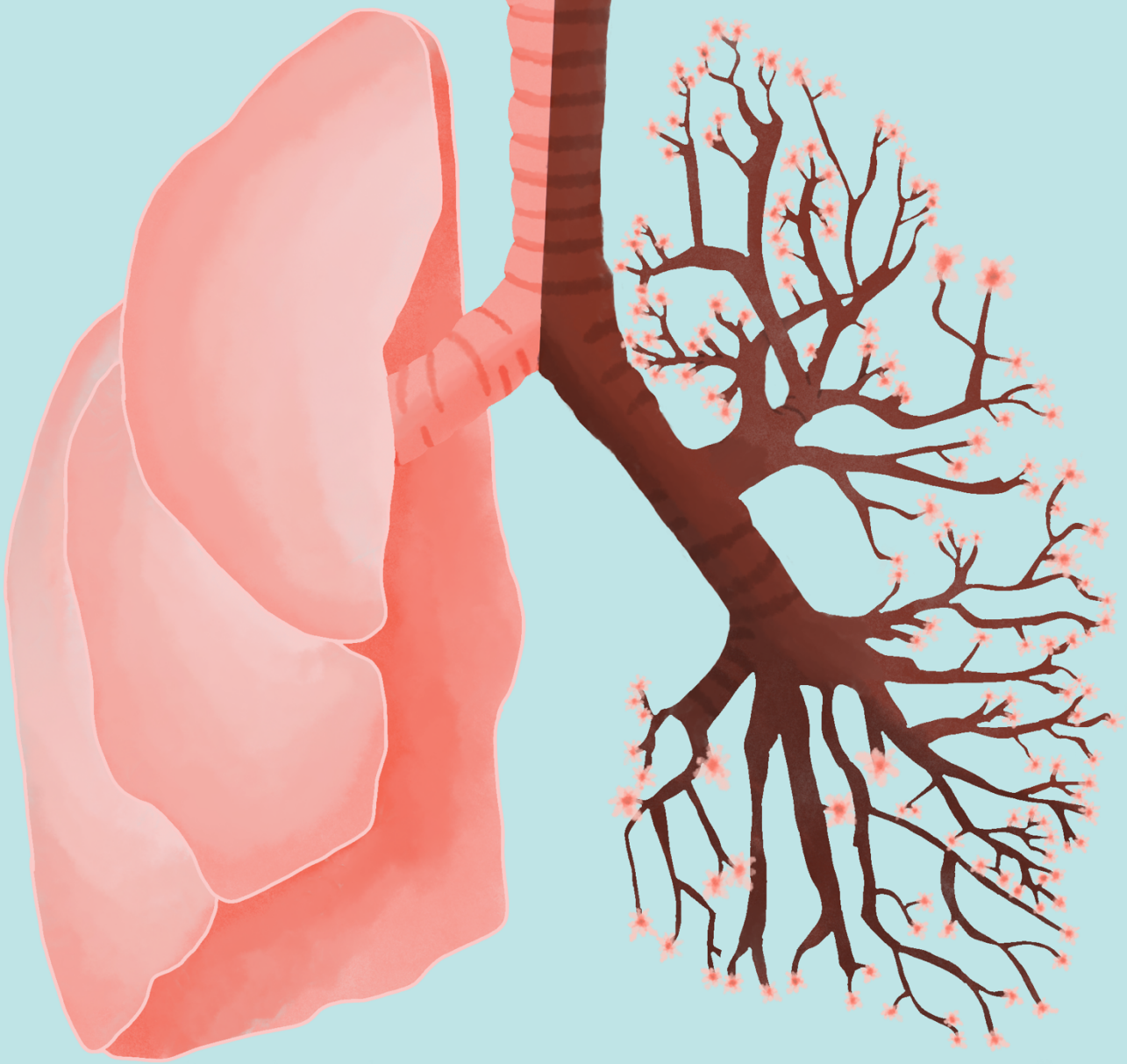
RESPIRATORY SYSTEM

MICROBIOLOGY



Title: Sheet # 1 – Influenza (flu)

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What's **written in bold** is what the doctor said in the video. **What's underlined is important.** (what's neither, is just written in the slides).

