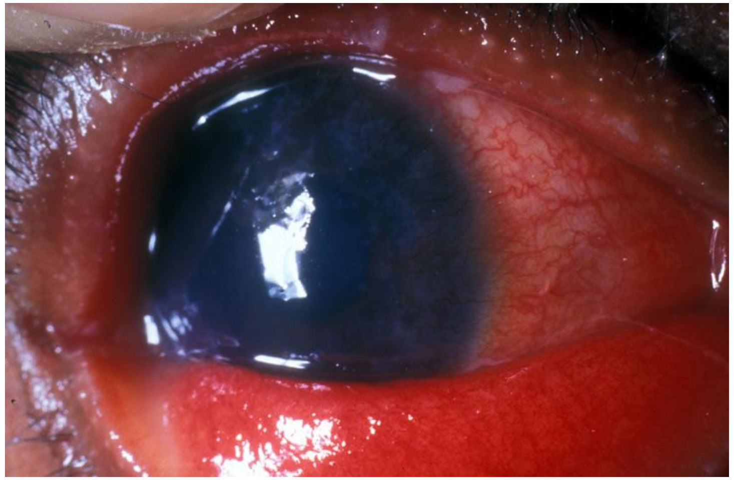
Exanthem:

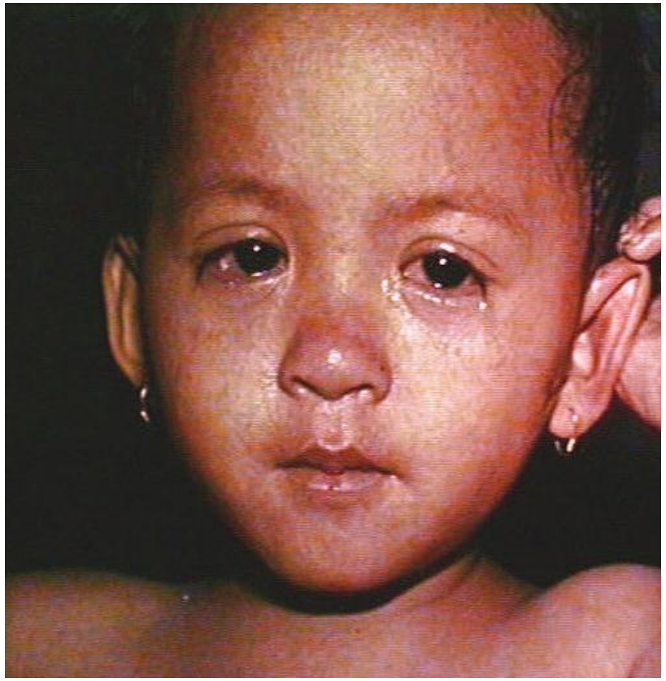
Erythematous, non-pruritic, maculopapular

- Upper lateral of the neck, behind ears, hairline, face → trunk → arms and legs →feet

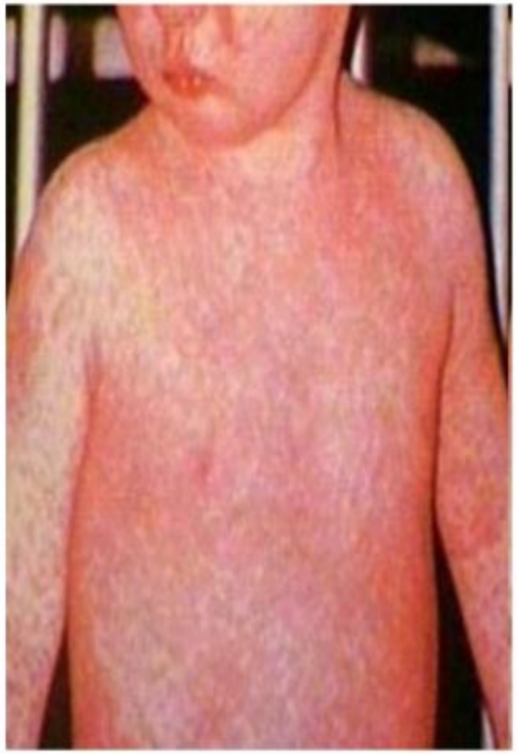
- The severity of the disease is directly related to the extent and confluence of the rash

Conjunctivitis





Maculopapular rash



CLINICAL MANIFESTATION

4. Recovery period

3-4days

Exanthem:

- Fades in order of appearance
- Branny desquamation and brownish discoloration

Entire illness – 10 days

Complications:

- **Pneumonia**, **otitis** media, optic neuritis
- Encephalitis, **subacute sclerosing panencephalitis (SSPE)**
- Hemorrhagic measles

Post - Measles Encephalitis

Immunity

- Infection confers **lifelong immunity** (after measles, permanent humoral immunity is formed). Recurrence is rarely observed.
- **Passive immunity** transmitted through the placenta in the form of IgG protects the child for 6 months after birth.
- Cell-mediated immunity causes rashes.
- Measles infection is accompanied by immunosuppression, which results in complications.

DIAGNOSIS

characteristic clinical picture:

- Measles contact
- Koplik spot
- Features of the skin rash
- The relation between the eruption and fever

Laboratory confirmation is rarely needed

LABORATORY DIAGNOSIS OF MEASLES

Clinically Koplick's spots are pathognomonic.

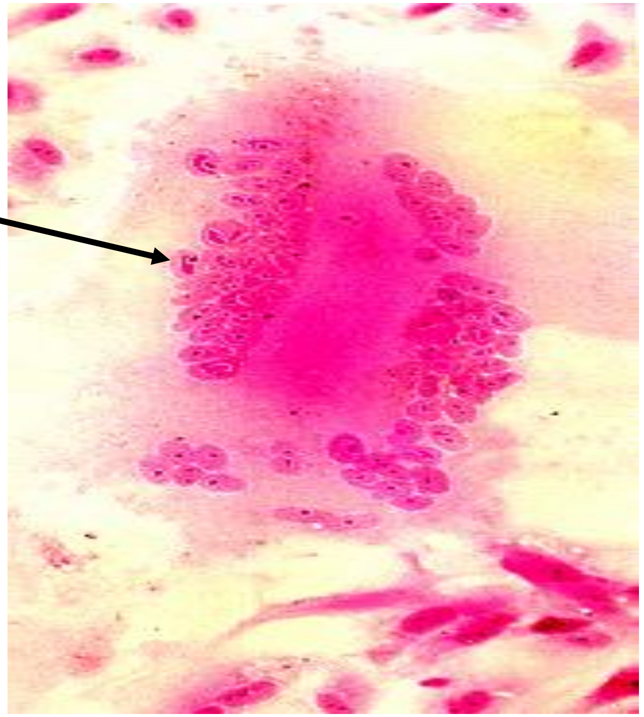
1.**Detection of antigen** from nasopharyngeal aspirates and throat swab **by IF**.

2. **The virus isolation** in cell culture.

CPE: giant multinucleated cells formation.

Identification: **HAI, IF, NT.**

3. **Serology**: **HAI, NT, CFT.**



TREATMENT

- Supportive, symptom-directed
  - Antipyretics for fever
  - Bed rest
  - Adequate fluid intake
  - Be protected from exposure to strong light
- Antibiotics for otitis media, pneumonia
- High doses Vitamin A in severe/ potentially severe measles/ patients less than 2 years
  - 100,000IU—200,000IU

PREVENTION

- 1. Quarantine period

  5 days after rash appears, longer for complicated measles

- 2. Vaccine

  The initial measles immunization is recommended at 8mo of age

  A second immunization is recommended routinely at 7yr of age

- 3. Postexposure Prophylaxis

  Passive immunization with immune globulin (0.25mL/kg) is effective for prevention and attenuation of measles within 5 days of exposure.

Measles

IT ISN'T JUST A LITTLE RASH

Measles can be dangerous, especially for babies and young children.

Protect your child from measles

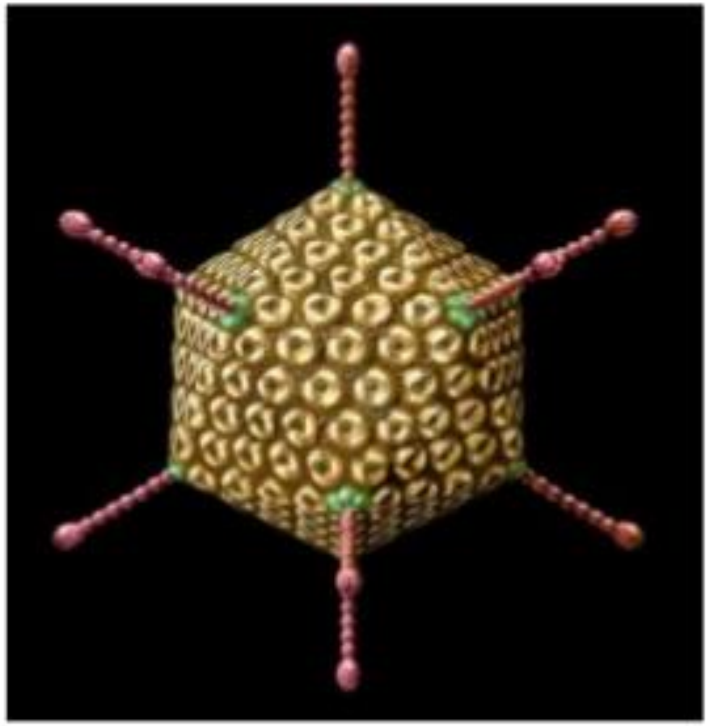
Give your child the best protection against measles with **two** doses of measles-mumps-rubella (MMR) vaccine:

