Paramyxoviruses (RSV, Measles and Mumps) and Rubella

and

Orthomyxoviruses (Influenza)

Medical Microbiology; PTM 4310 (MBChB)

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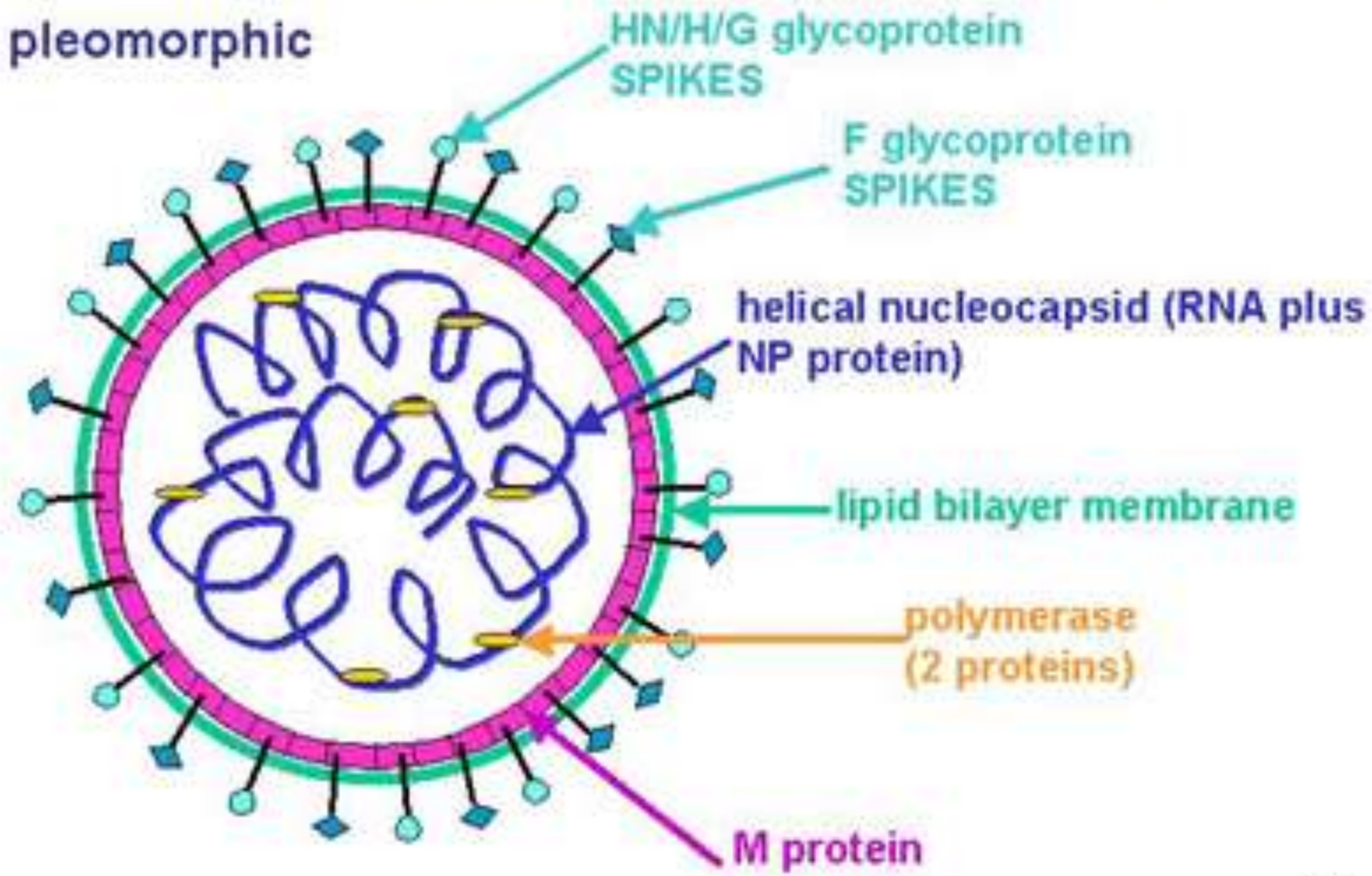
PARAMYXOVIRUSES



Paramyxoviruses

- Include most important etiologies of respiratory infections in infants and young children (RSV and Parainfluenza viruses) as well as common childhood contagious illnesses (measles and mumps)
- Acute respiratory infections and pneumonia are responsible for 4 million in children below 5 years annually world-wide (WHO)
- Paramyxoviruses initiate infection via respiratory tract
  - Parainfluenza viruses and RSV replicate in the respiratory tract
  - Measles and mumps viruses become disseminated systemically to produce generalised disease
  - Rubella virus (Togaviridae) can be considered a Paramyxovirus on epidemiological basis
- Outstanding features:
  - Antigenically stable (very good vaccine candidates)
  - Particles are labile yet very infectious