Overview about influenza and influenza viruses:

Influenza is an **acute** respiratory illness, caused by influenza viruses **(highly contagious, by droplets, airborne, as well as direct and indirect contact) (short incubation period: 1-3 days).**

Droplet: when someone coughs in front of you.

Airborne: when someone coughs and leave the place, but the virus particles are still in the air for a long time, then you come and inhale it. In this mode of transmission, virus particles can move with dust.

Acute viral respiratory illnesses account for one-half (50%) or more of all acute illnesses.

One of the diseases that **keep emerging and re-emerging. (because of antigenic variation).**

The season of the virus starts from late November until late March (when its season starts, people start taking vacations, and even children start missing schools).

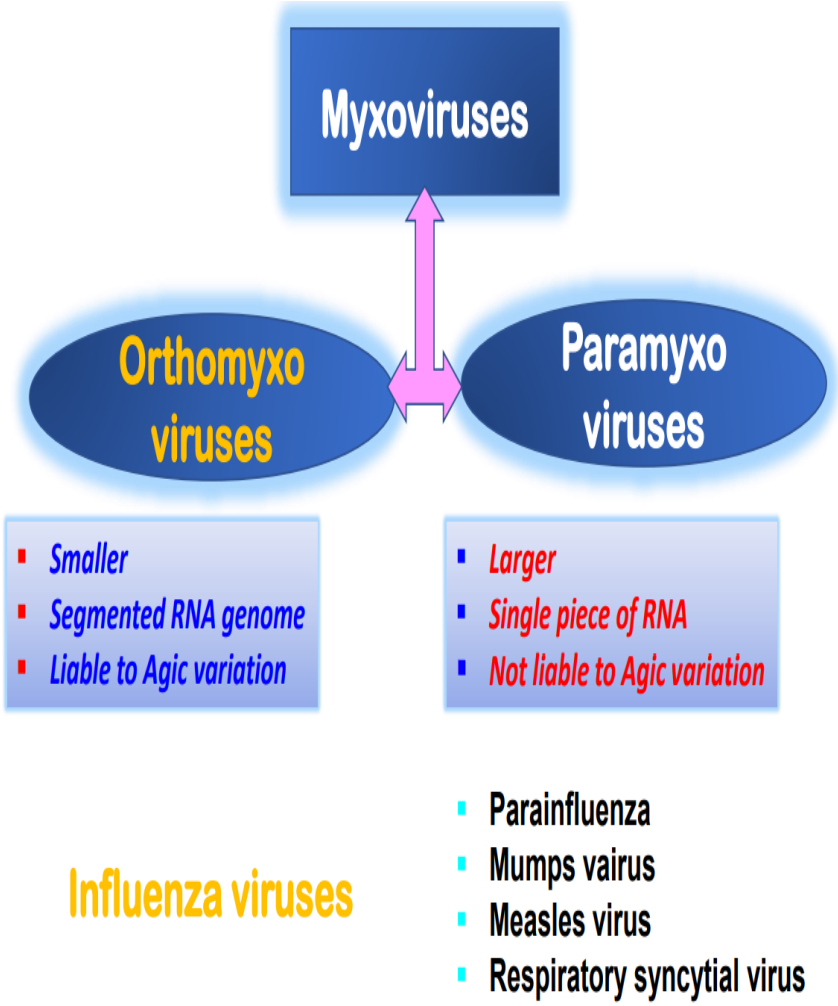
Influenza mainly affects upper respiratory tract, rarely involve lungs (lower respiratory tract), accompanied by **systemic** signs and symptoms such as fever, headache, myalgia (muscle pain), and weakness.

Outbreak (spread) of illness of variable extent and severity occur nearly every year. Such outbreaks result in significant morbidity rates in the general population and in increased mortality rates among certain high-risk patients, mainly as a result of pulmonary complications.