1st dose at **12-15 months**

2nd dose at **4-6 years**

Since vaccinations began in 1963, cases of **MEASLES** have dropped by **99%**

ADENOVIRUSES

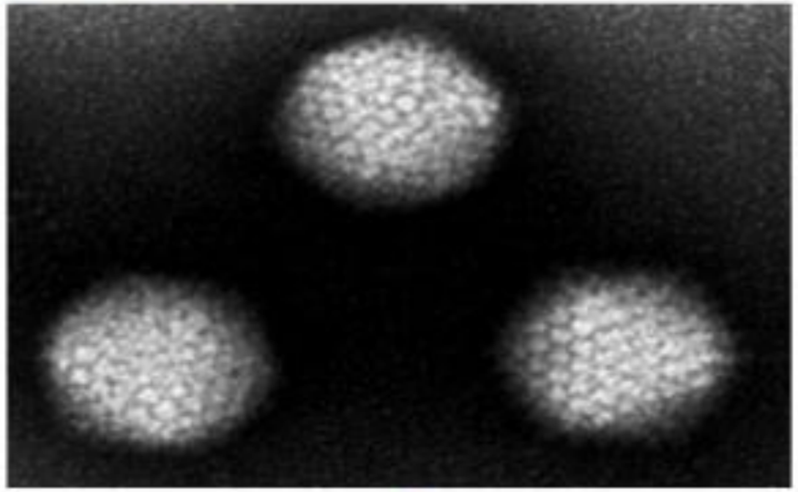


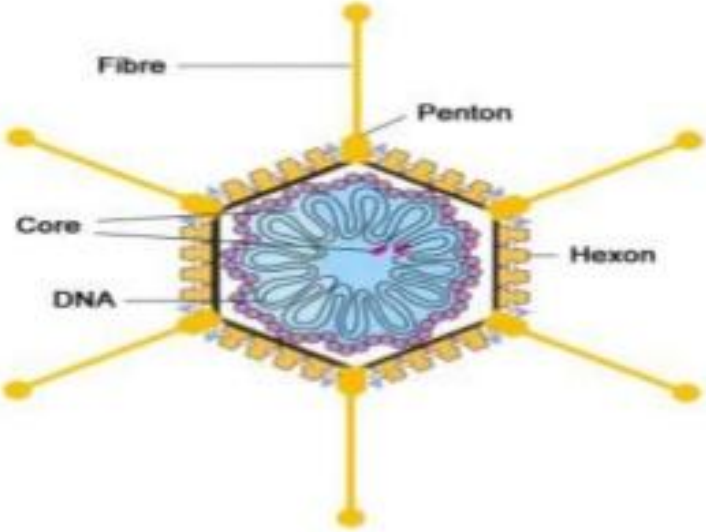
INTRODUCTION

- Adenoviridae is a group of medium sized, non-enveloped, double stranded DNA viruses that replicate and produce disease in the eye and in the respiratory, gastrointestinal and urinary tracts;
- Originally isolated from adenoids;
- At least 47 serotypes associated with human diseases;
- Most infections asymptomatic;
- Infections are common in children;

MORPHOLOGY

- Icosahedral, about 80-110 nm diameter;
- 252 capsomeres;
- Fibre projects from each of 12 vertices;
- Resemble a space ship;
- Genome is dsDNA;
- Replicates in nucleus;





CLASSIFICATION

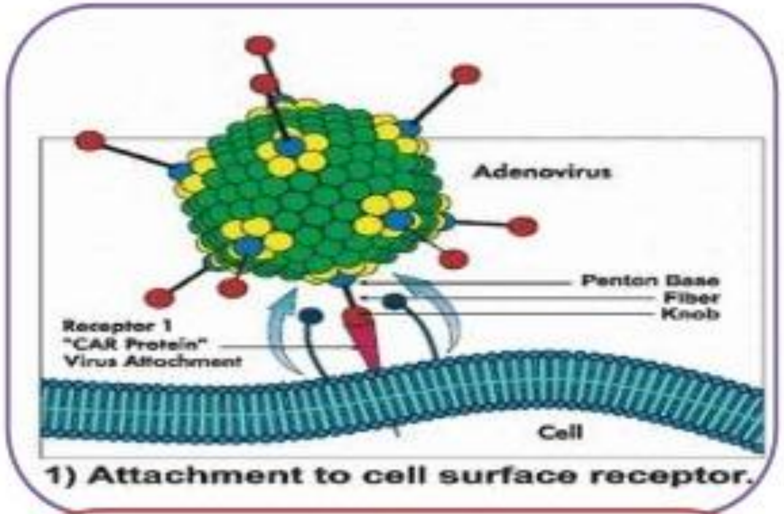
- Family Adenoviridae : 2 genera
  - Aviadenovirus : adenoviruses of birds;
  - Mastadenovirus : infect mammals;
    - **47 serotypes** of human origin;
    - **6 subgroups (A-F)** based on genome homology;
    - **Subgroup A : serotypes 12,18 and 31 : highly oncogenic;**

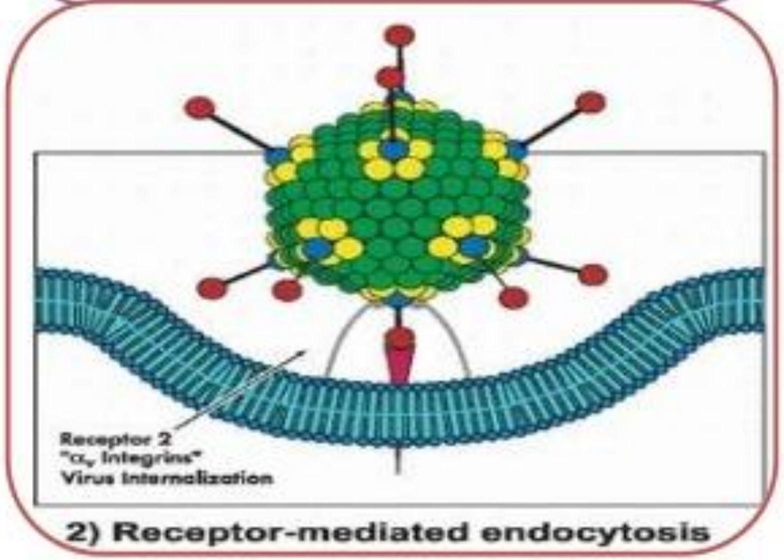
DISEASE ASSOCIATIONS

Serotype	Disease	At risk
1-7	**Acute febrile pharyngitis**	**Children**
3,7,14	Pharyngoconjunctival fever	Older children
8,9,37	**Epidemic keratoconjunctivitis (shipyard eye)**	**Adults**
3,4,11	Acute follicular conjunctivitis	Any age
40,41	**Diarrhoea**	**Infants, young children**
11,21	Hemorrhagic cystitis	Children

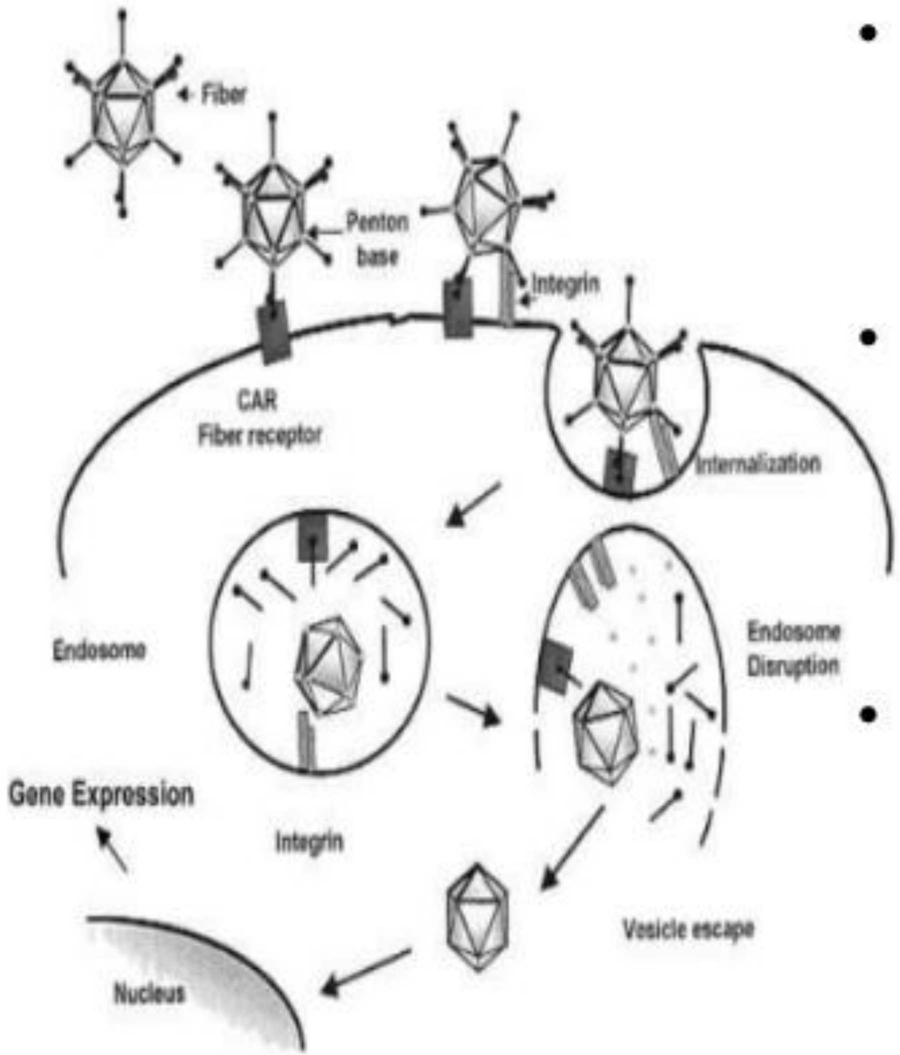
VIRAL REPLICATION

- Adenoviruses attach to surface of the cells by their fibers, then penetrate the cell, and once inside the cell, uncoat the viral DNA.
- The viral DNA is then transported into the nucleus of the cell and initiates replication cycle.
- Host cell DNA-dependent RNA polymerase transcribes the early genes leading to formation of functional mRNA.





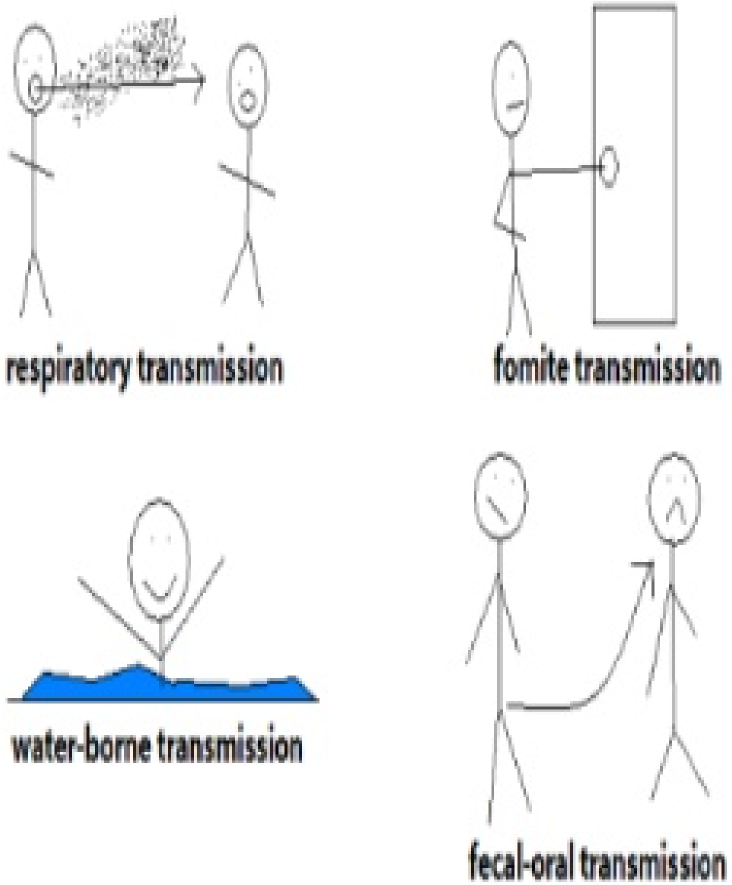
VIRAL REPLICATION



- Then in the cytoplasm, the early mRNA is translated into nonstructural proteins.
- In the nucleus, after viral DNA replication, late mRNA is transcribed and then translated into structural virion proteins.
- This is followed by assembly of virions in the nucleus and release of virions by lysis of the cells, but not by budding.

