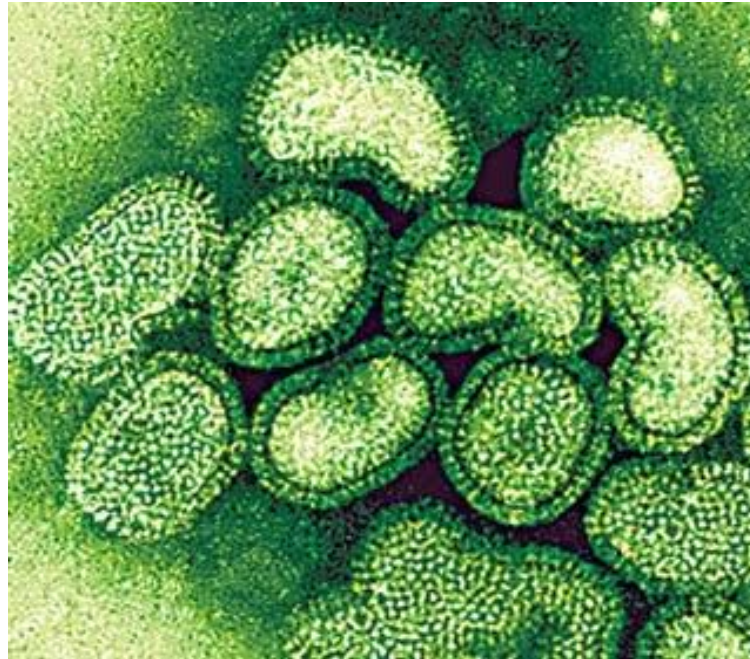


Influenza Viruses



Epidemiology of Influenza viruses

- **Influenza is an acute respiratory tract infection that usually occurs in epidemics.**
- **These viruses received their name from their special affinity to mucous.**
- **Three immunologic types of influenza viruses are known designated:**
 - **A, B and C**
 - **based on different ribonucleoprotein antigens**

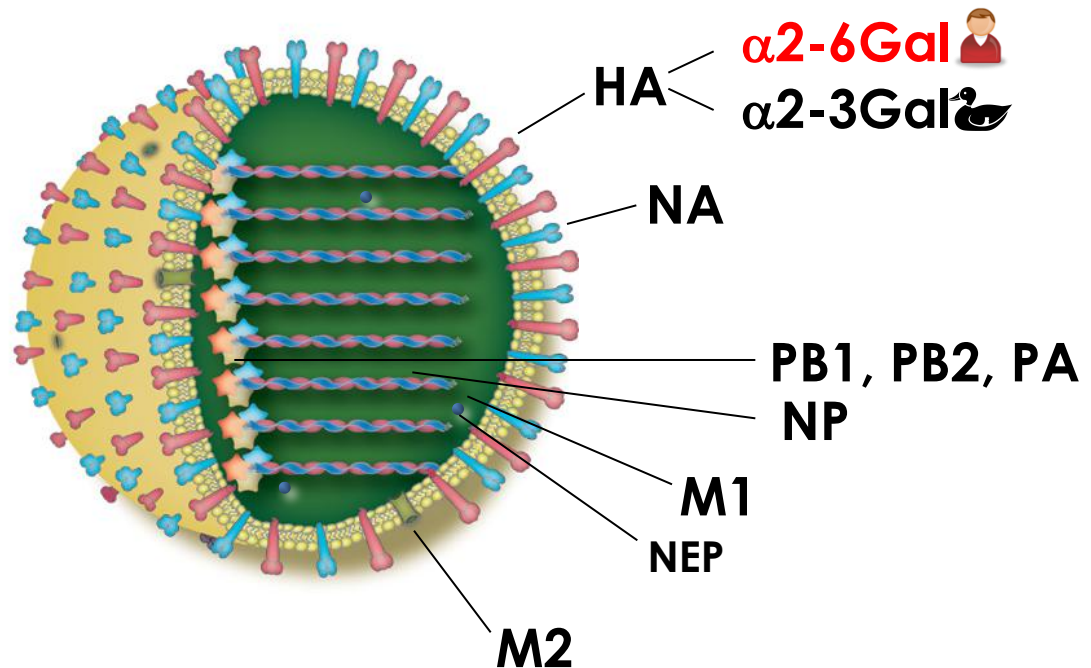
Epidemiology of Influenza viruses

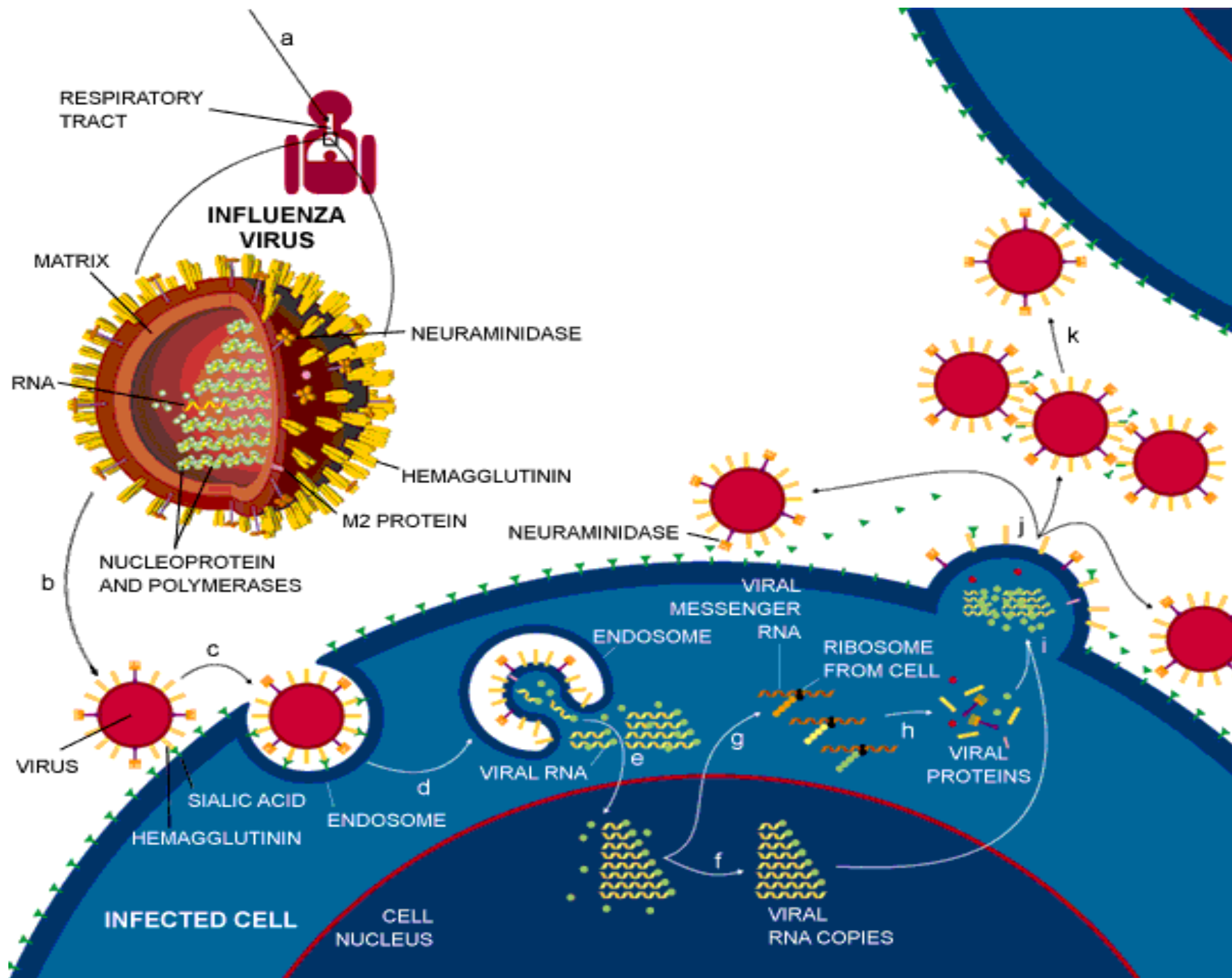
- **Influenza A strains are also known for:**
 - aquatic birds (e.g. ducks, turkeys, chickens, geese),
 - pigs
 - & horses.