Properties of Paramyxoviruses

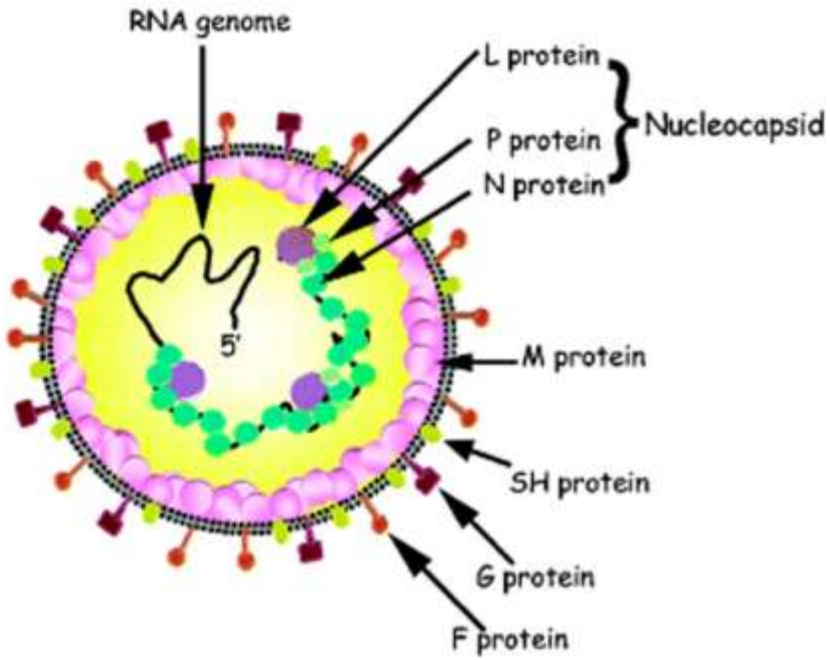
- Virion
  - Spherical, pleomorphic, 150nm in diameter, helical necleocapsid of about 13-18nm
- Composition
  - RNA (1%), protein (73%), lipid (20%), carbohydrate (6%)
- Genome
  - Negative sense, single-stranded, non-segmented RNA genome of about 15kb
- Envelop
  - Contains glycoproteins (G, H or HN) which sometimes has hemmaglutinin or neuraminidase activity and fusion protein F; very fragile
- Replication
  - Cytoplasm; particles bud from plasma membrane

Genome of Paramyxoviruses

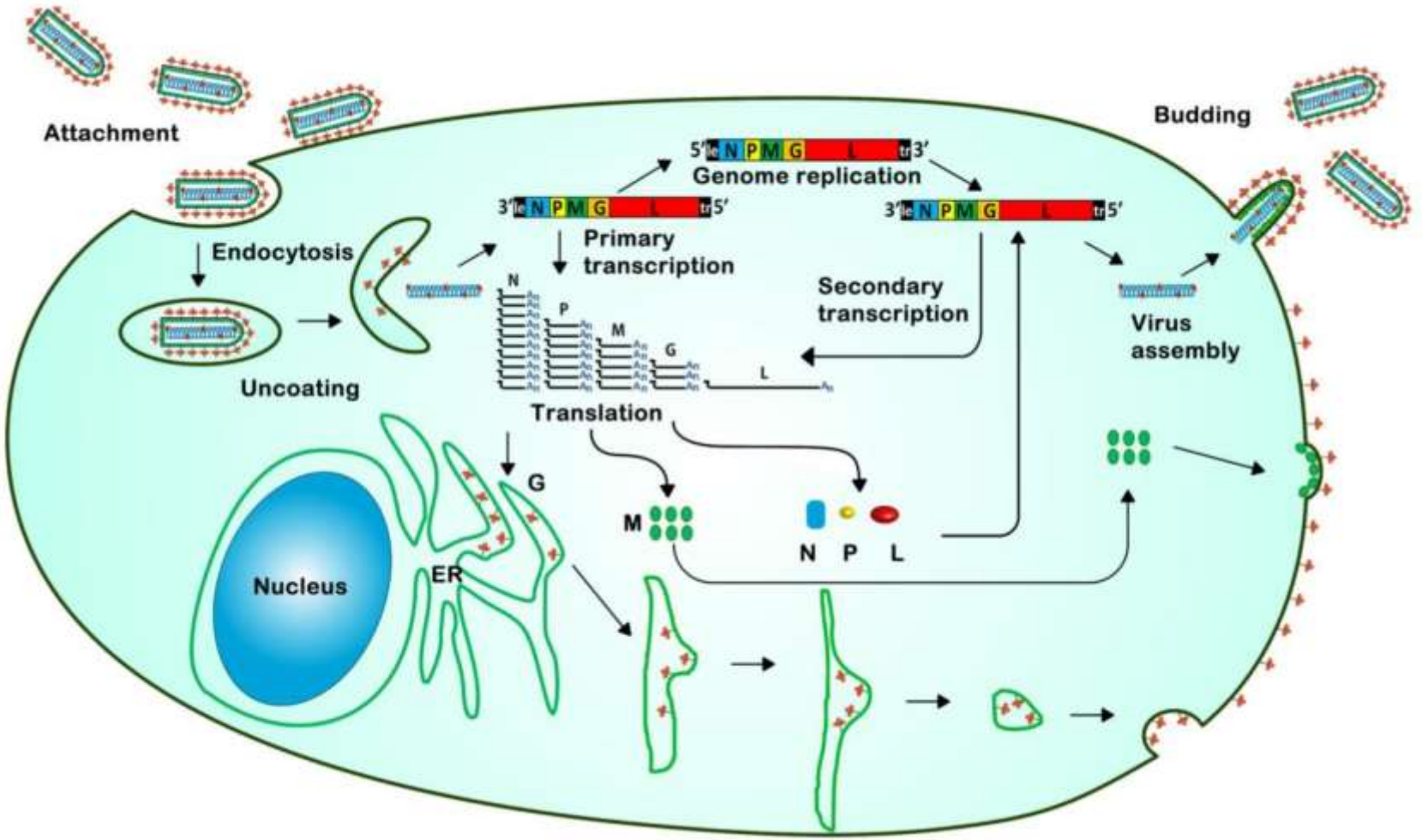
(a)