PATHOGENESIS

- Adenoviruses are transmitted mainly by respiratory or feco-oral contact from humans.
- They infect the conjunctiva or the nasal mucosa.
- They may multiply in conjunctiva, pharynx, or small intestine, and then spread to preauricular, cervical and mesenteric lymph nodes, where epithelial cells are infected.



PATHOGENESIS

- Adenoviruses may cause three different types of interaction with the infected cells.
- These are (a) lytic infection, (b) latent infection, and (c) transforming infection.
- lytic infection: Adenoviruses infect mucoepithelial cells in the respiratory tract, gastrointestinal tract, and conjunctiva or cornea, causing damage of these cells directly.
- After local replication of the virus, viremia follows with subsequent spread to visceral organs.
- Dissemination occurs more commonly in immunocompromised patients than in the immunocompetent individuals.

PATHOGENESIS

- latent infection: The adenovirus has a unique ability to become latent in lymphoid and other tissues such as adenoids, tonsils, and Payer's patches.
- The exact mechanism of latency of adenoviruses in these tissues is not known.
- These latent infections can be reactivated in patients infected with other agents or in the patients who are immunocompromised.

PATHOGENESIS

- Oncogenic transformation: Some adenoviruses belonging to groups A and B have the property for oncogenic transformation in rodent cells.
- During oncogenesis the multiplication of adenovirus is inhibited followed by integration of viral DNA into the host DNA.
- After integration adenoviruses produce E1A proteins which target rodent cells by altering cellular transcription, finally leading to transformation of rodent cells.
- However, oncogenesis of human cells has not been demonstrated.

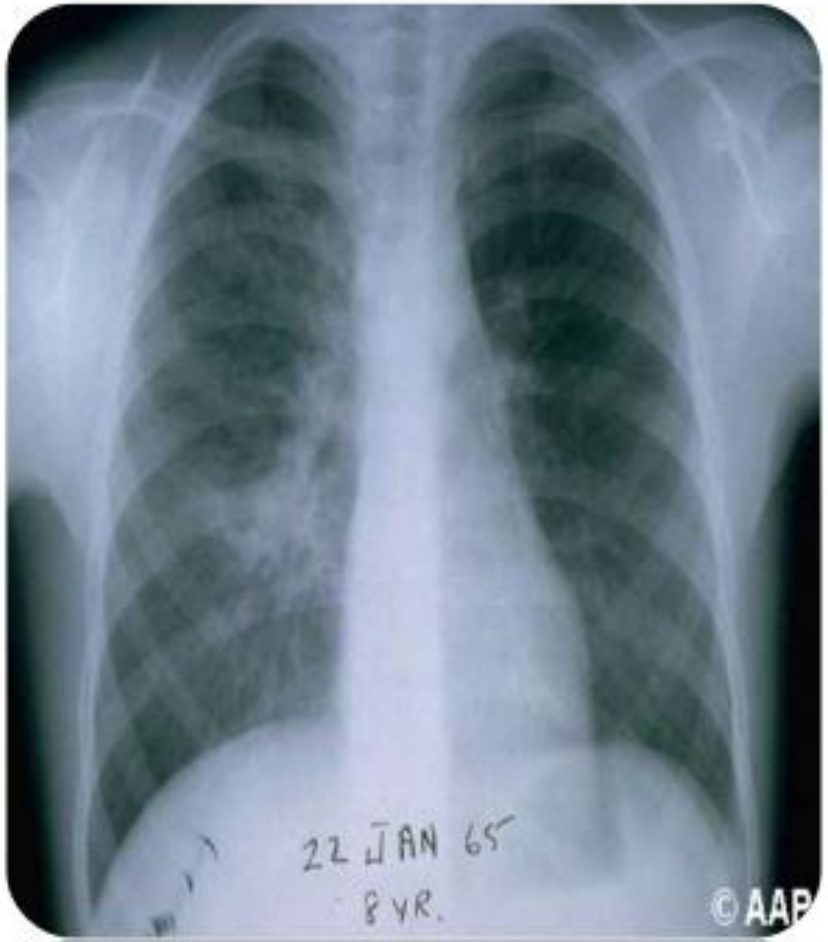
CLINICAL SYNDROMES

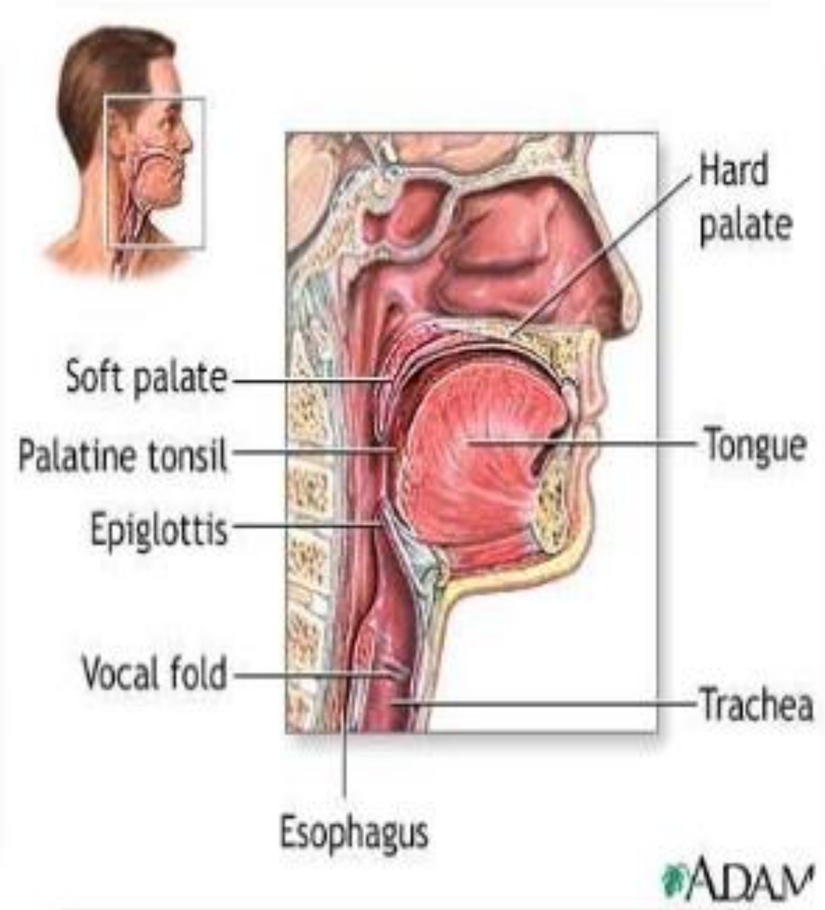
- Adenoviruses primary infect children.
- Adults are also infected.
- More than one serotypes of virus may produce the same clinical syndrome and one serotype of virus may cause clinically different diseases.
- Adenoviruses 1-7 are the common serotypes worldwide and are responsible for most cases of adenovirus-associated infections.
- Incubation period varies from 5 to 8 days.
- The major clinical syndromes caused by the human adenoviruses are as follows.

CLINICAL SYNDROMES

- Respiratory diseases
- **Acute respiratory diseases:** This disease is caused by adenovirus serotypes 4 and 7.
- Fever, rhinorrhea, cough, and sore throat are the typical symptoms which last for 3-5 days.
- This syndrome most often affects military recruits living in crowded conditions.
- **Pharyngoconjunctival fever:** This syndrome occurs primarily in school-going children.
- Fever, sore throat, coryza, and red eye are the classic presentations of the condition.

CLINICAL SYNDROMES





CLINICAL SYNDROMES

- These symptoms may precede ocular findings, or they may not be present.
- Acute conjunctivitis may occur as a separate entity with or without pharyngitis.
- The condition is usually self-limiting.
- This condition is caused predominantly by serotypes 3, 4, and 7.
- **Other respiratory tract diseases:** Bronchiolitis, croup, laryngitis, and cold-like symptoms are the other respiratory tract diseases caused by adenoviruses.

CLINICAL SYNDROMES

- **Epidemic keratoconjunctivitis:**
- This is a highly contagious condition and has an insidious onset of unilateral red eye.
- Subsequently, both the eyes are involved.
- Patients complain of photophobia, tearing, and pain.
- Fever and lymphadenopathy may be present in some children.
- Inflammation of the conjunctiva may persist for a week, accompanied with residual scarring and visual impairment.
- The condition may occur as an epidemic, which is usually caused by serotype 8 and less often by serotypes 19 and 37.