**INFLUENZA TYPES B AND C
ARE
RESTRICTED TO HUMANS**

Morphology:

- Spherical virus, (filamentous forms occur).
- Helical nucleocapsid
- Segmented single stranded RNA (eight segments), to which protein capsomeres are attached.
- Enveloped
- Two virus encoded glycoproteins are inserted into the envelope, and are exposed as spikes: HA & NA





Influenza virus Genome

- **Each of the RNA eight segments encode a certain viral protein.**
 - **Segment 4 encodes the haemagglutinin**
 - **Segment 6 encodes the neuraminidase, representing the two envelope spikes.**
- **The two surface antigens of influenza undergo antigenic variation independent of each other.**
- **Four HA (HA1-2-3-5)**
and
- **Two NA (NA1 &2) subtypes have been recovered from humans.**

Properties of Orthomyxoviruses:

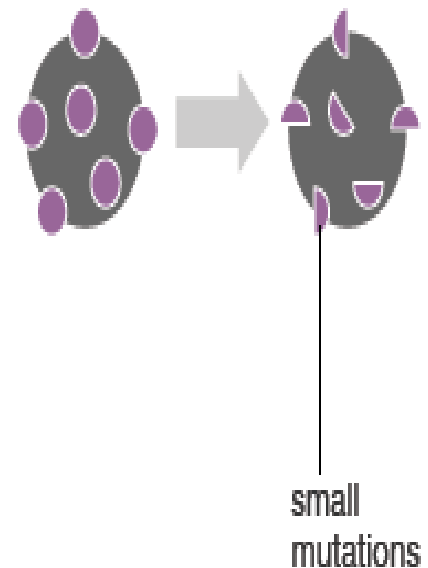
- **Mutability and high frequency of genetic reassortment are characteristics of orthomyxoviruses:**
 - **Antigenic Drift**
 - **Antigenic Shift**

“Antigenic Drift”

- Antigenic changes within major subtypes can involve both the “H” and “N” antigens
- They result from as little as **A SINGLE MUTATION IN THE VIRAL RNA**, which leads to **gradual changes of antigenic properties** of the strain.
- **NEW STRAINS** showing minor differences from the structure of previous years emerge,

Mutation

Antigenic drift

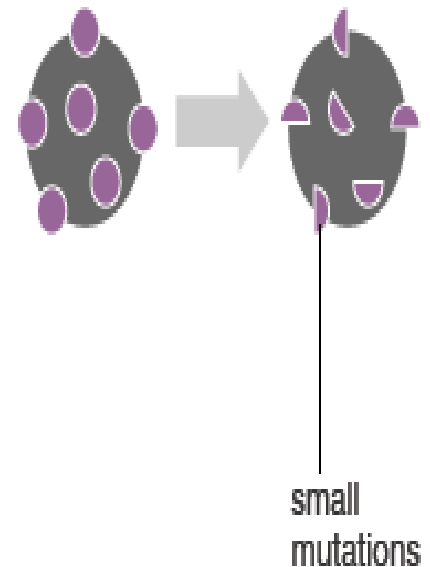


Result of continuous “*Antigenic Drift*”s

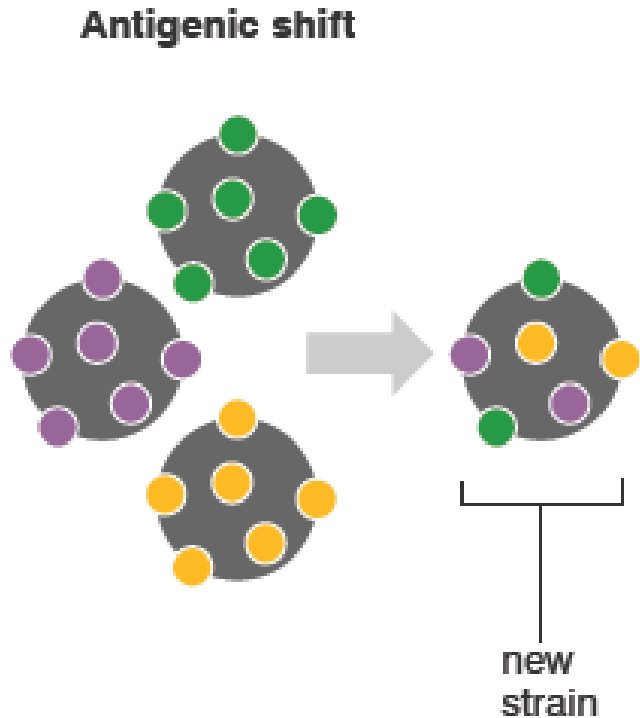
- These drifts from season to season, allow some degree of infection to continue.
- Thus infectivity persists because **TYPE-SPECIFIC IMMUNITY** is not entirely protective against drifting strains.

Mutation

Antigenic drift



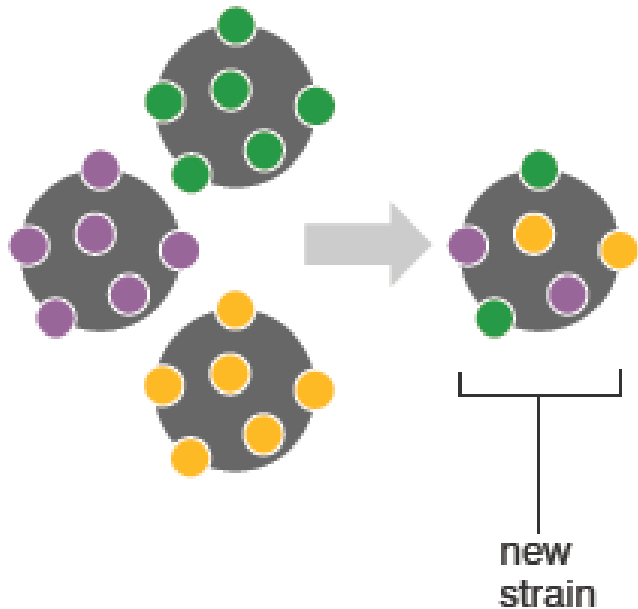
“Antigenic Shift”.



- However, in type “A” strains, a major interruption of these progressive changes can occur at long intervals varying from 10-40 years,
- A sudden and unpredictable appearance of an entirely new subtype may occur.
- This process is drastic and abrupt and is described as “*antigenic shift*”.

“Antigenic Shift”.