Myxoviruses (order) has 2 main families: orthomyxo viruses & paramyxo viruses.

Influenza virus is a negative sense RNA virus, and has <u>8 segmented RNA genome</u> (v. important), its family is the orthomyxo viruses.

Paramyxo viruses are: measles virus, mumps virus, para influenza virus, and respiratory syncytial virus (most common cause of bronchiolitis - inflammation of bronchioles).



"Myxo" (Greek word for mucus) **because of their high affinity to mucin.**

We have 4 types of influenza: A, B, C and D (D is the only type that doesn't affect humans).

Typing is based on 2 things:

1. **Nucleocapsid (ribonucleo protein)**
2. **Matrix protein**

Subtypes are based on 2 things: <u>hemagglutinin (HA) and neuraminidase (NA).</u>

The nowadays circulating subtypes are: <u>H3N2 and H1N1</u>. (H5N1, H7N9 from avian, not really efficient in its transmission between humans).

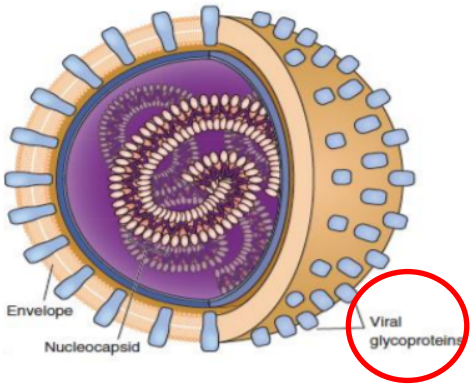
C type has only 7 segments (lacks the neuraminidase segment).

8 segments in A and B.

Almost all influenza viruses originate from birds. One of the exceptions is HA (17 and 18) AND NA (10 and 11) that originate from bats.

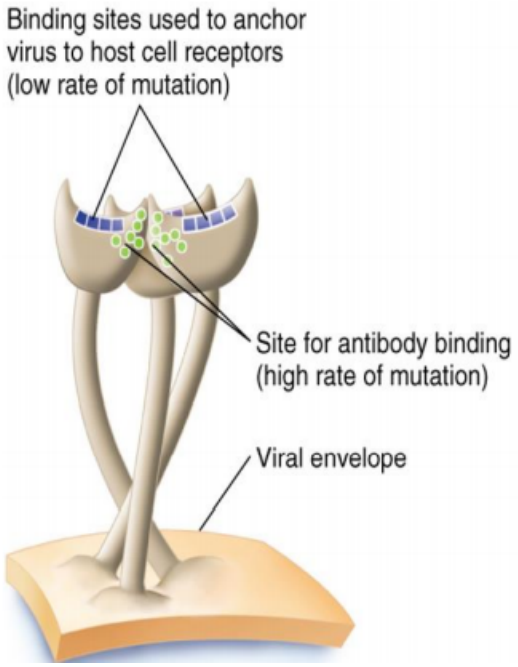
Influenza Structure and components:

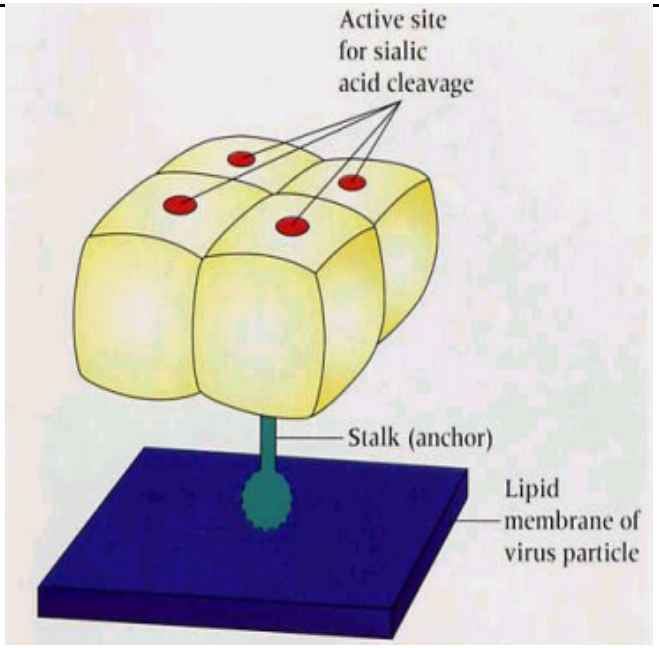
This picture is taken by an electron microscope, its spherical in shape, and has projection spikes (used to determine subtype of Influenza virus).