Genome		Protein size(aa)	MW (kDa)	Function
3' UTR				
NS1	NS1	124	22	Non-structural proteins: Viral replication activity
NS	NS2	139	18	
N	N	391	44	Nucleocapsid protein: Transcriptional activity -binds tightly to RNA
P	P	241	34	Nucleocapsid phosphoprotein: Transcriptional activity chaperonin and polymerase cofactor
M	M	256	28	Matrix protein: Viral assembly
SH	SH	64	7.5-30	Small hydrophobic protein: Function unknown?
G	G	298	90	Attachment protein: Viral attachment to the cell
F	F	574	70	Fusion protein: Viral entry and syncytium formation
M2	M2-1	194	22	Transcription regulation: Transcription elongation factor
	M2-2	90	11	Transcription regulation: Regulation of viral transcription
L	L	2165	~200	Polymerase protein: RNA polymerase
5' UTR				

(b)

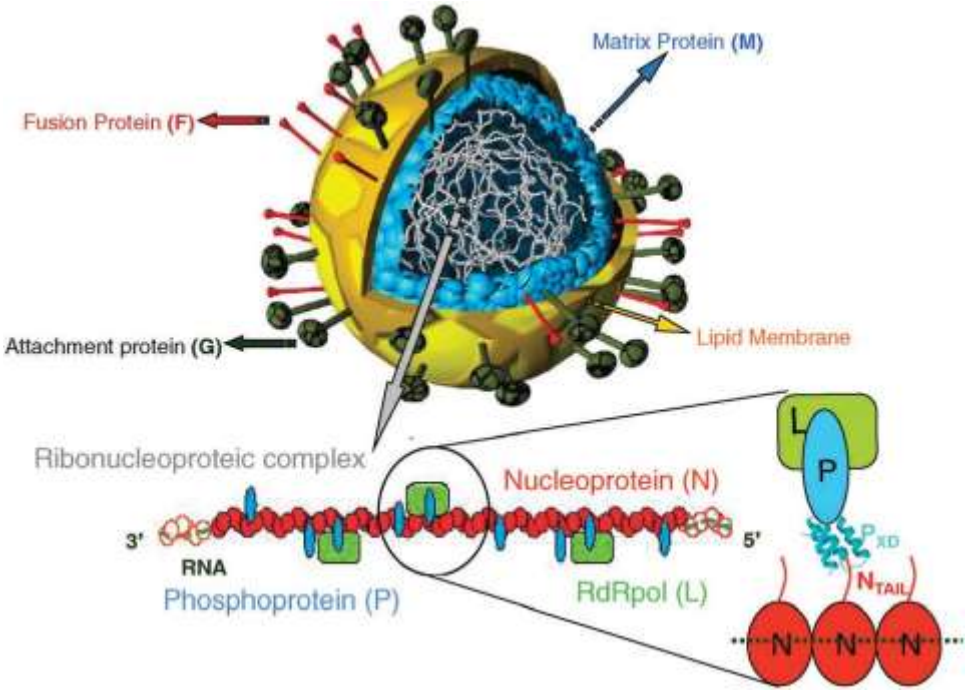


Replication of Paramyxoviruses



Classification of Paramyxoviruses

- Paramyxovirinae
  - Respirovirus
    - Parainfluenza 1, 3
  - Rubulavirus
    - Mumps
    - Parainfluenza 2, 4a, 4b
  - Morbilivirus
    - Measles
  - Henipavirus
    - Hendra, Nipah
- Pneumovirinae
  - Pneumovirus
    - Respiratory Syncytial virus (RSV)
  - Metapneumovirus
    - Human metapneumovirus