Antigenic shift



- **VIRUSES REASSORT READILY IN DOUBLY INFECTED CELLS.**
- **So, the mechanism for shift is genetic reassortment between human,avian, or swine influenza viruses.**

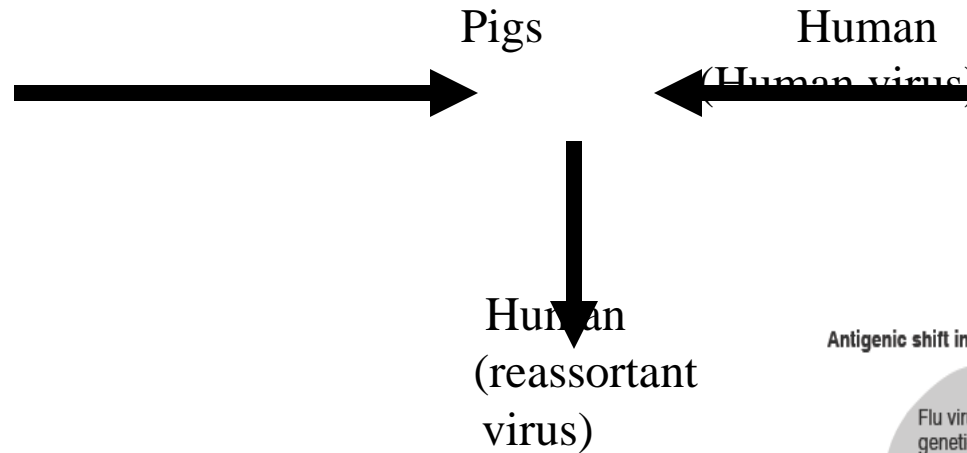
Avian influenza A virus (H5N1)

- **The first documented infection of humans by avian influenza A virus (H5N1) occurred in:
1997 in Hong Kong .**
- **The source was domestic poultry.**
- **The virus did not appear till now to be transmissible from human to human.**
- **Isolates from human cases contained all eight RNA gene segments from avian viruses indicating that the avian virus had jumped directly from birds to humans.**

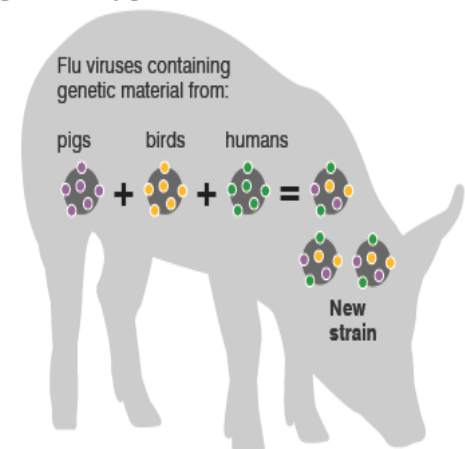
Pigs Serve As Mixing Vessels for reassortants

- **Pig cells contain receptors for both human and avian viruses.**

Aquatic birds
and domestic
Poultry
(Avian virus)