CLINICAL SYNDROMES

- **Gastroenteritis and diarrhea**
- These conditions are most commonly associated with serotypes 40 and 41, but other serotypes may also be involved.
- The entecic adenovirus infection is a common cause of infantile diarrhea in day-care centers.
- The condition manifests as fever and watery diarrhea, which resolves within 1-2 weeks.
- **Other syndromes** :
  - Acute hemorrhagic cystitis (11,21)
  - Generalised exanthems
  - Mesenteric adenitis
  - Intussusception

LABORATORY DIAGNOSIS

- Specimens : from throat, eye, urine, feces;
- Isolation of virus:
  - Inoculation into cell cultures;
  - human embryonic kidney/HeLa/HEp2
  - CPE : cell rounding and aggregation into grape like clusters;
  - Other tests : HA, Neutralization, CF

LABORATORY DIAGNOSIS

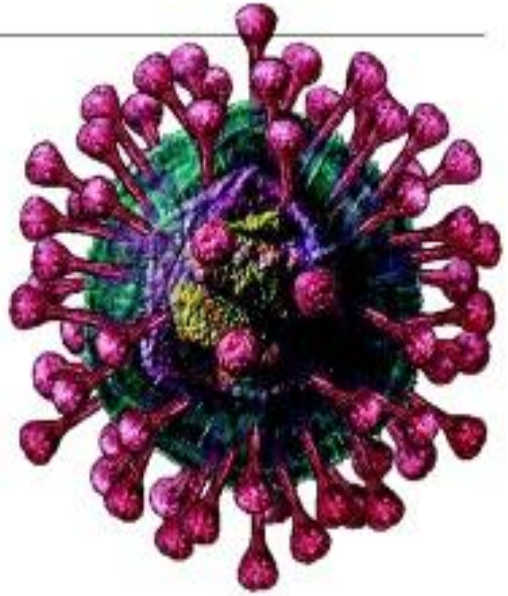
- Serology : rise in titre of antibodies in pared sera; (single specimen – not useful)
- Electron Microscopy : for stool to see virus
- Immunofluorescence : antigen detection in Nasopharyngeal /occular specimens

PROPHYLAXIS

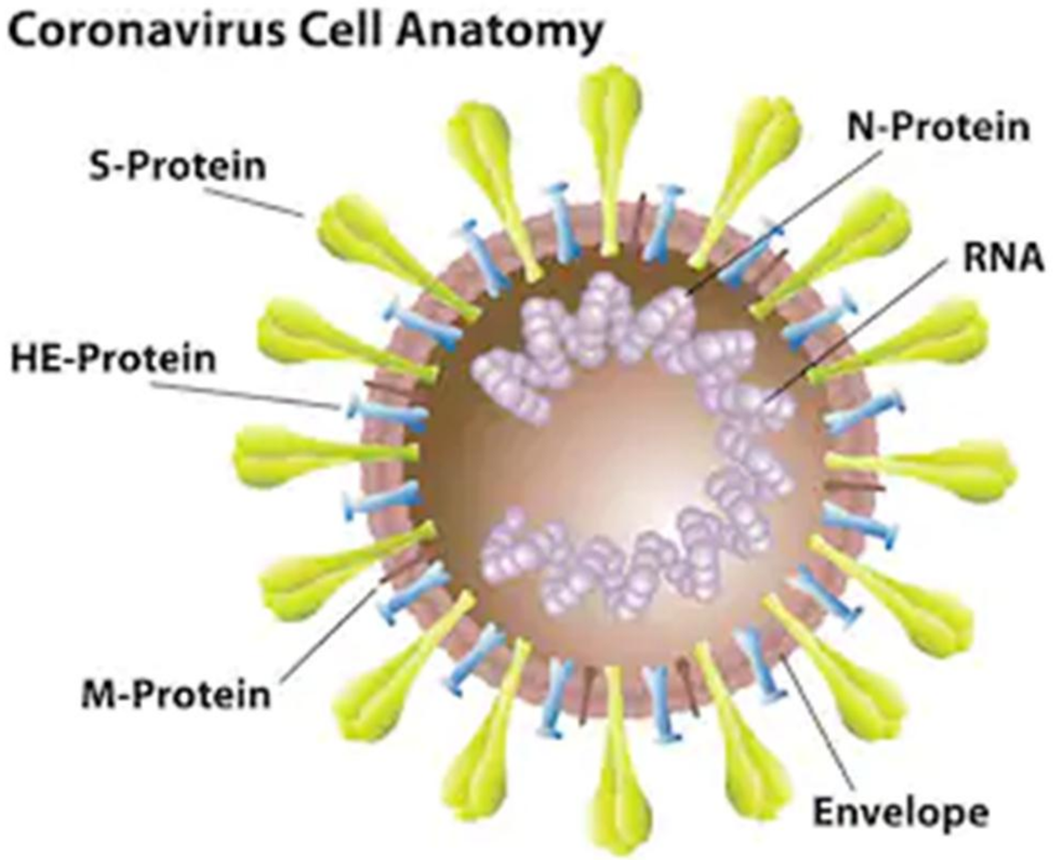
- Specific prevention – only for control of outbreaks in closed communities;
- Killed and live vaccines tried in Acute Respiratory Disease(ARD);
- No vaccine for general use;

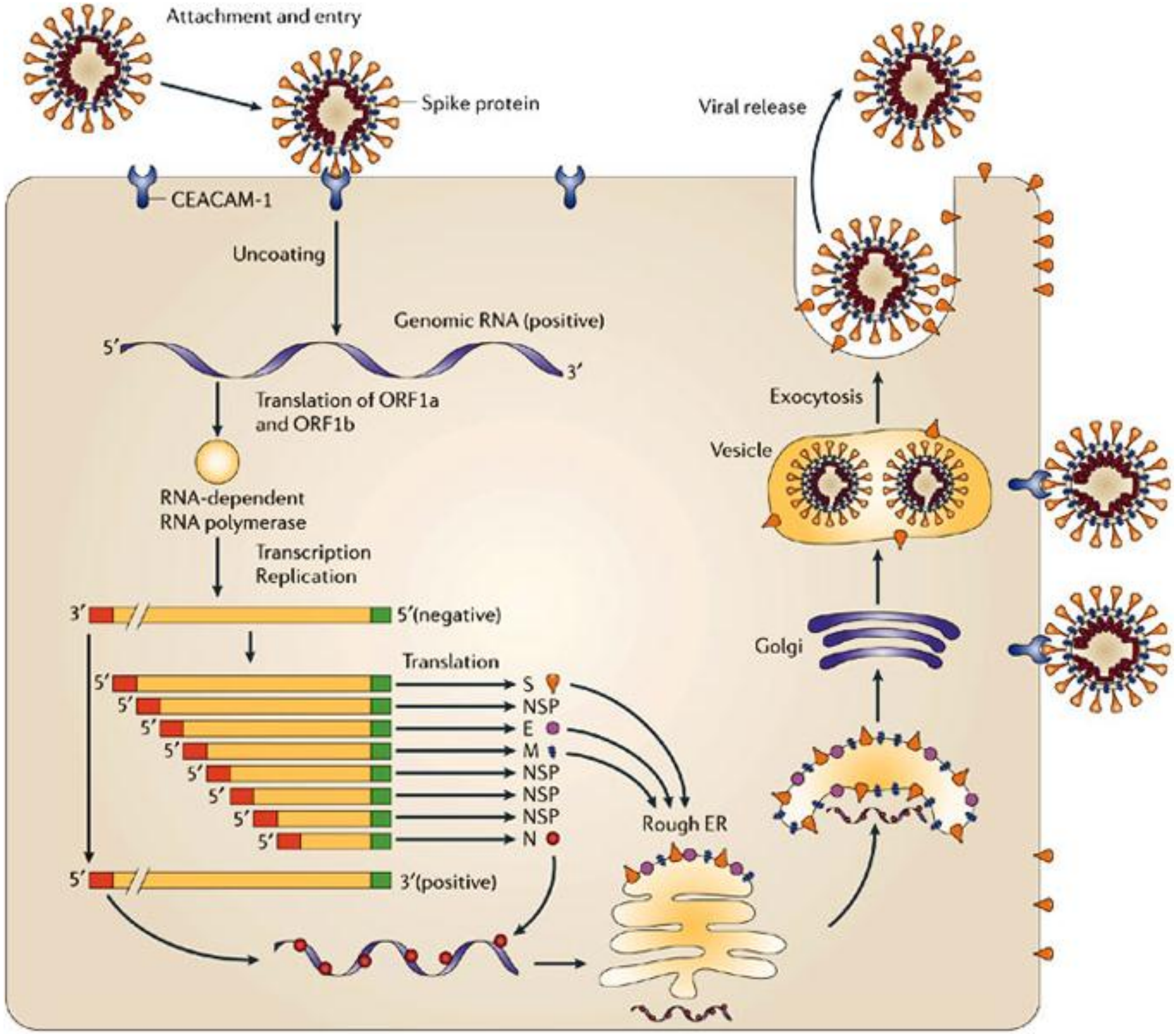
Classification :

- Family: Coronaviridae
- · Gender: Coronavirus
- · Genome: linear single-stranded RNA +
- · pleomorphic, Wrapped
- · 80 to 220 nm
- · 30 serotypes

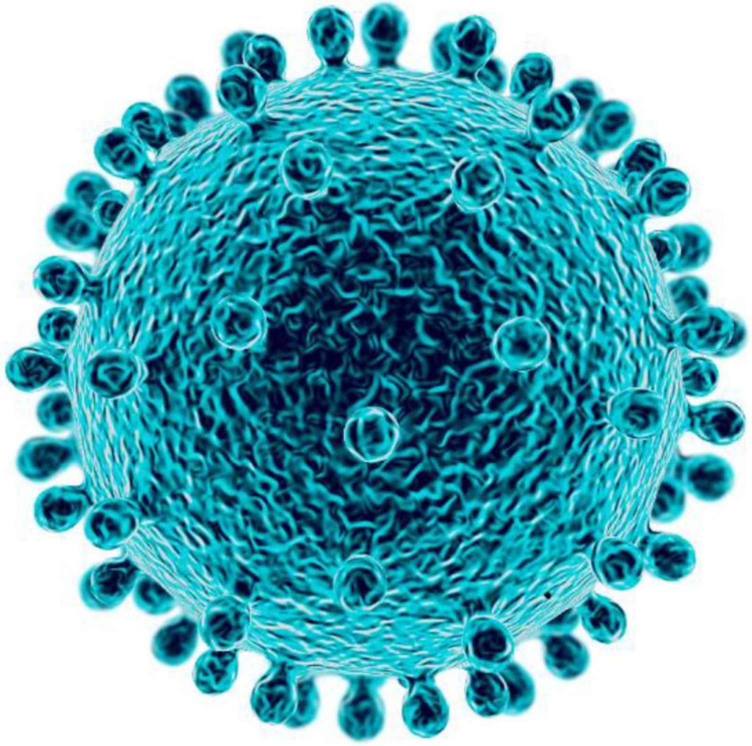


Reproductive scheme of coronaviruses





What Do We Know About Coronaviruses?



Coronaviruses have a crown-like appearance under the microscope

Coronaviruses are a large family of viruses - some cause illness in people, and others only infect animals.