Respiratory Syncytial Virus (RSV)

- Most important cause of lower respiratory tract (RT) infection in infants and young children
  - Outranking all microbial pathogens as a cause of brochiolitis and pneumonia in infants younger than 1 year of age
  - Causes about 25% of paediatric hospitalizations cause by respiratory disease in the developed countries

- Pathogenesis and Pathology
  - Initial replication in epithelial cells of the naso-pharynx
  - Virus spreads to the lower RT and cause brochiolitis and pneumonia
  - Viral antigens detected in shed epithelial cells
  - Viremia rarely occurs

Pathogenesis and Pathology

- Incubation period of 3-5 days
- Viral shedding continues up to 1-3 weeks
  - Adults shed virus for only 1-2 days
  - High viral titers in nasal secretions in children
- Intact immunity (cell-mediated) is important to resolve RSV infections
  - Other with impaired cell-mediated immunity may have persistent RSV infection and can shed virus for months
- Only a subset of infected children develop disease
  - Susceptibility brochiolitis may be linked polymorphisms in innate immunity genes

Clinical Features

- Spectrum of clinical disease ranges from inapparent infection or common cold through pneumonia in infants to bronchiolitis in very young children

- Bronchiolitis is a distinctive feature of RSV infection
  - 1/3 of primary RSV infection involves the LRT severely enough to require medical attention
  - Wheezing is a common feature
  - Progression may be rapid culminating in death; high mortality in children with underlying congenital heart complications
  - Pneumonia develops in immunocompromized children and adults more especially in transplant patients receiving immunosuppressants
  - RSV infection may also cause otitis media

  - Reinfection is common but restricted to the URT resulting in colds (mild disease)

Immunity to RSV Infection

- High levels of maternal IgG present in the first few months of life are crucial in protection from LRTIs
- Severe LRTIs occur after 2-4 months of life as maternal antibody levels fall
  - Infection and reinfection can occur despite viral antibodies
  - Serum neutralizing antibodies correlate with protection from LRTIs but not URTIs
- RSV is not a strong inducer of interferon
- Primary infection result in heterotypic immunity and cross protection from the other serotype
  - Reinfection is common but severity of disease is lower
  - Cell-mediated immunity is key in recovery and it is unclear whether secretory nasal IgA is important in preventing secondary infection
  - IgE has been associated with severe brochiolitis (IgE is involved in allergy)

Laboratory diagnosis of RSV

- Nucleic acid detection
  - RT-PCR to detect viral RNA in nasal pharyngeal washes or nose or throat swabs
  - Not available in all settings
  - Nucleic acid sequencing is important in typing and epidemiological investigations
- Antigen detection
  - Indirect Immunoflourescence to detect viral proteins from nasal secretions
- Serology
  - Detection of serum antibodies
  - Not very important in clinical decision making

Epidemiology of RSV

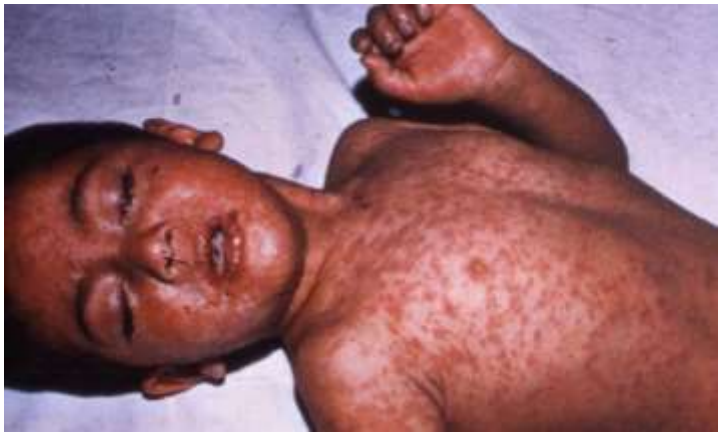
- World wide distribution
- 70% of children are infected by age 1 and almost all by age 2
- Pneumonia and brochiolitis is common from 6wks to 6months peaking at 2 months
- Infection is by large droplets and direct contact
  - Portal of entry is nose and eye
  - Virus is labile but can survive in the environment for up to 6 hours
  - Spread very common in the winter or rainy season
  - Nosocomial infections in nurseries and paediatric wards (infection from hospital staff); common in healthy adults living in crowded conditions e.g. military recruits and prisons