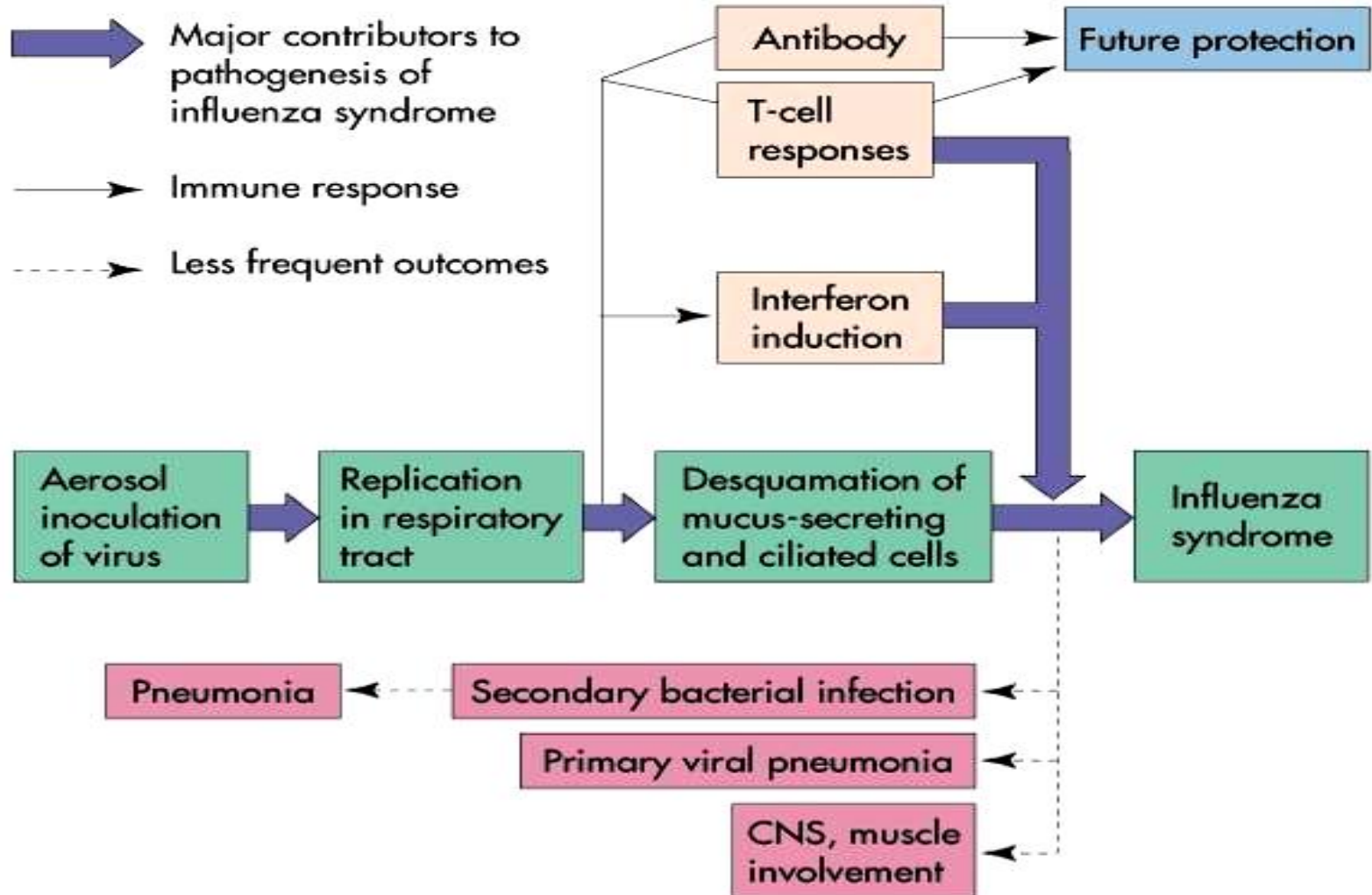


Antigenic shift in pigs

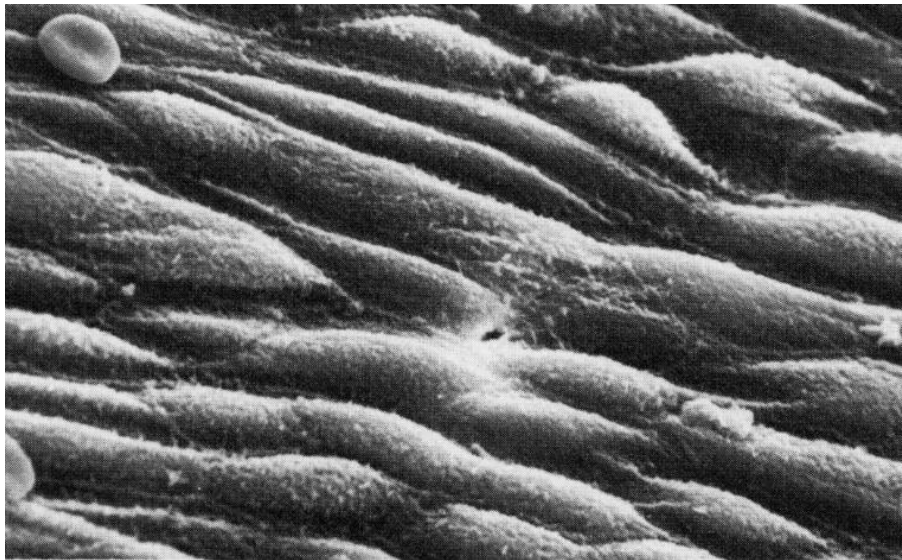
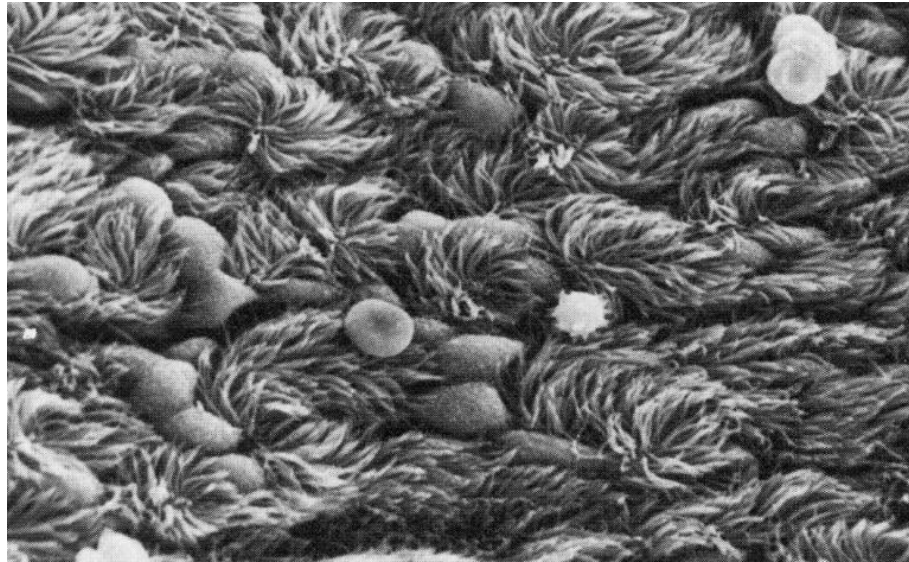


Influenza pathogenesis

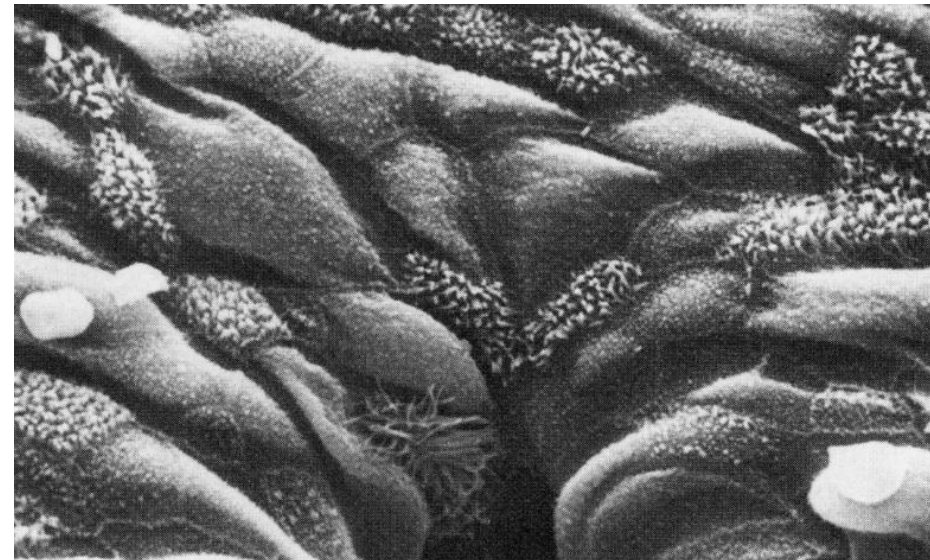
Key:



NORMAL TRACHEAL MUCOSA



3 DAYS POST-INFECTION



7 DAYS POST-INFECTION

SYMPTOMS

- FEVER
- HEADACHE
- MYALGIA
- COUGH
- RHINITIS
- OCULAR SYMPTOMS



CLINICAL FINDINGS

- SEVERITY
 - VERY YOUNG
 - ELDERLY
 - IMMUNO-COMPROMISED
 - HEART OR LUNG DISEASE



PULMONARY COMPLICATIONS

- CROUP (YOUNG CHILDREN)
- PRIMARY INFLUENZA VIRUS PNEUMONIA
- SECONDARY BACTERIAL INFECTION
 - *Streptococcus pneumoniae*
 - *Staphylococcus aureus*
 - *Hemophilus influenzae*

NON-PULMONARY COMPLICATIONS

- myositis (rare, > in children, > with type B)
- cardiac complications
- recent studies report encephalopathy
 - studies of patients <21 yrs in Michigan - 8 cases seen last season
- liver and CNS
 - Reye syndrome
- peripheral nervous system
 - Guillian-Barré syndrome

Reye's syndrome

- liver - fatty deposits
- brain - edema
- vomiting, lethargy, coma
- risk factors
 - youth
 - certain viral infections (influenza, chicken pox)
 - aspirin

Laboratory Diagnosis of Influenza Virus infections

- **Direct detection of viral antigens in infected cells**
Immuofluorescent staining
- **Isolation:**
 - **Inoculation into** the amniotic cavity of the chick embryo. detect HA then confirm & type by HAI
 - Primary Monkey Kidney cell lines
 - Detect HA & confirm and type by HAI of culture supernatant
 - Haemadsorption affinity of Tissue culture cells confirm and type by Haemadsorption Inhibition
 - **Serology:**
 - haemagglutination-inhibition (HAI)
 - ELISA
 - Complement fixation

Prevention and Treatment:

- Amantadine hydrochloride and one of its analogues, rimantadine, are antiviral drugs for systemic use in prevention of influenza “A”.
- They induce 70% protection against influenza “A” and should be considered in high risk groups.
- They also modify the severity of influenza “A” if administration is begun within 24-48 hours after onset of illness.

Prevention and Treatment:

- The **neuraminidase inhibitors** zanamivir (given by inhalation) and oseltamivir (orally) were approved in 1999 for treatment of both influenza A and B.
- To be maximally effective the drugs must be administered very early in the disease.