Those viral glycoproteins are HA (hemagglutinin), and NA (neuraminidase).

HA	NA
Has the ability to agglutinate RBCs from various animal species. It's the target of neutralizing antibodies.	**Acts on neuraminic acid (component of mucus in the respiratory tract). Also increase the rate of spreading by lowering the viscosity of the mucous film in the respiratory tract. Minimal role in immunity.**
Fusion and initiation of infection because it binds with a glycoprotein on epithelial cells (mucus secreting cells) – the sialic acid(receptor). HA is synthesized as a precursor protein and requires cleavage by host cell **proteases** to gain its fusion capacity. These proteases are mainly found in the upper respiratory tract. That's why influenza viruses usually infect upper respiratory tract	**Cleavage and spreading of infection between infected cell and susceptible cell.**
Structure: **Trimer of dimers with a binding site at the top for the receptor.**	**Structure:** **Box- shaped tetramer of identical monomers, with stalk that anchors it to the cellular membrane.**
We have 18 known Has.	**We have 11 known NAs.**

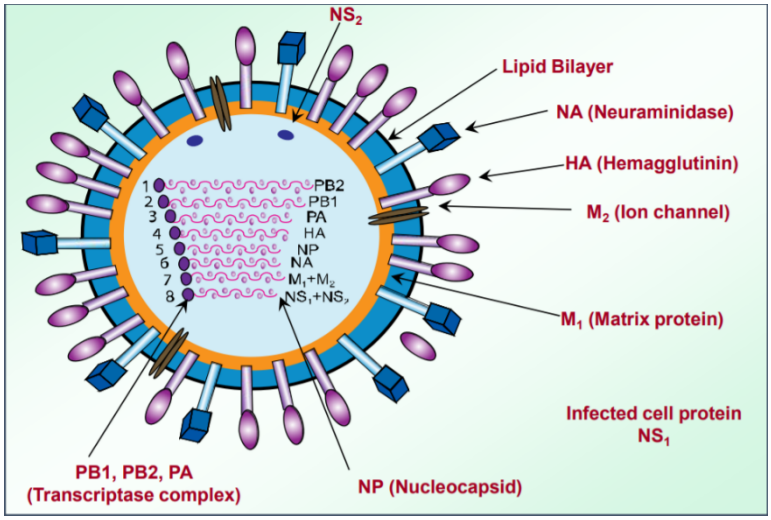




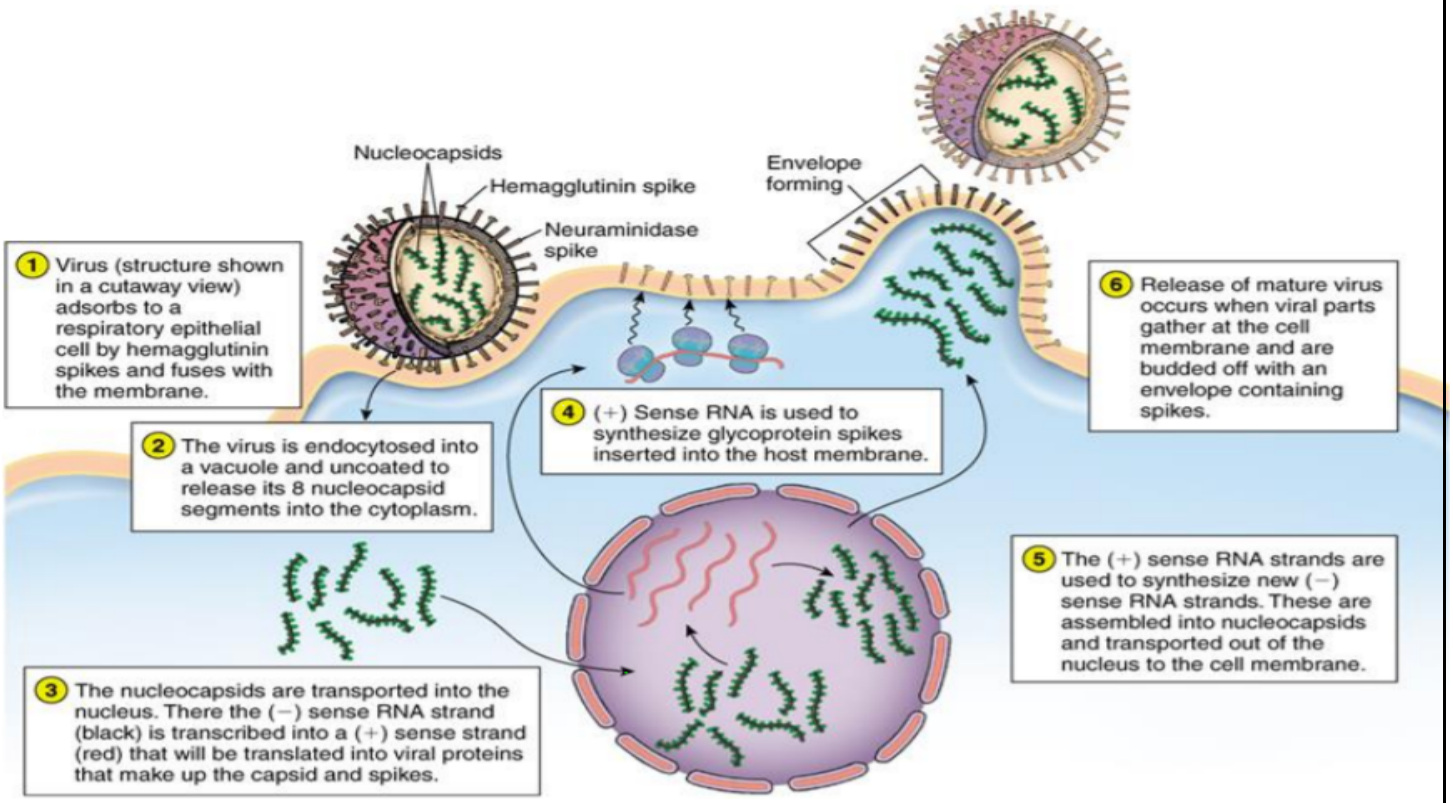
As you can see in this picture, we have 8 RNA segments (except for type C).

PB1, PB2, PA are polymerases (work in transcription).

On the envelope we have the spikes (HA & NA) and what connects them to the core is the matrix protein.



Influenza virus' replication cycle



Receptor mediated endocytosis: Here we have 6 steps, which are:

- **HA binds with sialic acid (a surface receptor).**
- **It gets inside the cell as an endosome, RNA segments get released in the cytoplasm.**
- **They get transported to the nucleus, where replication takes place. (influenza viruses, in addition to retro viruses – like HIV- are the only viruses whose replication takes place in the nucleus).**
- **HA and NA synthesis take place in the ER.**
- **Final assembly takes place in host's cell membrane.**

Types of Influenza viruses:

A. **Type A virus: most common, most dangerous because of the probability to create an antigenic variations called antigenic shift, (happens specifically in this type because the host reservoir is very wild) (Results in major changes in the subtype) as well as antigenic drift (minor changes in the subtype).**
   antigenic shift: It is the process in which the genetic segment encoding for envelope glycoproteins (HA&NA) is replaced by another one from a different strain through genetic reassortment causing replacement of the original HA or NA by a new one.
   It affects humans as well as animals (swine & avian – birds).
   Can result in pandemics (because of the antigenic shift).
   Was the cause of 8-10 pandemics in the last 100 years (e.g. Spanish Flu).
   The antigenic shift creates a completely new sub-type, and because your immune system hasn't faced this before, that's why it has high mortality rate.