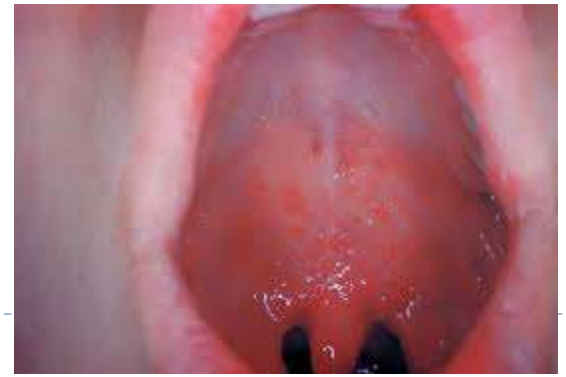
Prevention and Treatment of RSV

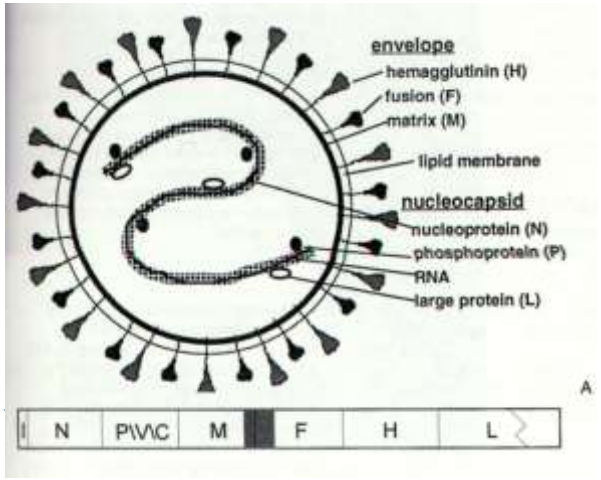
- Control
  - Prevention of nosocomial infections through hand washing , contact isolation, and restriction of visitors)
- Vaccination
  - Still a challenge
  - Formalin inactivated virus was tested in the 1960s
  - One approach is to vaccinate pregnant women so that maternal antibodies could be transferred in-utero
- Treatment
  - Supportive care
    - Removal of secretions, administration of oxygen
  - Antiviral drugs (Ribavirin as an aerosol, oral Ribavirin not useful)
  - Immunoglobulin (human monoclonal antibodies)

Measles

- Most common childhood illness
- Characteristic maculopapular rash, coryza and conjunctivitis
- Reduced incidence due to effective vaccine
  - Still problematic in developing countries