WHOM TO TREAT

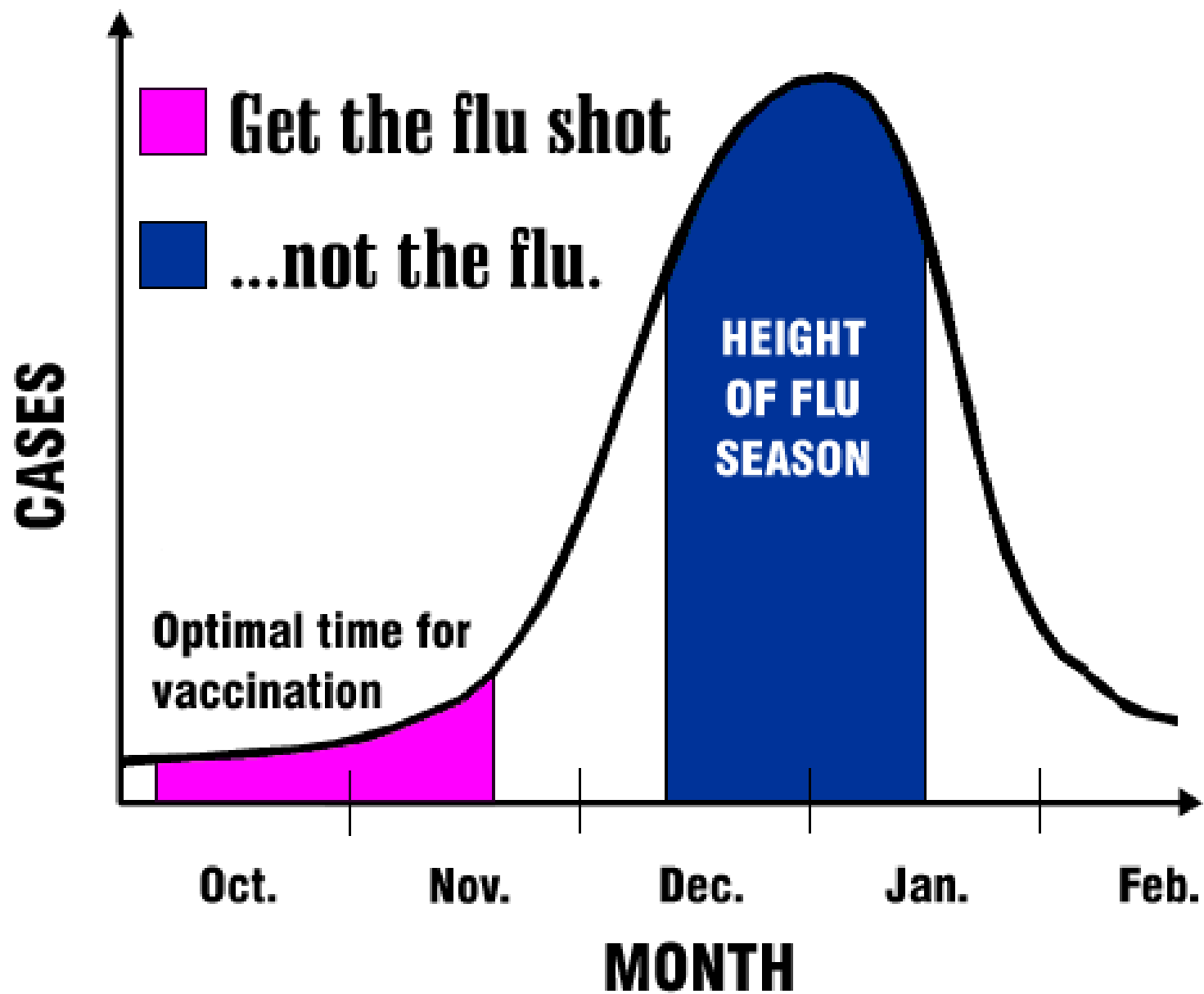
- Antiviral treatment with oseltamivir or zanamivir is recommended for all patients with confirmed or suspected influenza virus infection
 - **WHO ARE HOSPITALIZED**
 - or
 - **WHO ARE AT HIGHER RISK FOR INFLUENZA COMPLICATIONS**

Prophylaxis

Because of:

- **the short incubation period**
- &**
- **high attack rate**

The best to be done, is to use a suitable vaccine, and to immunize those at risk.



Inactivated influenza vaccines

- Is a cocktail containing **one or two type A viruses and a type B virus** of the strains isolated in the previous winter's outbreak.
- **Vaccines are either whole virus (WV) vaccine** which contains intact, inactivated virus
- **or subvirion (SV) vaccine:**
 - contains purified virus disrupted with detergents.
 - **Surface antigen vaccines (subvirion vaccine)** contain purified HA and NA glycoproteins.

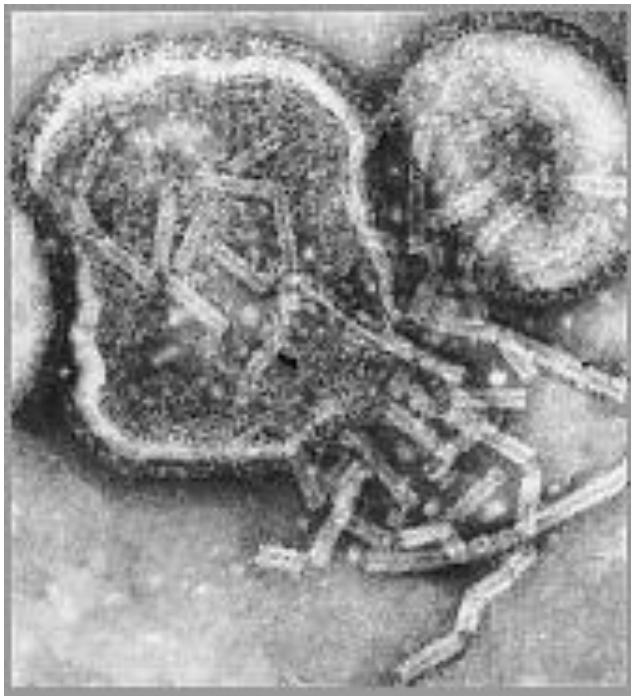
ALL THE ABOVE ARE EFFICACIOUS.

Live influenza vaccines:

- **A cold-adapted donor virus (able to grow at 25°C but not at 37°C)** introduced intranasally should replicate in the nasopharynx but not in the lower respiratory tract, its multiplication stimulate the local production of IgA. (Flumist)
- Approved for healthy people between 2-49yrs

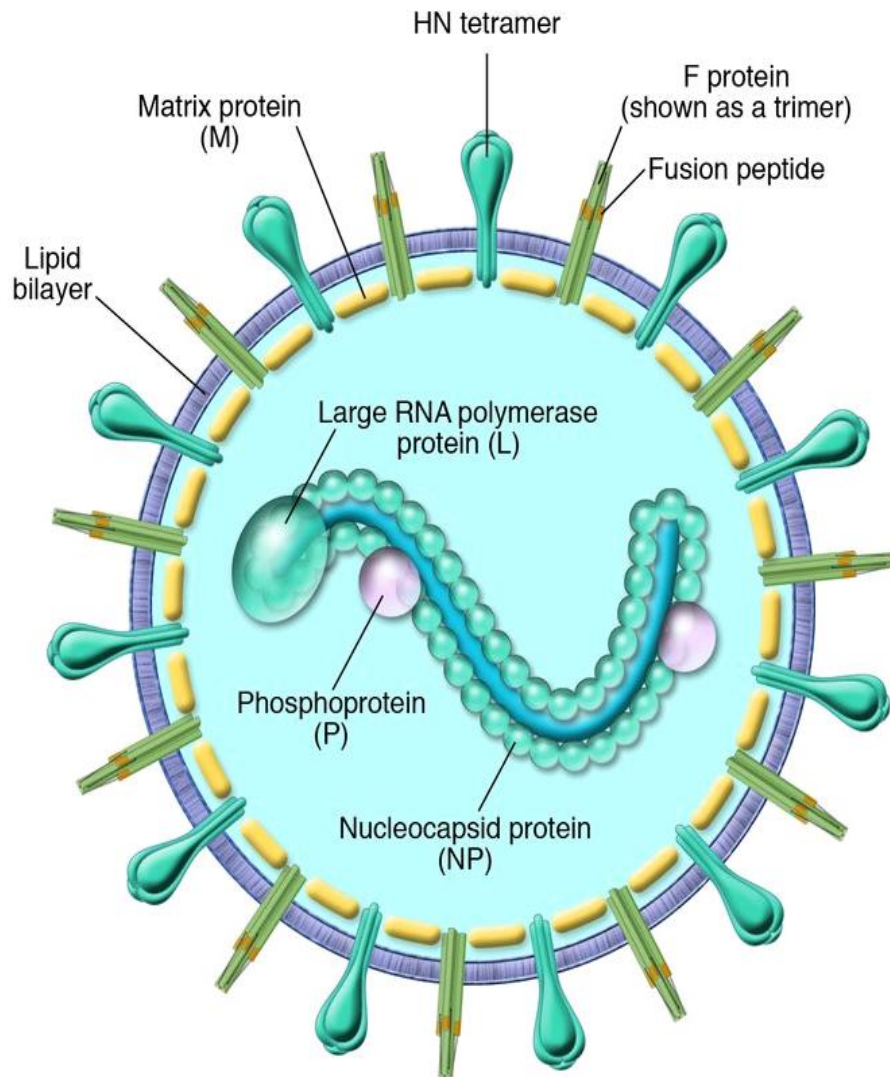
	TYPE A	TYPE B	TYPE C
severity of illness	++++	++	+
animal reservoir	yes	no	no
human pandemics	yes	no	no
human epidemics	yes	yes	no (sporadic)
antigenic changes	shift, drift	drift	Drift, stable
segmented genome	yes	yes	yes
amantadine, rimantidine	sensitive	no effect	no effect
zanamivir	sensitive	sensitive	
surface glycoproteins	2	2	(1)