Some coronaviruses infect animals then spread to people, and then spread person to person such as:

- Middle East Respiratory Syndrome (MERS)
- Severe Acute Respiratory Syndrome (SARS)
- **Coronavirus Disease 2019 (COVID-19)**

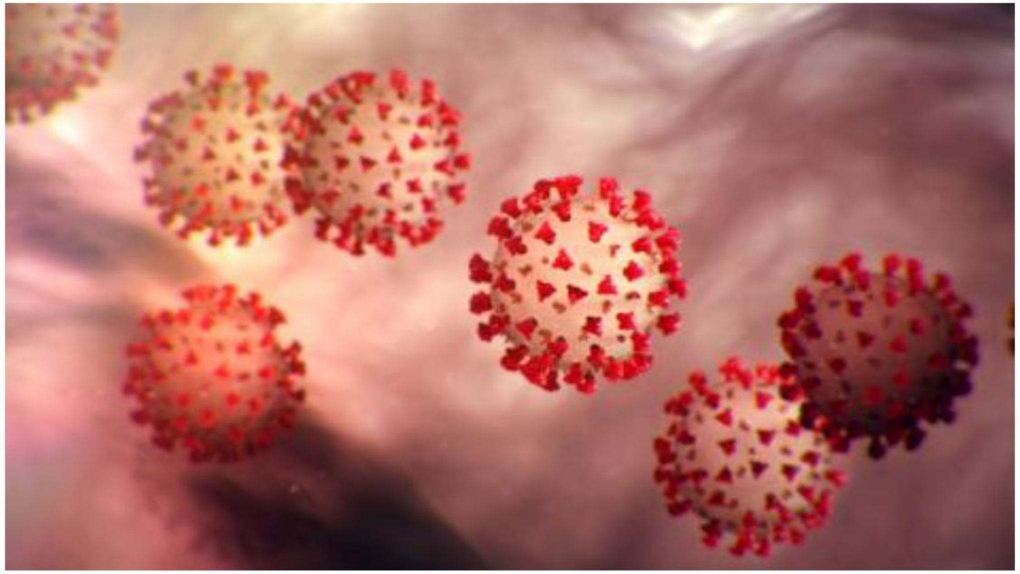
Common coronaviruses include some that cause mild upper-respiratory illnesses, like the common cold.

What is COVID-19?

Coronavirus disease 2019 or COVID-19 is a respiratory illness that can spread from person to person.

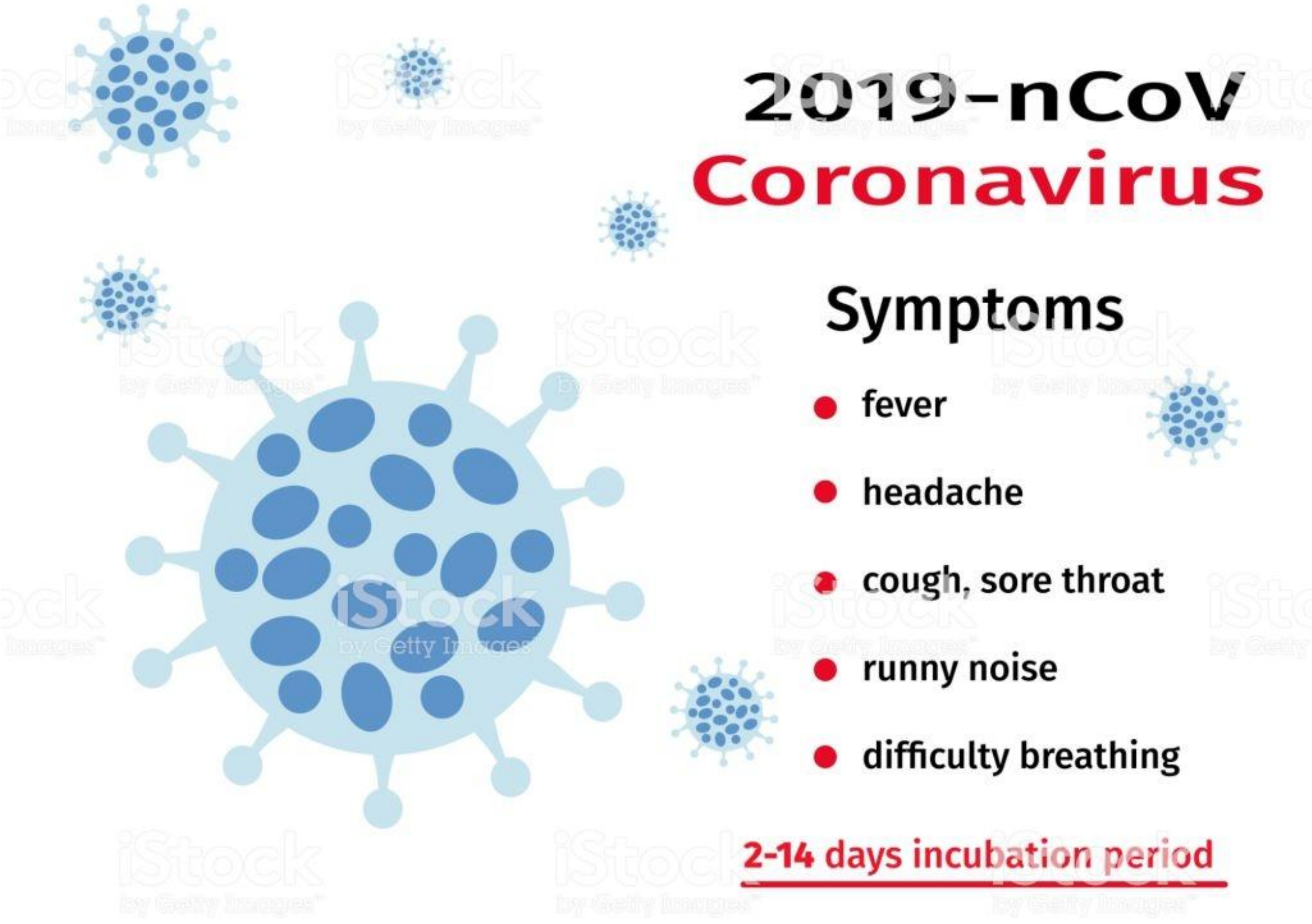
The virus that causes COVID-19 is a **new** coronavirus first identified during an investigation into an outbreak in Wuhan, Hubei Province, China.

Initial case-patients reported visiting a large seafood and live animal market in Wuhan.



Symptoms

- Corona viruses primarily infect the upper respiratory and gastrointestinal tract of humans.
- Most people who got infected with the novel corona virus developed severe acute respiratory illness with symptoms of **fever, cough**, and **shortness of breath**. Some people were reported as having a mild respiratory illness


2019-nCoV
Coronavirus
Symptoms
fever
headache
cough, sore throat
runny noise
difficulty breathing
2-14 days incubation period

Microbiological diagnosis of coranovirus infections

- Viruses can be detected in respiratory secretions by ELISA, IFR and PCR.
- RNA for SARS and Covid-19 virus can also be found in the blood.
- Since the virus is difficult to obtain in cell cultures, the main diagnostic method is PCR.
- An increase in the antibody titer in the double serum studied by ELISA confirms coronavirus infection.

Testing, testing

Promising drugs to treat covid-19

Drug	Current use	Original mode of action	Being tested?
Chloroquine	Antimalarial	Heme polymerase inhibitor	Yes
Kaletra (ritonavir + lopinavir)	HIV	Protease inhibitor	Yes
Interferon alfa-2b	Hepatitis-C	Immune modulator	Yes
Remdesivir	Experimental	Nucleotide analogue	Yes
Favipiravir	Influenza	RNA polymerase inhibitor	Yes
Actemra (tocilizumab)	Rheumatoid arthritis; covid-19	Anti-inflammatory	Approved*
Kevzara (sarilumab)	Rheumatoid arthritis	Anti-inflammatory	Trials expected

Source: WHO, adapted from landscape analysis, 17th February 2020

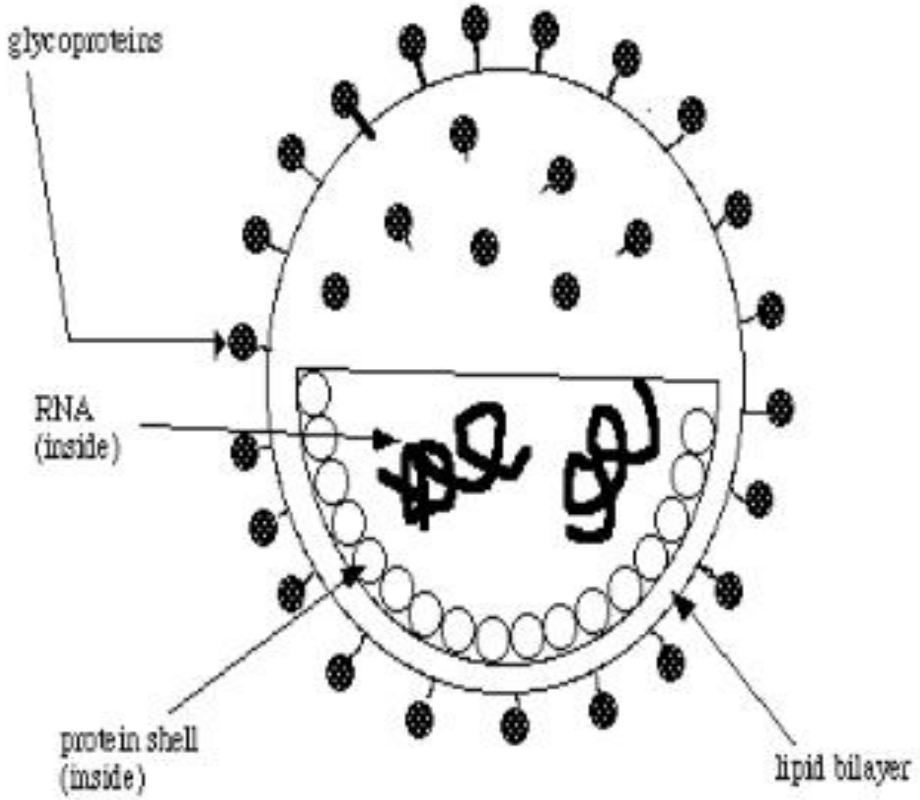
*For use on covid-19 in China, March 2020

Rhinoviruses

- Rhinoviruses (from the Greek (gen.) "nose") are the most common viral infective agents in humans and are the predominant cause of the common cold. Rhinovirus infection proliferates in temperatures between 33–35 °C (91–95 °F), and this may be why it occurs primarily in the nose. Rhinovirus is a species in the genus Enterovirus of the **Picornaviridae family of viruses.**

Rhinovirus

- Picornoviridae family
- Size
- Single stranded
- Incubation period of 1 to 3 days
- Optimum growth occurs between 33 and 34 deg Celsius (93 deg F)
- Not stable below the pH of 5-6



Rhinoviruses are ..

- Rhinoviruses are the most commonly isolated viruses from persons with mild upper respiratory illness.
- Rhinoviruses are a genus of picornaviridae
- In contrast to enteroviruses they do not replicate in the intestinal tract, they have an extreme species specificity and more fastidious growth requirements

Properties of Rhinoviruses.

- Gross appearance like Entero viruses,
- Acid Labile destroyed at 3.0 pH.
- Grown in

  Human Cell lines,

  WI – 38

  MRC -5
- Cultivated at 33^0 c

Transmission of Rhinoviruses

- There are two modes of transmission: via aerosols of respiratory droplets and from contaminated surfaces, including direct person-to-person contact.