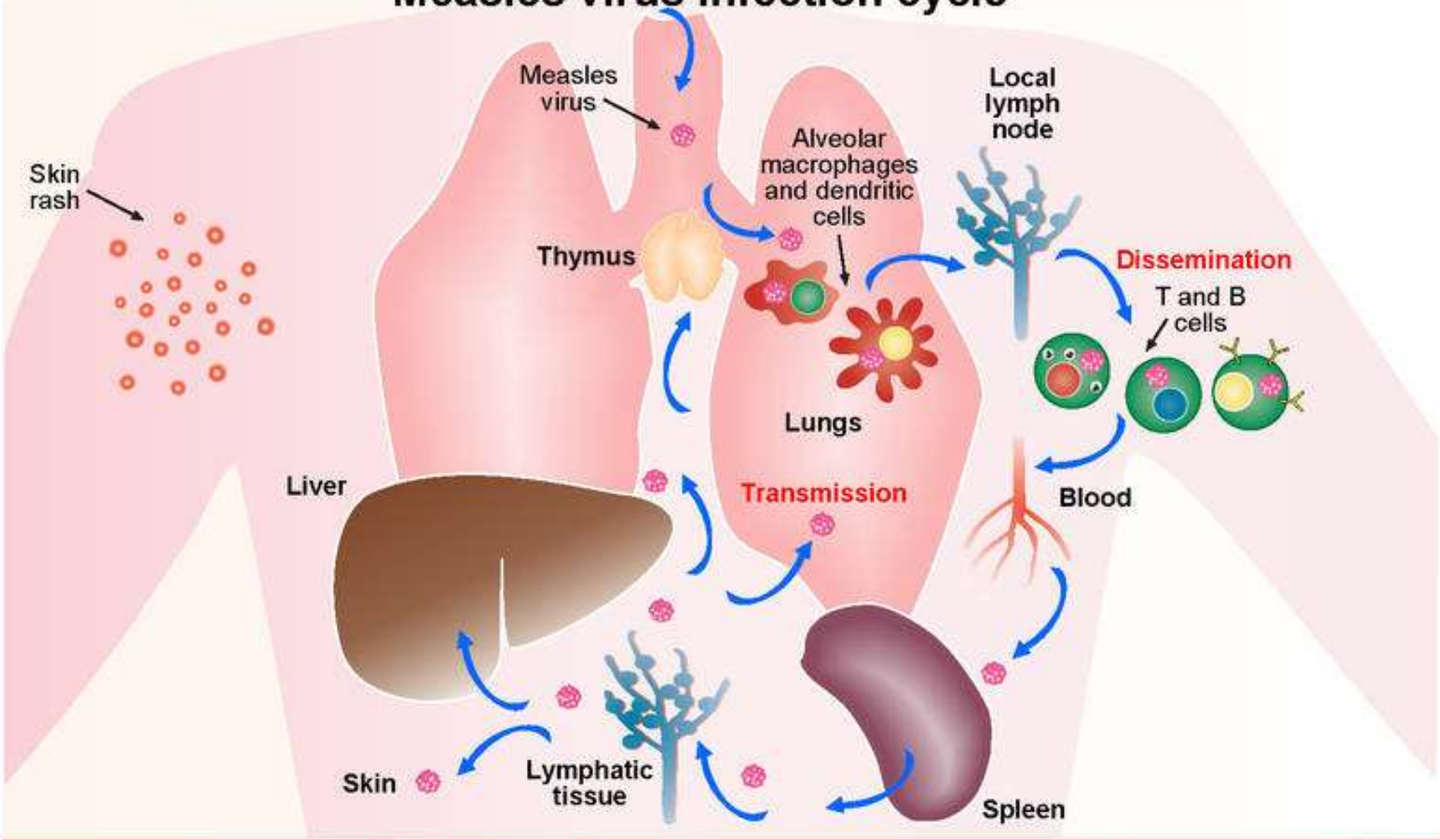




Measles

- Pathogenesis and Immunity
  - Infection via the respiratory route
  - Virions are transported to local lymph nodes and the spleen via macrophages
  - Viremia follows and the virus spreads to epithelial cells of the skin, conjunctiva, respiratory tract etc
  - Necrosis of skin epithelia and conjunctiva
  - Mucosal foci ulcerate giving rise to Koplik's spots in the mouth
  - Skin rush is as a result of cell-mediated immunity to the virus antigens (delayed hypersensitivity)
  - Malnutrition and immunocompromise may lead to secondary bacterial infection (pneumonia)

Measles virus infection cycle



Measles virus is transmitted between humans by aerosal inhalation or contact with respiratory secretions. The main target cells are immune cells such as T and B cells, macrophages and dendritic cells that express CD150 (or SLAM) which serves as an entry receptor. CD46 expressed on most cells can also be used by some wild-type strains, but mainly vaccine strains. Measles virus infects epithelial cells using nectin-4. Endothelial cells and neurons are also infectable, but the entry receptors are unknown. It is thought that the first cells infected in the lungs are alveolar macrophages and dendritic cells that transport virus to regional lymph nodes where T and B cells become infected. Dissemination to other sites including spleen, lymphatic tissue, liver, thymus, skin and lungs follows. The characteristic skin rash is immune-mediated due to infection of dermal capillary endothelial cells and immune complex formation. Measles virus can also penetrate the brain, but is usually controlled. Infection of pulmonary epithelial cells permits transmission to other hosts.