Parainfluenza Virus



- ssRNA virus
- enveloped, pleomorphic morphology
- 4 serotypes: 1, 2, 3, 4 and 2 subtypes 4a and 4b

Morphology – Parainfluenza



- HPIVs are negative-sense, single-stranded RNA viruses that possess fusion and hemagglutinin-neuraminidase glycoprotein "spikes" on their surface. There are four serotypes types of HPIV (1 through 4) and two subtypes (4a and 4b).

Para Influenza Viruses types

- Four Types are present.
- Type 1 Acute croup, pharyngitis and tracheobronchitis. Fall months
- Type 2 Acute Laryngo tracheo bronchitis. Fall months
- Type 3 Lower Respiratory infection in children (Bronchitis, pneumonia and croup)
- Type 4 Upper Respiratory infection. Least common

Clinical Manifestations

- Croup (laryngotracheobronchitis) - most common manifestation of parainfluenza virus infection. However other viruses may induce croup e.g. influenza and RSV.
- Other conditions that may be caused by parainfluenza viruses include Bronchiolitis, Pneumonia, Flu-like tracheobronchitis, and Corza-like illnesses.

Laboratory Diagnosis

- **Detection of Antigen** - a rapid diagnosis can be made by the detection of parainfluenza antigen from nasopharyngeal aspirates and throat washings.
- **Virus Isolation** - virus may be readily isolated from nasopharyngeal aspirates and throat swabs. Monkey kidney cell cultures
- **Serology** - a retrospective diagnosis may be made by serology. EIA and IF.
- PCR

Management

- No specific antiviral chemotherapy available.
- Severe cases of croup should be admitted to hospital and placed in oxygen tents.
- No vaccine is available.

Respiratory Syncytial Virus (RSV)

- ssRNA enveloped virus.
- belong to the genus Pneumovirus of the Paramyxovirus family.
- Considerable strain variation exists, may be classified into subgroups A and B by monoclonal sera.
- Causes a sizable epidemic each year.
- Present world wide, yearly epidemics.
- Appears in Nov. or Dec. persists till Apr. or May.
- A strain predominant , the two strains circulate.
- Strain variation does not significantly affect the clinical severity.
- Peak incidence 2-5 months.
- In the 1st two years of life : one or more RSV infections
- More severe : Boys , lower socioeconomic classes.
- Reinfection throughout life is common.
- Milder than primary infection.

RSV : Pathogenesis & Immunity

- Incubation period :2-8 days.
- Ocular, nasal contact with infected secretions.
- Upper airway : cough & rhinorrhea.
- 50% primary infection spreads to lower tract.
- Bronchiolitis: lymphocyte infiltrate & epithelial proliferation.
- Obstruction: mucus & epithelium .
- Hyperinflation.
- Interstitial infiltrates: Pneumonia.

Clinical Manifestations

- Most common cause of severe lower respiratory tract disease in infants, responsible for 50-90% of Bronchiolitis and 5-40% of Bronchopneumonia
- Other manifestations include croup (10% of all cases).
- In older children and adults, the symptoms are much milder: it may cause a corza-like illness or bronchitis.

Infants at Risk of Severe Infection

1. **Infants with congenital heart disease** - infants who were hospitalized within the first few days of life with congenital disease are particularly at risk.
2. **Infants with underlying pulmonary disease** - infants with underlying pulmonary disease, especially bronchopulmonary dysplasia, are at risk of developing prolonged infection with RSV.
3. **Immunocompromized infants** - children who are immunosuppressed or have a congenital immunodeficiency disease may develop lower respiratory tract disease at any age.

Diagnosis:

- Young Children:
 - Season
 - Typical history
 - Physical examination
- Children & Adults:
 - Signs & Symptoms are less specific.
 - Chest x ray nonspecific
- Chest X rays:
 - Hyperinflation
 - Peribronchial thickening
 - Increased interstitial markings
 - Consolidation, Atelectasis

RSV: Diagnosis

- Infants:
 - Nasal wash
- Children & adults:
 - Swab from nasal turbinates+pharynx
- or bronchoalveolar lavage are the most likely to be positive
Specimens obtained by endotracheal tube
- Specimens for culture should be placed in viral culture media & kept cold during transport.
- RSV grows in multiple cell lines (Hep-2 & HeLa)
- Typical pattern: syncytial & giant cell , 3-7
- Fluorescein-labeled Ab are applied to cultures.

Treatment and Prevention

- Aerosolised ribavirin can be used for infants with severe infection, and for those at risk of severe disease.
- There is no vaccine available.
- RSV immunoglobulin can be used to protect infants at risk of severe RSV disease.

Common Cold Viruses

- Common colds account for one-third to one-half of all acute respiratory infections in humans.
- Rhinoviruses are responsible for 30-50% of common colds, coronaviruses 10-30%.
- The rest are due to adenoviruses, enteroviruses, RSV, influenza, and parainfluenza viruses, which may cause symptoms indistinguishable to those of rhinoviruses and coronaviruses.