Pathogenesis

Epithelial destruction hypothesis:

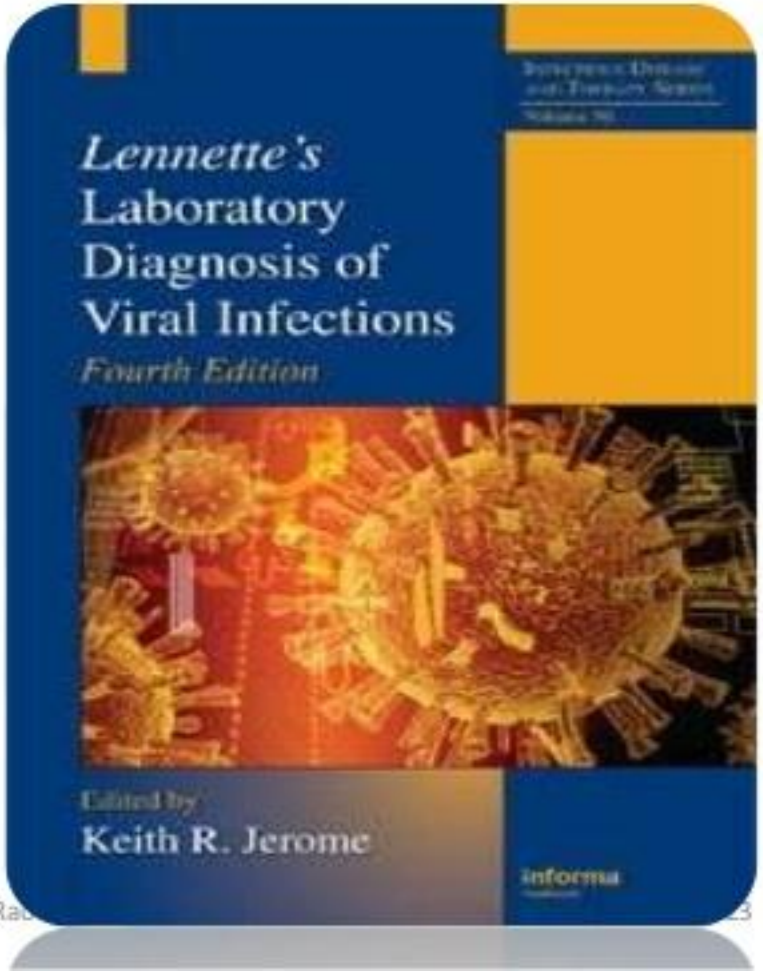
- intact nasal epithelium *(Winther et al)*
- rhinovirus replication in extremely small number of cells *(Arrunda et al)*
- viral quantity does not influence duration nor severity of colds *(Arrunda et al)*

Pathogenesis - Rhinoviruses

- Entry through Respiratory tract.
- Nasal Mucosa, can infect Lower Respiratory tract.
- Chilling, wearing wet cloths do not produce infection.
- But common cold starts with chills.
- Local inflammation and cytokines may be responsible for the symptoms of common cold.
- **Interferon production occurs early and specific antibody appears in nasal secretions**

Laboratory Diagnosis

- Isolation of virus may be obtained from nasal or throat swabs collected early in infection.
- Culturing on MRC5 or W 138
- Appearing of CPE



Prevention and Control

- No specific treatment.
- Vaccines are unsuccessful.
- Intranasal spray of Gamma Interferon for 5 Days

Poxviridae family

- The family Poxviridae (lat., pox - pustula) consists of large-sized, complex-structured DNA-containing viruses that cause diseases in insects and vertebrates.
- Viruses that cause diseases in vertebrates belong to the subfamily Chordopoxvirinae.
- Four of the six genera of this subfamily - Orthopoxvirus, Parapoxvirus, Molluscipoxvirus and Yatapoxvirus - cause various diseases in humans.
- Orthopoxvirus genus includes vaccinia virus, natural smallpox virus, viruses of cows, monkeys, camels, mice, etc. including smallpox viruses.
- Parapoxviruses include Orf (ecthyma contagion), bovine papulosis stomatitis virus, paravaccin (false cowpox) virus, etc. includes.
- Molluscipoxvirus genus includes molluscum contagiosum virus.Yaba virus and Tana virus from the genus Yatapoxvirus are pathogenic viruses for humans.

Poxviridae family

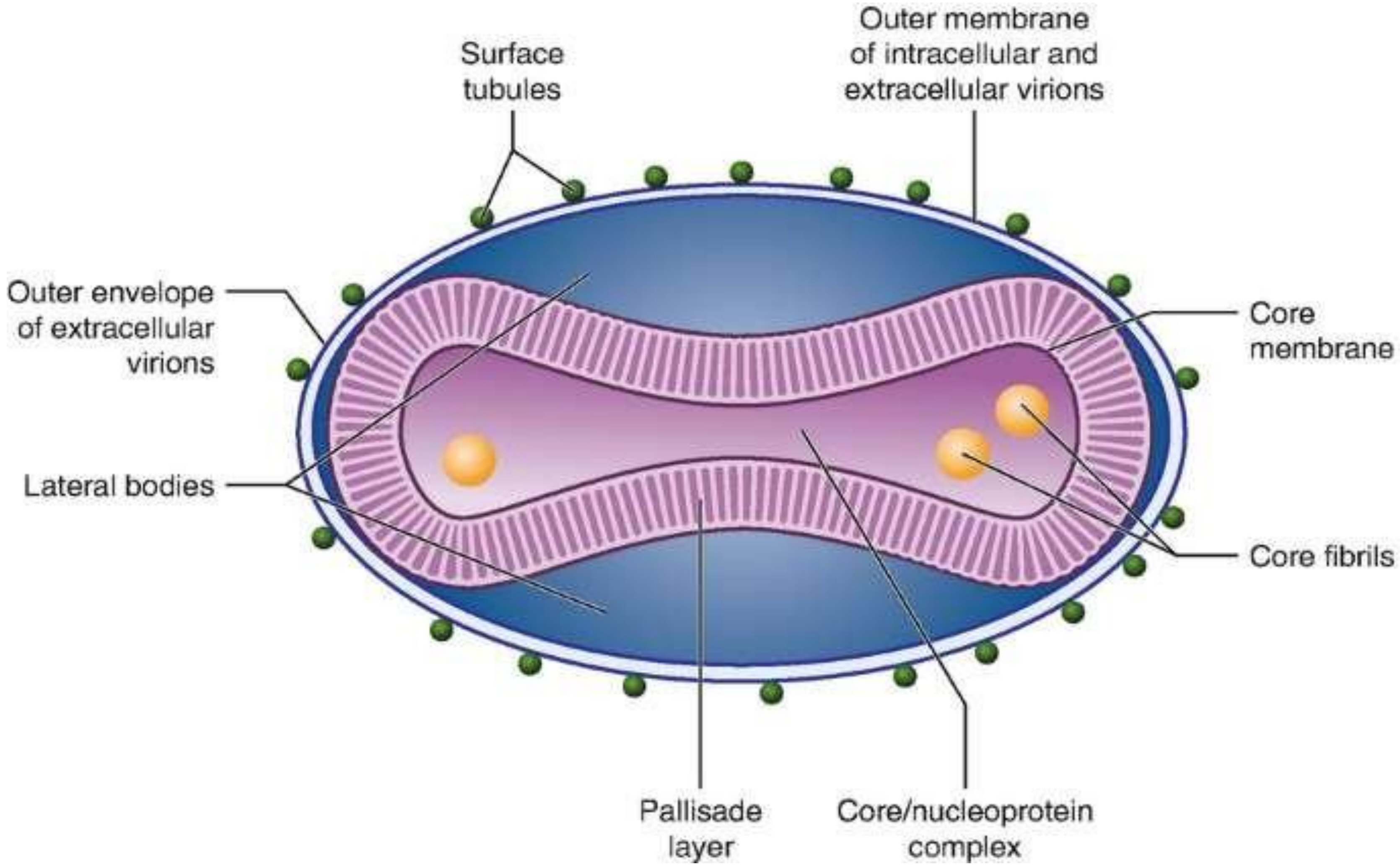
- Poxviruses are the largest viruses, measuring 230x400 nm. The virion is ovoid in shape, composed of a membrane, an outer membrane and a core (DNA and proteins) located between the lateral bodies. the virion's genome consists of double-stranded linear DNA. Reproduction takes place only in the cytoplasm, Guarinieri appendages are observed inside the infected cells. Virions bud from the plasma membrane and are released when the cell lyses.

Is MonkeyPox the next pandemic ?