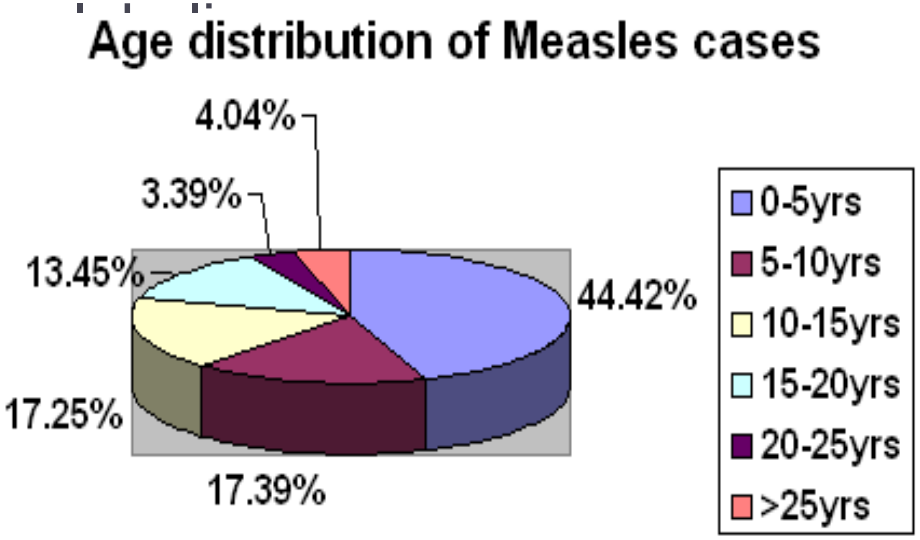
Measles

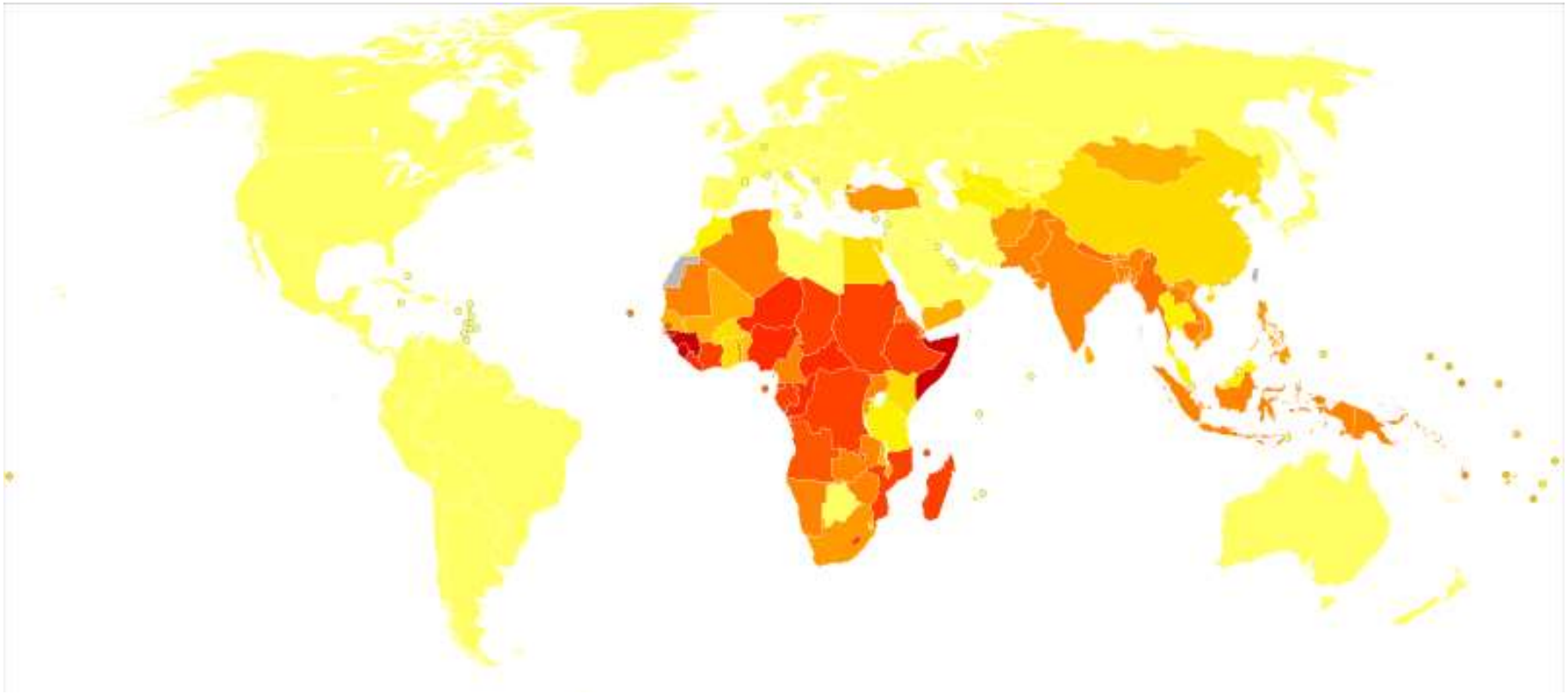
- Pathogenesis and Immunity
  - CNS involvement may occur leading to encephalitis
  - Most common in children under age 2 (1 in 1000 cases in older children)
  - Pathogenesis involves autoimmune demyelination

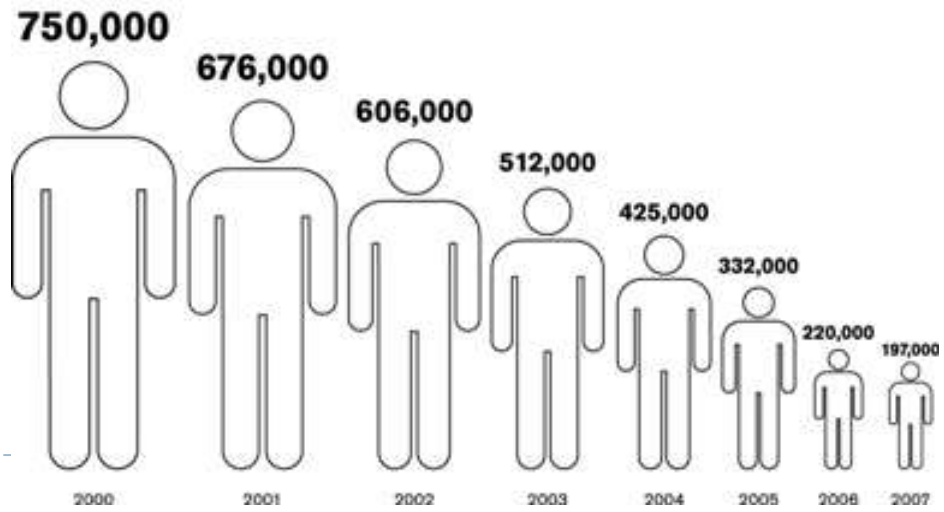
- **Diagnosis**
  - Clinical diagnosis
  - IgM ELISA