RHINOVIRUSES

Rhinoviruses are the **most important cause** of :

- the common cold
 - and upper respiratory infection (URI).
- > 100 serotypes have been identified by:
- **neutralization with specific antisera.**



RNA VIRUSES

SINGLE STRANDED positive sense

ENVELOPED

ICOSAHERAL

FLAVIVIRIDAE
TOGAVIRIDAE
RETROVIRIDAE

HELICAL

CORONAVIRIDAE

NONENVELOPED

ICOSAHERAL

PICORNAVIRIDAE
CALICIVIRIDAE

SINGLE STRANDED negative sense

ENVELOPED

HELICAL

ORTHOMYXOVIRIDAE
PARAMYXOVIRIDAE
RHABDOVIRIDAE
FILOVIRIDAE
BUNYAVIRIDAE
ARENAVIRIDAE

DOUBLE STRANDED

NONENVELOPED

ICOSAHERAL

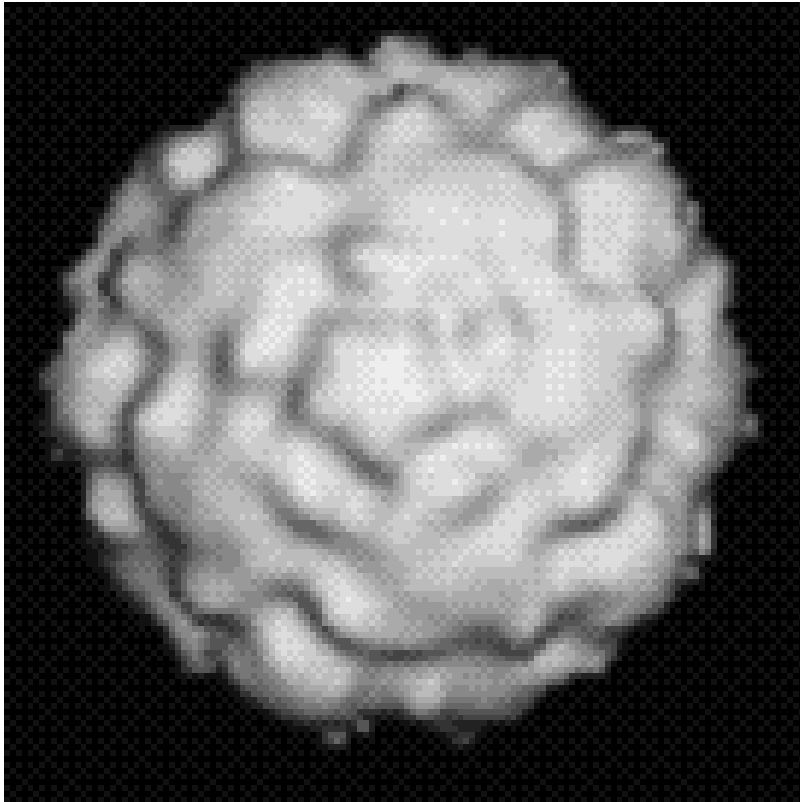
REOVIRIDAE

Genus
enteroviruses

Genus rhino
>100 antigenic
types

Genus hepatovirus:
Hepatitis A virus

Rhinovirus



Reconstructed Image of rhinovirus particle (Institute for Molecular Virology)

- ssRNA virus
- Belong to the picornavirus family
- Small 20-30nm
- Icosahedral symmetry
- Non enveloped-Ether resistant
- acid-labile
- at least 100 serotypes are known

Pathogenesis:

- In contrast to enteroviruses, rhinoviruses are **unable to replicate in the gastrointestinal tract.**
- Rhinoviruses grow best at **33 °C**, which may partly account for their **predilection** for the cooler environment of the nasal mucosa.
- Most viral replication occurs in the nose, and the **severity** of symptoms correlates with the **quantity (titer) of virus in nasal secretions.**
- The receptor for rhinovirus is glycoprotein intercellular adhesion molecule 1 (ICAM-1).

Epidemiology:

- **Rhinoviruses can be transmitted by two mechanisms:**

Aerosols

NOT THE MAJOR ROUTE

and

Contact: (DIRECT & INDIRECT)

Clinical Syndromes:

- URIs caused by rhinoviruses usually begin with sneezing, followed soon by rhinorrhea.
- The rhinorrhea increases and is then accompanied by symptoms of nasal obstruction.
- Mild sore throat occurs along with
- headache, malaise and the “chills” (rigors).
- The illness peaks in three to four days or longer.

Laboratory Diagnosis

Culture:

- Nasal washing is the best clinical specimen for recovering the virus.
- Rhinoviruses grow **ONLY IN** in vitro on:
 - **Cells of primate origin,**
 - **Human diploid fibroblast cells**

Virus isolation –

Rhinoviruses are best isolated in **human embryo lung fibroblasts** or a sensitive continuous cell line such as **HeLa**.

Samples should be inoculated into triplicates and rolled at 33oC.

The virus CPE, which consists of the **rounding of cells** similar to that induced by enteroviruses should appear within **8 days of inoculation**.

The identity can be confirmed by acid lability tests. (pH3)

Direct detection of rhinovirus antigen –

an ELISA has been developed for the detection of rhinovirus antigen in nasal washings.

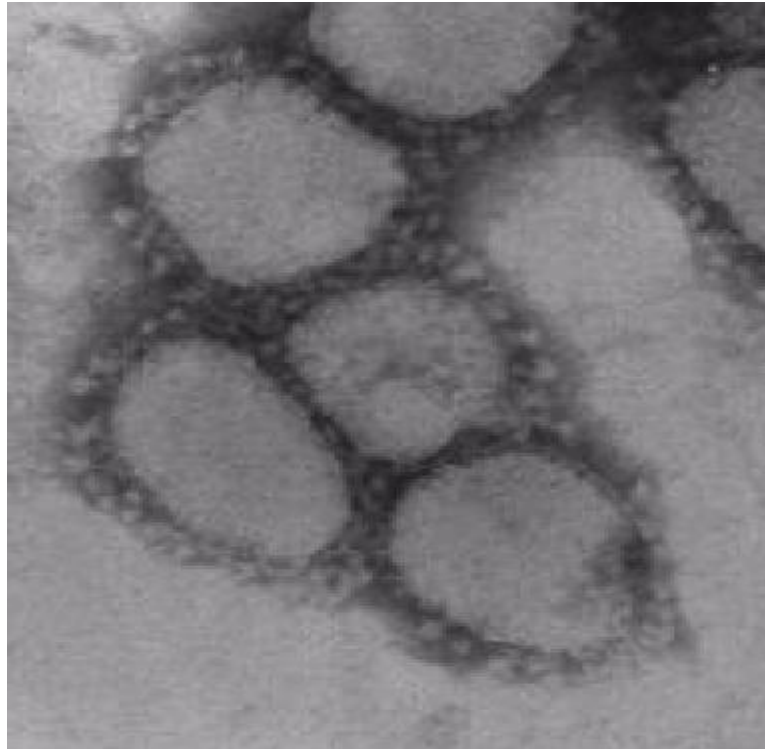
Serology - virus neutralization tests remain the best method.