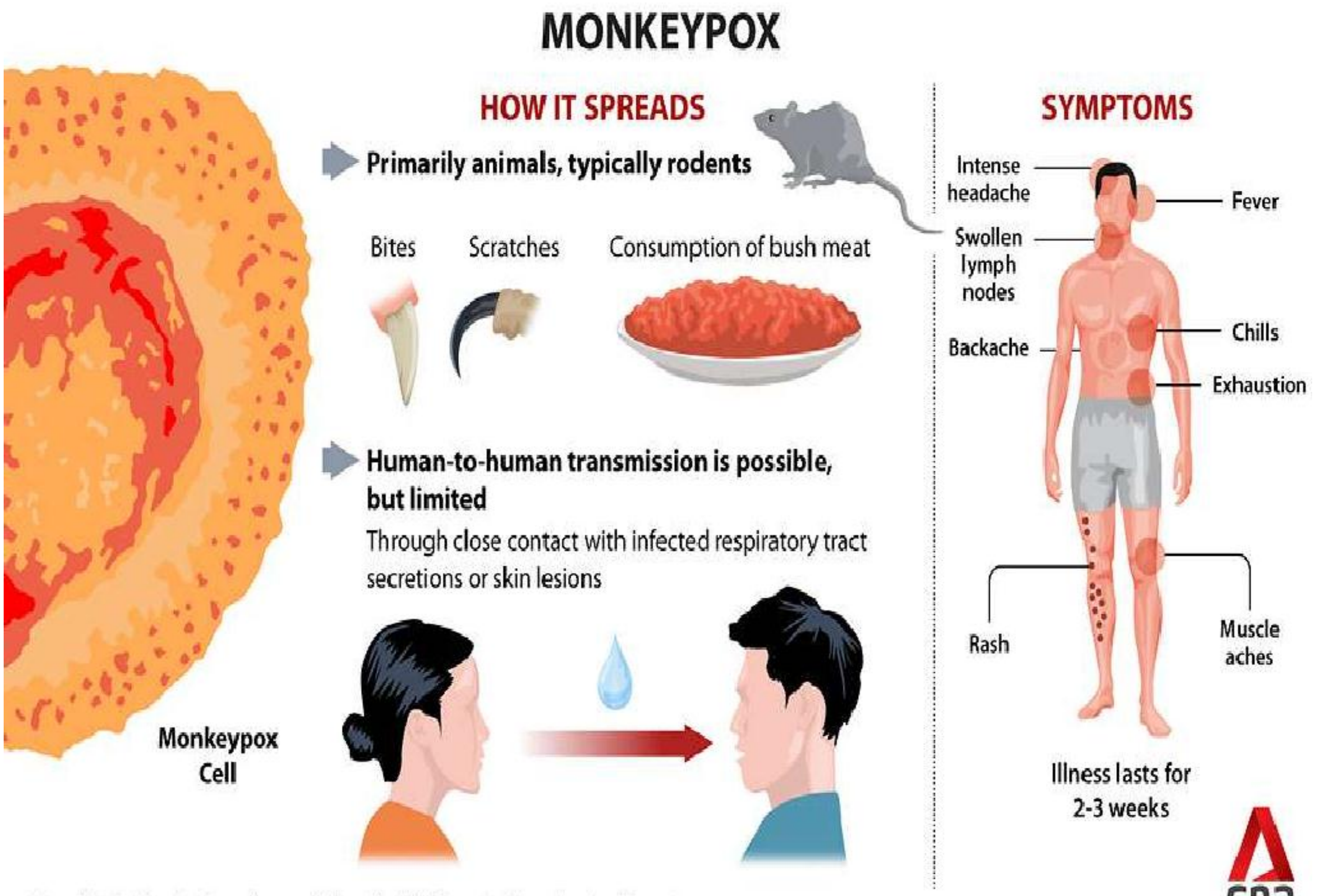
Monkeypox

- Monkeypox virus is an enveloped double-stranded DNA virus with a genome size of around 190 kb.
- Monkeypox is a viral zoonotic disease that occurs primarily in tropical rainforest areas of Central and West Africa and is occasionally exported to other regions.

Structure

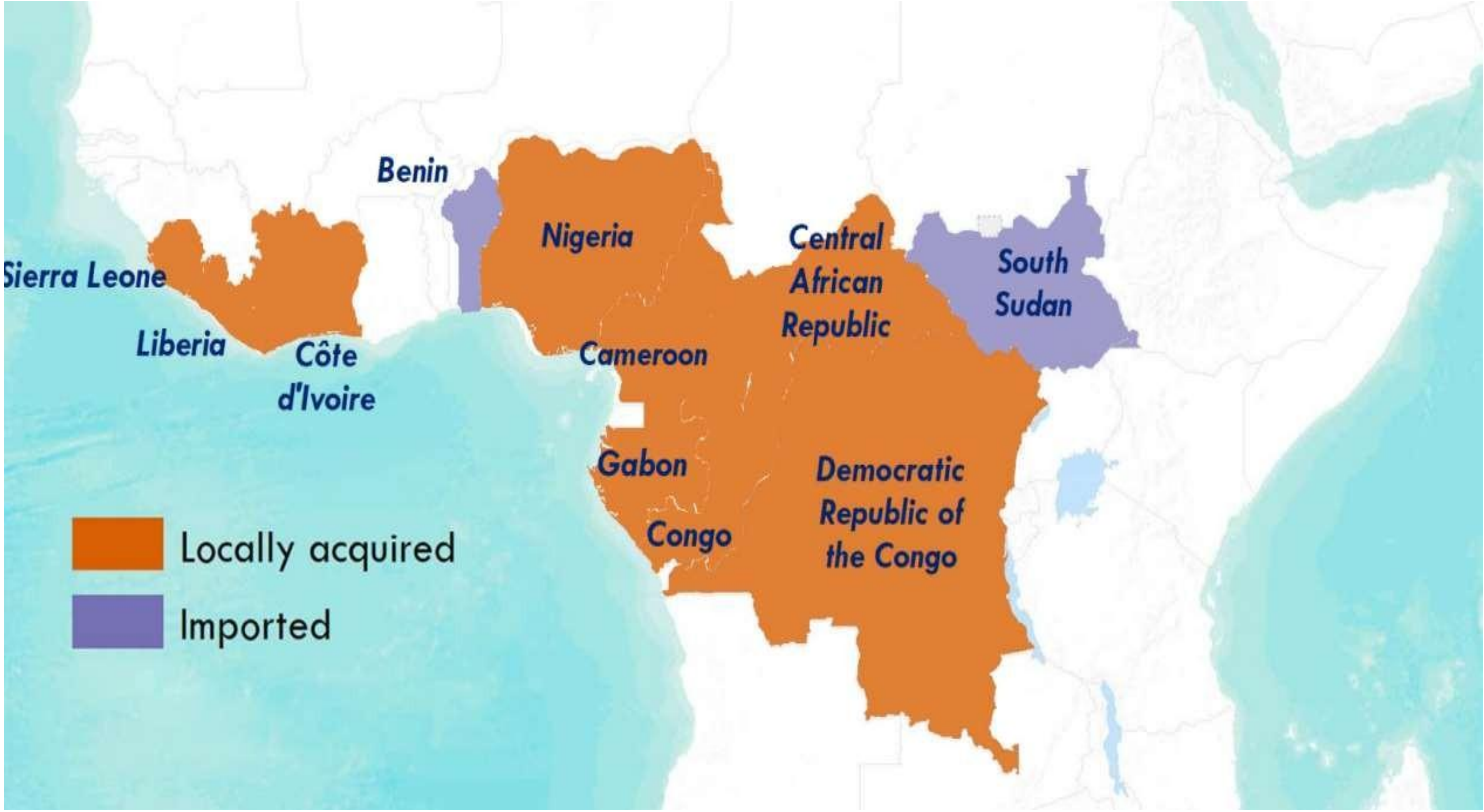


Surface tubules
Outer membrane of intracellular and extracellular virions
Outer envelope of extracellular virions
Core membrane
Lateral bodies
Core fibrils
Pallisade layer
Core/nucleoprotein complex

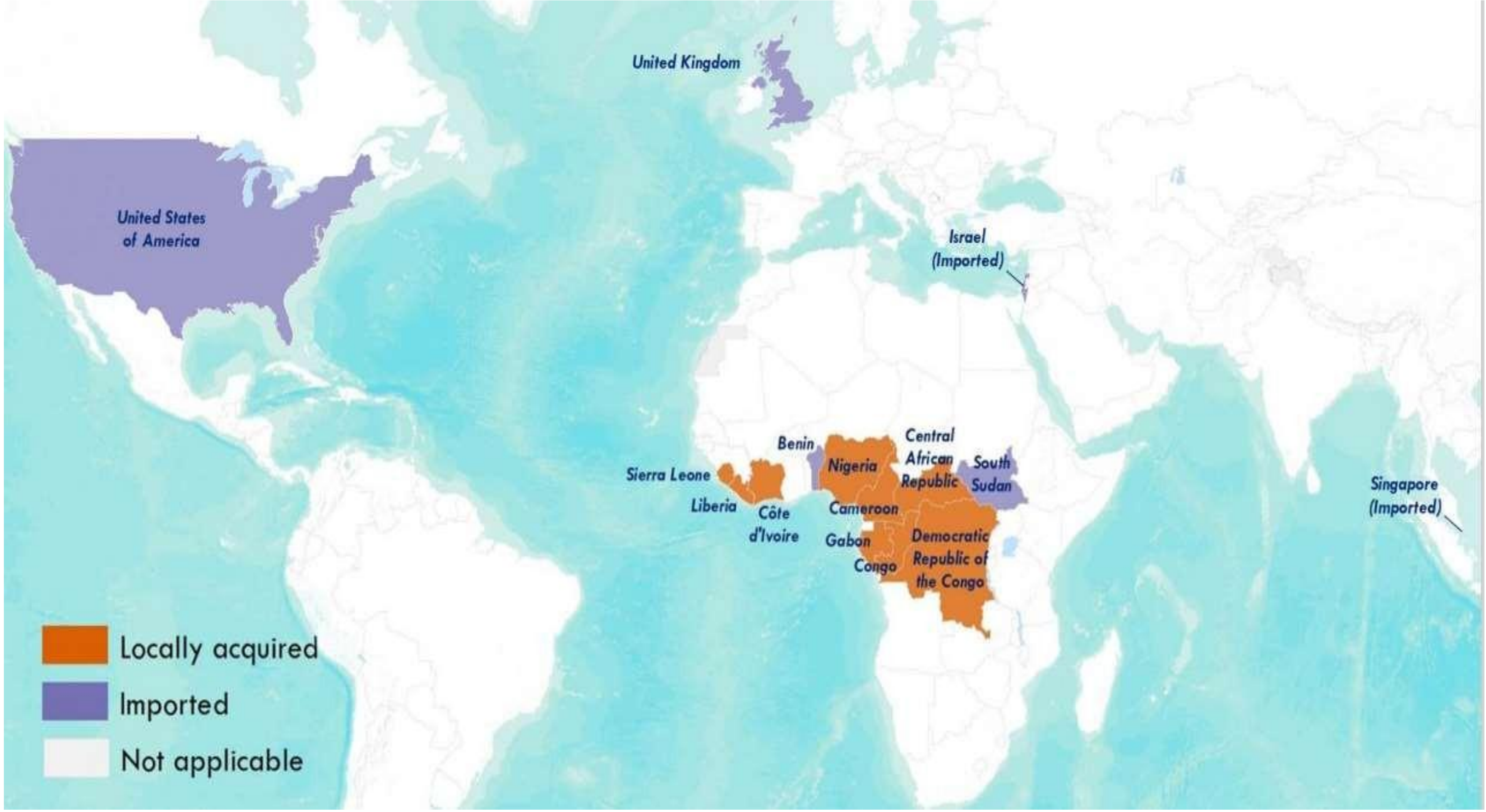


Infographic by Rafa Estrada   Source: Singapore Ministry of Health, US Centers for Disease Control and Prevention