B. **Type B virus: affects only humans (can be seen in seals, but still not as wild as type A).**
   Minor outbreaks, sometimes results in epidemics, but never results in pandemics.

C. **Type C virus: affects only humans (can be seen in swine, but still not as wild as type A).**
   Only sporadic cases (infrequently and irregularly).

In terms of severity: Type A > Type B > Type C

In terms of antigenic stability: Type C > Type B >Type A

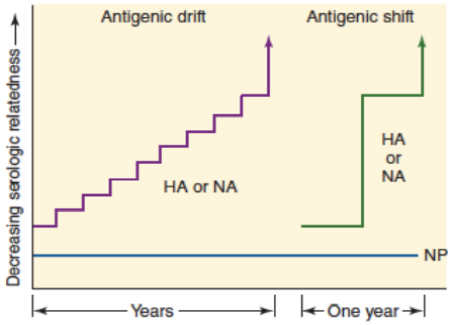
Pigs are susceptible to avian, human & swine influenza viruses and they potentially may be infected with influenza viruses from different species. If this happens, it is possible for the genes of these viruses to mix and create a new virus.

Antigenic shift: It is the process in which the genetic segment encoding for envelope glycoproteins (HA&NA) is replaced by another one **from a different strain through genetic reassortment causing replacement of the original HA or NA by a new one.**
It's characterized by Major changes, new subtypes and May result in pandemic.
Genetic reassortment: is the exchange of genetic material "a whole segment" between viruses inside a host cell.

Example: if human influenza virus and duck influenza virus infect a peg at the same time, the duck HA could go inside the human influenza virus producing a new subtype which is highly virulent because our immune system doesn't have a recall for duck HA.
Antigenic drift: Caused **by point mutations in gene, minor change of an amino acid** sequence of HA or NA.
It's characterized by minor changes, same subtype and may result in epidemics.
Antigenic drift could result in a new subtype, but it needs a lot of time.

Notice that the antigenic drift (minor changes) is very slow and takes many years to result in a new subtype.

While antigenic shift (major changes) on a sudden onset which results in a new subtype immediately.



This table shows the most important 4 pandemics in the last 100 years, Spanish Flu is the most known among all. 20 million documented deaths were caused by it. (400-100 million are the estimated number, but it's Not documented)

Name of pandemic	Date	Deaths
Spanish Flu	1918-1920	40 -100 million
Asian Flu	1957-1958	1 - 1.5 million
Hong Kong Flu	1968-1969	0.75 - 1 million
Swine Flu	2009-2010	0.15-0.6 million

Here is a table to revise the differences between the 3 types:

	Severity	Stability	Number of segments	Animals and humans	Shifting and drifting	Pandemics and epidemics
Type A	Most severe	Least stable	8	Both	Both	Both
Type B	Intermediate	Intermediate	8	Just humans	Only drifting	Only epidemics
Type C	Least severe	Most stable	7 (lacks NA)	Just humans	Only drifting	Sporadic cases

Nomenclature:

The standard nomenclature system for influenza virus isolates includes the following information: **type, host of origin, geographic origin, strain number, and year of isolation.** Antigenic descriptions of the HA and the NA are given in parentheses **for type A. The host of origin is not indicated for human isolates**, such as A/Hong Kong/03/68(H3N2), but it is indicated for others, such as A/swine/Iowa/15/30(H1N1).

Pathogenesis:

1. **Viral NA degrades the protective mucin layer, Allowing the virus to enter the cells.**
2. **Replication inside the cells.**
3. **Cilia damage.**
4. **Epithelial desquamation.**

Symptoms appear due to cytokines production. (symptoms are systemic, like fever, chills, myalgia, in addition to minor respiratory symptoms like a runny nose, cough).