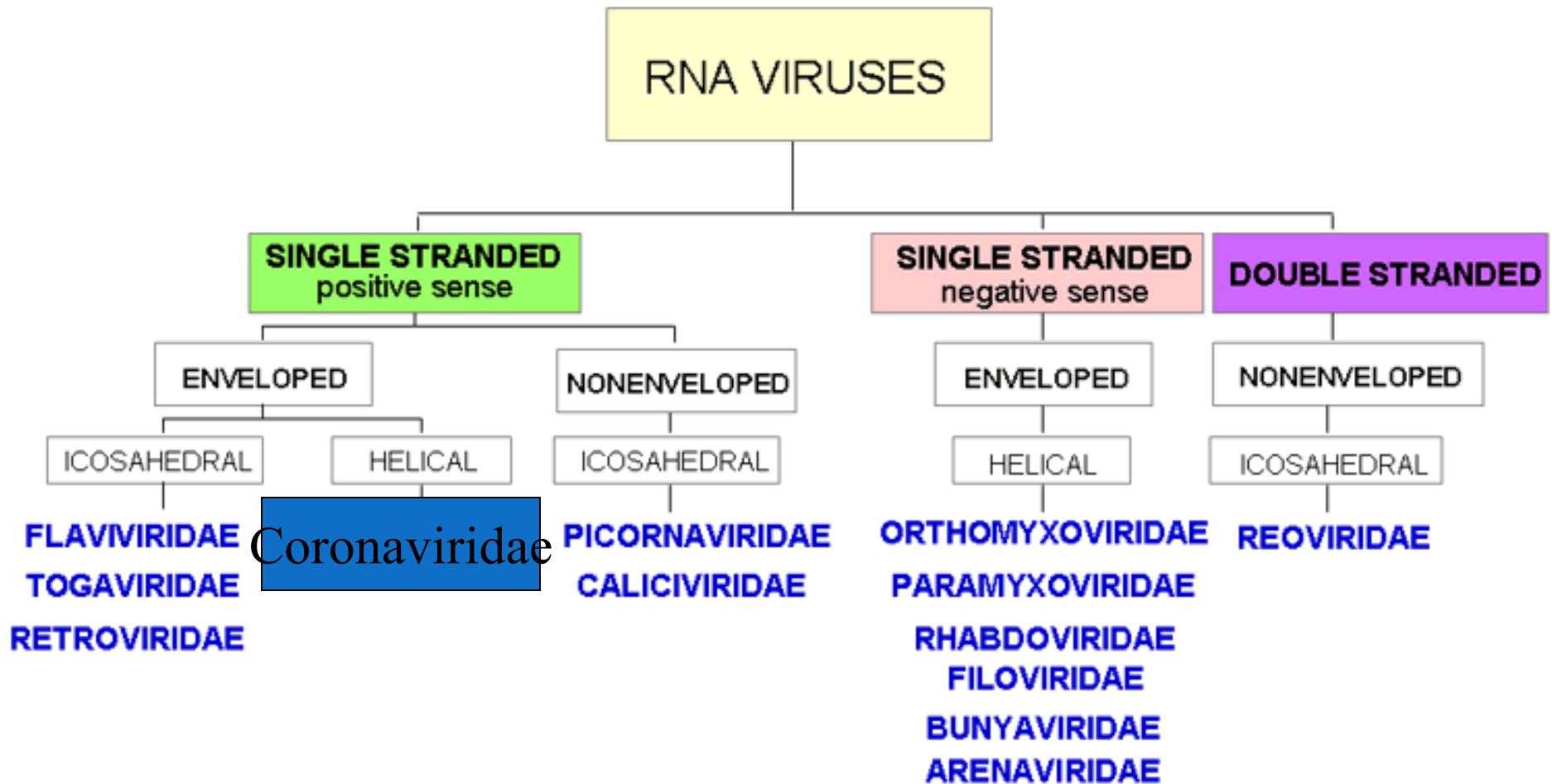
ELISAs have been described.

Prevention and Treatment:

- **No antiviral drug** has been proved useful.
- **No vaccine.** The multiplicity of serotypes and the fleeting immunity pose major problems for the development of vaccines.
- **Hand Hygiene** is the most potent method of prevention and control

CORONAVIRUS

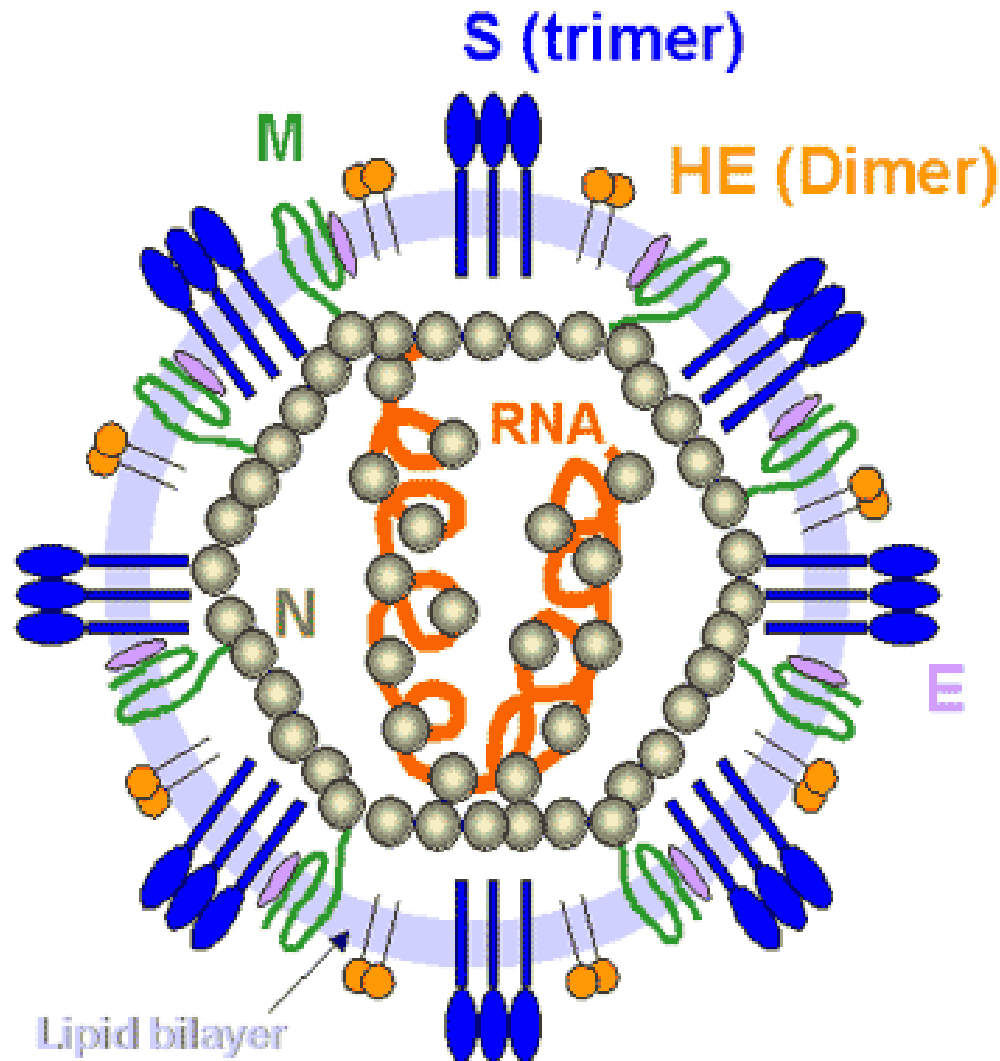




CORONAVIRUSES

The genome

- SS linear non segmented +ve sense RNA
- The largest among RNA viruses.
- Enveloped, RER and Golgi origin
- 2 serogroups: OC43 and 229E



The family coronaviridae is composed of two genera:

- **Genus Coronaviruses**
- **Genus Torovirus:**
 - **widespread in horses & cattle**
 - **associated with gastroenteritis.**

Genus Coronaviruses

- **Genus Coronaviruses are difficult to isolate in cell culture**
- **So infections with this virus are rarely diagnosed in clinical practice**

Relationship to human infections

- **Based on serologic studies, coronaviruses cause respiratory tract infections and pneumonia in humans.**
- **Electron microscopy links coronaviruses to gastroenteritis in infants, children and adults (tropism to epithelial cells)**

Genetic variation & evolution of new strains

a high frequency of:

- **deletion mutations**
- **high frequency of recombination during replication which is unusual for an RNA virus with unsegmented genome**

Clinical picture & epidemiology

- **Upper respiratory infections, similar to “colds” caused by rhinoviruses, but with a longer incubation period (average three days).**
 - **15-30% of respiratory illness in adults during winter months but lower respiratory infections were rare.**
 - **Antibodies appear early in childhood and are found in 90% in adults**
 - **CORONAVIRUSES may be associated with gastroenteritis which occurs year-round.**