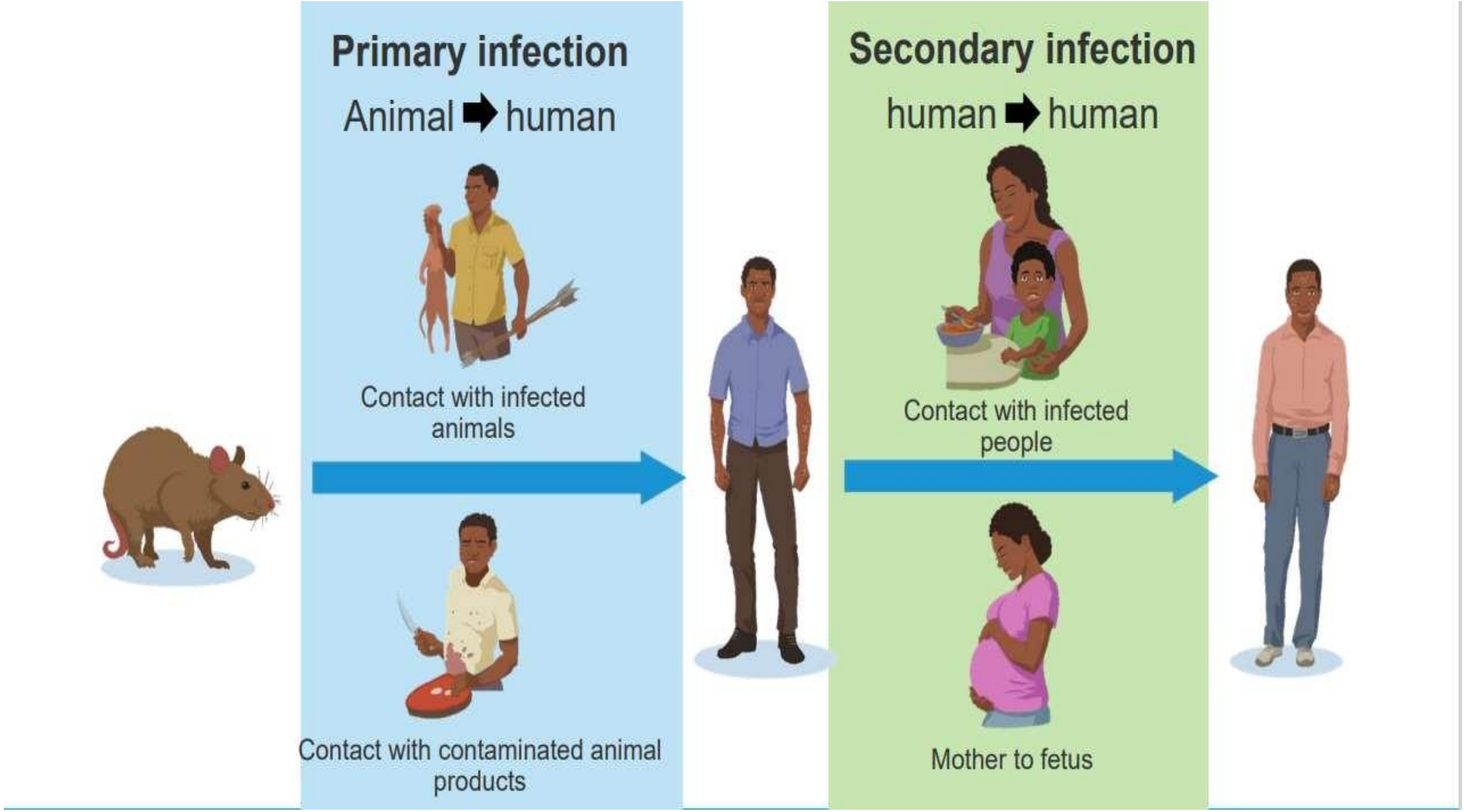
Confirmed human monkeypox cases Africa, 1970-2021



Confirmed human monkeypox cases Worldwide, 1970-2021



Monkeypox transmission - an overview



Monkeypox – modes of transmission

- Unprotected contact with:
- respiratory droplets
- lesion material
- body fluids
- contaminated materials and surfaces The virus can enter through:
- respiratory tract
- mucous membranes (eyes and mouth)
- broken skin (e.g. animal bites

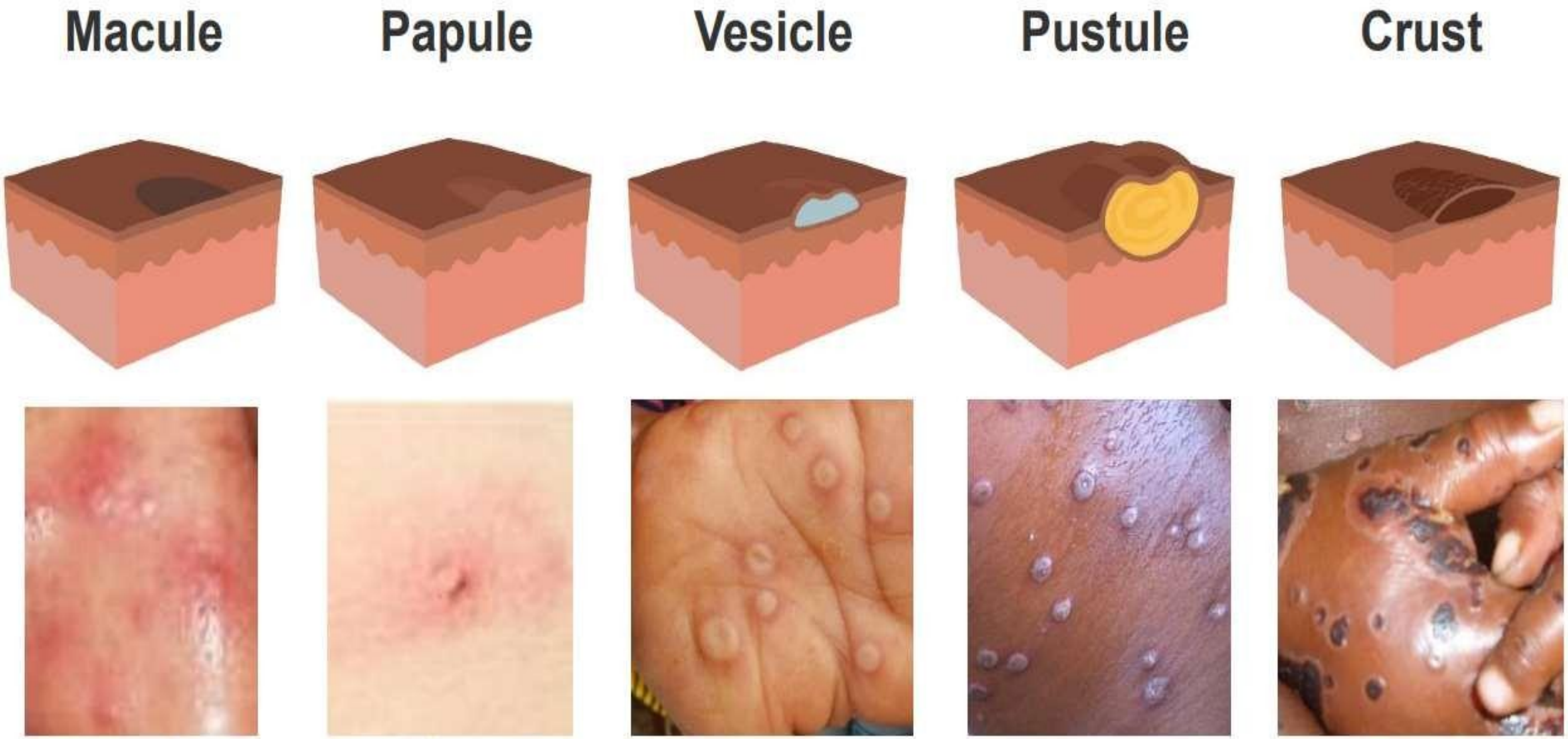
Disease progression I – Incubation

- Duration: 5-21 days
- No symptoms
- Virus present in bloodstream (viremia) at the end of the incubation period

Disease progression II – Febrile stage

- 1-4 days
- Fever + other symptoms: swollen lymph nodes (lymphadenopathy), headache, chills, sore throat, malaise, fatigue
- Virus in the blood
- Small lesions in the mouth (enanthem) appear towards the end

Disease progression III – Rash stage



Credit: Emerg Infect Dis / N. Erez et al., 2018. Retrived from:https://wwwnc.cdc.gov/eid/article/25/5/19-0076-f1

Credit: NEJM/ D.Kurz et al .2004 Retrived from: https://www.nejm.org/doi/full/10.1056/NEJMoa032299

Credit: Andrea McCollum / CDC

Credit: Toutou Likafi/ Kinshasa School of Public Health

Credit: P. Mbala /Institut Nationale de recherche biomedicale. DRC

Disease progression III – Rash stage

- Virus may be in the blood early in this stage
- Virus is present in skin lesions
- Antibodies are produced and become detectable

Disease progression IV – Recovery

- The patient has recovered
- Specific antibodies are present in the blood
- Scars may remain

Monkeypox symptoms – an overview

Monkeypox and other common rash illnesses

Symptoms		Monkeypox	Chickenpox	Measles
Symptoms	Fever	1-3 days before rash	1-2 days before rash	3-5 days before rash
	Rash appearance	Lesions often in one stage of development	Lesions often in multiple stages of development	Lesions often in multiple stages of development
	Rash development	Slow	Rapid	Rapid
	Rash distribution	More dense on face; present on palms and soles	More dense on trunk; Absent on palms and sole	Starts on face and spreads, sometimes reaching hands and feet
	Lymphadenopathy	Present	Absent	Occasional
	Death	Up to 10%	Rare	Varies widely

Clinical Symptoms

- Monkeypox typically presents clinically with fever, rash and swollen lymph nodes and may lead to a range of medical complications.
- Monkeypox is usually a self-limited disease with the symptoms lasting from 2 to 4 weeks.
- Severe cases can occur.
- In recent times, the case fatality ratio has been around 3-6%.
- Monkeypox is transmitted to humans through close contact with an infected person or animal, or with material contaminated with the virus.
- Monkeypox virus is transmitted from one person to another by close contact with lesions, body fluids, respiratory droplets and contaminated materials such as bedding.

Prevention

- Raising awareness of risk factors and educating people about the measures they can take to reduce exposure to the virus is the main prevention strategy for monkeypox.
- Scientific studies are now underway to assess the feasibility and appropriateness of vaccination for the prevention and control of monkeypox.
- Some countries have, or are developing, policies to offer vaccine to persons who may be at risk such as laboratory personnel, rapid response teams and health workers.

Treatment

- At this time, there are no specific treatments available for be monkeypox infection, but monkeypox outbreaks can controlled.

- Smallpox vaccine, cidofovir, ST-246, and vaccinia immune globulin (VIG) can be used to control a monkeypox outbreak.
- CDC guidance was developed using the best available information about the benefits and risks of smallpox vaccination and drug use for the prevention and management of monkeypox and other orthopoxvirus infections.

Monkeypox and Smallpox Vaccine

- One vaccine, JYNNEOS™ (also known as Imvamune or Imvanex), has been licensed in the United States to prevent monkeypox and smallpox.
- Because monkeypox virus is closely related to the virus that causes smallpox, smallpox vaccine can also protect people from getting monkeypox.
- Past data from Africa suggests that smallpox vaccine is at least 85% effective in preventing monkeypox.
- The effectiveness of JYNNEOS™ against monkeypox was concluded from a clinical study on the immunogenicity of JYNNEOS and efficacy data from animal studies.
- Experts also believe that vaccination after a monkeypox exposure may help prevent the disease or make it less severe.