In influenza infection, we have no viremia – no infection in the blood.

Duration of shedding:

1 day before symptoms and signs, and until the 7th day (could reach 10 days). (that's what the doctor said regarding of what's written in the slides), **peak of the shedding is in the first 48 hours after symptoms and signs appear. (short incubation period: 1-3 days).**

Clinical Findings:

Systemic symptoms and signs. (Fever, arthralgia, myalgia, chills)

Respiratory symptoms may appear, like cough (usually nonproductive "NO sputum"), shortness of breath, and runny nose.

Systemic findings are more obvious.

Evidence of lower respiratory tract disease with opacities, consolidation, and infiltrates noted on chest imaging.

More severe infections (i.e. pneumonia) are sometimes associated with Influenza because of the increased susceptibility to other infections as a result of a damaged airway.

Complications:

Influenza itself is not fatal, it is the complications (mainly pneumonia) that we are afraid of (especially in high risk group) .

Pneumonia can be viral (influenza virus directly involves the lungs), and can be secondary bacterial (Streptococcus pneumoniae**, Staphylococcus aureus,** and Haemophilus influenzae)**, can be mixed viral and bacterial as well.**

Usually in secondary bacterial pneumonia, patients get better then become sick again (there is a space between pneumonia symptoms and Flu symptoms). However, in primary influenza pneumonia, this time (space) isn't existed.

The most common cause of superimposed infection post influenza infection is staph aureus.

Other complications: Septic shock, Respiratory failure, Acute respiratory distress syndrome, Refractory hypoxemia, Acute renal dysfunction, Multiple organ dysfunction, Rhabdomyolysis, **Encephalopathy (Reye syndrome) "don't give children aspirin"**, bacterial and fungal infections such as ventilator associated pneumonia and blood stream infection sometimes by multi drug resistant bacteria.

This table shows the high risk groups of people, the doctor only mentioned **children under 2 years, pregnant women or postpartum, adults above 65 years, and resident of nursing homes and other chronic care facilities, these high risk groups should be treated immediately.**

▪ Children <2 years*
▪ Adults ≥65 years of age
▪ Persons with chronic pulmonary (including asthma), cardiovascular (except hypertension), renal, hepatic, hematologic (including sickle cell disease), metabolic (including diabetes mellitus), neurologic, neuromuscular, and neurodevelopmental disorders (including disorders of the brain, spinal cord, peripheral nerve and muscle such as cerebral palsy, epilepsy, stroke, intellectual disability [mental retardation], moderate to severe developmental delay, muscular dystrophy, or spinal cord injury)
▪ Immunosuppression (including immunosuppression caused by medications or by human immunodeficiency virus)
▪ Women who are pregnant or postpartum (within 2 weeks after delivery)
▪ Children <19 years of age and receiving long-term aspirin therapy
▪ Native Americans and Alaskan Natives
▪ Morbidly obese (body mass index [BMI] ≥40 for adults or BMI >2.33 standard deviations above the mean for children)
▪ Residents of nursing homes and other chronic care facilities

Laboratory Diagnosis:

In epidemics, very easy diagnosis by clinical picture (all patients complain from the same symptoms and signs)

1. **Definitive diagnosis is isolation (culture) of the virus. (the problem is that it takes a long time – 3-10 days).** Classically, **embryonated eggs and primary monkey kidney cells have been the isolation methods of choice** for influenza viruses, although some continuous cell lines may be used in the presence of

trypsin, which cleaves and activates the HA so that replicating virus will spread throughout the culture. Cell cultures can be tested for the presence of virus by hemadsorption 3-5 days after inoculation, or the culture fluid can be examined for virus after 5-7 days by hemagglutination.
At the past, we weren't able to do influenza test for anyone has allergy to eggs because the only way was to use an egg-based agar.

2. **Because of that, most countries –including Jordan- use PCR tests to diagnose influenza (high sensitivity, high specificity, and very rapid– 3 to 4 hours to diagnose if the patient has influenza and which subtype).**

3. **Serology can also be used, but also takes a long time to give results.** Antibodies to several viral proteins (hemagglutinin, neuraminidase, nucleoprotein, and matrix) are produced during infection with influenza virus. The immune response against the HA glycoprotein is associated with resistance to infection. Routine serodiagnostic tests in use are based on hemagglutination inhibition (HI) and enzyme linked immunosorbent assay. **Paired acute and convalescent sera are necessary because normal individuals usually have influenza antibodies. A three to four fold or greater increase in titer must occur to indicate influenza infection.** Human sera often contain nonspecific mucoprotein inhibitors that must be destroyed before testing by HI.

Treatment and Prevention:

We depend a lot on vaccine. It's an annual vaccine. (High risk group mentioned above, are the people who must be vaccinated).

It can be a whole vaccine (W) "grown in embryonated eggs" **, or a subunit vaccine (S) (includes NA and HA)**

1. **Flu shot or inactivated (killed) vaccine: (given with a needle) Trivalent used in Jordan (2 subtypes of type A Influenza and 1 subtype of type B influenza).**

America uses quadrivalent vaccine (2 type A, 2 type B)

2. **Live attenuated (Flu mist): intra nasal, (Tri and Quad available). Not given to pregnant women.**
   Previously, the ABSOLUTE contraindication for this type of vaccines was giving it to persons who have egg proteins allergy.
3. Split virus vaccines: purified HA (lessen the side effects)

If there is NO contraindications, live attenuated vaccines are better than the killed ones.

Treatment:

Matrix protein (M2) inhibitors: amantadine, not given anymore (All viruses became resistant).

NA inhibitors: ends with 'vir', (e.g. oseltamivir, zanamivir). Note that this type of drugs can't be used to treat type C influenza viruses because they don't NA proteins.

Treatment is indicated for any patient requires hospitalization or at increased risk of complications. Early treatment is the key and you shouldn't wait for confirmatory tests to treat. Post exposure prophylaxis shouldn't be used unless for high risk group persons with close unprotected exposure. Post exposure prophylaxis isn't used at all if more than 48 hours have passed after exposure.

Habits to decrease influenza spreading:

When Healthy:

- Avoid close contact with those who are sick.
- Wash your hands often .
- Avoid touching your eyes, nose and mouth to decrease the spread of germs .

When ill:

- Cover your mouth and nose with a tissue (or upper sleeve) when you sneeze or cough.
- Stay home from work or school when you are sick.

Other types of influenza:

Avian influenza: the disease is in the birds NOT humans.

In wild birds especially migratory waterflow (e.g. ducks and geese) are considered the natural reservoirs "the infection is silent (that's why we can't eradicate influenza)". However, domestic poultry like chickens and turkeys develop clinical disease (that's why we use chicken to predict the next circulating strains).

Transmission: their feces (virus can live there for 3 months), direct contact and droplets to a less degree.

High pathogenic and low pathogenic (both affect humans) H5N1 and H7N9 are NOT effective to circulate between humans, but they are bad and affect lower respiratory tract (Lungs).

It's thought that H5N1 infects lower respiratory tract because the proteases that is needed to activate its HA are found mainly in the lower portions of the tract.

The virus should have 3 criteria to initiate a pandemic:

1.New subtype (Antigenic Shift).

2.Efficient human to human transmission.

3.Produce severe disease in humans.

Swine influenza: (unlike avian flu, the wild swine show signs and symptoms)

Swine Influenza (swine flu) is a respiratory disease of pigs caused by type A influenza viruses (H1N1 subtype) that causes regular outbreaks in pigs. People do not normally get swine flu, but human infections can and do happen. Swine flu viruses have been reported to spread from **person to person**, but in the past, this transmission was limited and not sustained beyond three people.

Good Luck