Measles

- Epidemiology and Control
  - Live attenuated measles vaccine
  - Only one serotype circulating
  - Administered in childhood (15 months)
  - Need for a cold chain (challenge in the developing world)
  - Passive immunization of immunocompromised individuals with immur

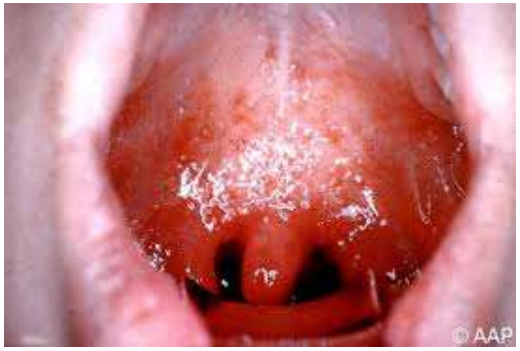


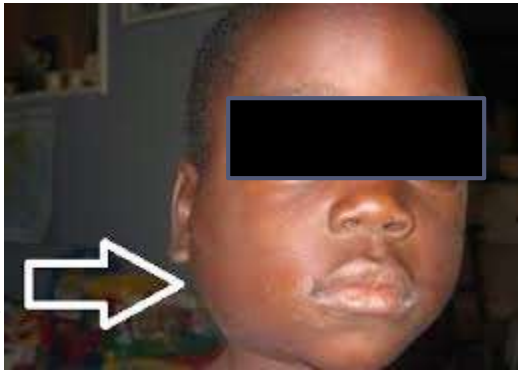



750,000
676,000
606,000
512,000
425,000
332,000
220,000
197,000
2000
2001
2002
2003
2004
2005
2006
2007

Mumps

- Painful edematous enlargement of the paratoid and other salivary glands
- Other glands may be involved (pancreas, ovaries, thyroid etc)
- Meningitis (10% of cases)
- Mumps encephalitis (less common)





Mumps

- **Diagnosis**
  - Clinical diagnosis
  - Meningoencephailitis: virus isolation from saliva, urine or CSF
  - Serology; IgM capture EIA
- **Epidemiology**
  - Transmission by direct contact with saliva or droplets
  - Decline after introduction of live attenuated mumps vaccine
- **Control**
  - Live attenuated mumps vaccine
  - Combinational vaccine; measles, mumps and rubella (MMR)
  - Given in childhood and a second booster at college age

Rubella (German Measles)

- **Togaviridae (Togaviruses)** (arboviruses, alphaviruses,)
- Enveloped polyhedral viruses (40-60nm)
- Single stranded positive sense RNA viruses
- Transmitted by arthropods (arboviruses)
  - Cause chikungunya virus infection
  - Rubella is not an arbovirus; **transmitted via respiratory route**
  - Epidemiologically similar to Paramyxoviruses

Mumps Globally

- Mumps occurs worldwide (annual average of ~500,000 reported cases)
- As of 2018, mumps vaccine introduced in 122 (63%) countries
- Dramatic declines in mumps incidence in countries with routine mumps vaccination programs

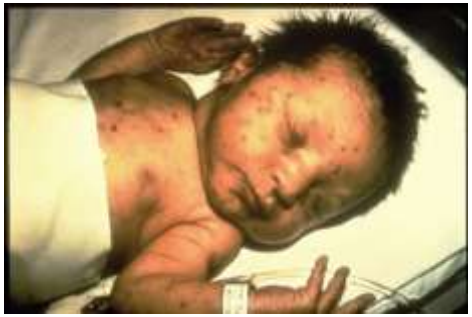
  - Starting in mid-2000s, mumps epidemics have occurred in countries even with high mumps vaccination coverage
  - Mumps incidence is much higher in countries without a routine mumps vaccination program

- In 2018–2019, there have been reports of mumps outbreaks in Central American countries
  - In Sept 2018, Honduran officials declared a medical state of emergency following a mumps outbreak with > 5000 cases
  - In April 2019, El Salvador Pediatric Association released an alert about mumps outbreaks in April 2019
  - Central American countries did not have a routine mumps vaccination program until the mid-1990's

Sources: [1] World Health Organization. Immunization, vaccines and biologicals: mumps; [2] World Health Organization. Mumps Reported Cases [3] Beleni AI, Borgmann S. Mumps in the Vaccination Age: Global Epidemiology and the Situation in Germany. Int J Environ Res Public Health. 2018 Jul 31;15(8). [4] http://outbreaknewstoday.com/honduras-reports-5000-mumps-cases-year-92438/ [5] https://asopedes.org/cominicado-sobre-paperas-parotiditis/

Rubella

- Childhood disease
- Associated with blindness in children born from mothers infected with Rubella in the first trimester
  - Other congenital defects including deafness, mental retardation and cardiac abnormalities



- Clinical features
  - Mild disease in adults
  - Fine, pink discrete macules of erythematous rash (face, trunk and limbs)
  - Fever, enlarged cervical lymph nodes, mild polyarthritis
  - Rare complications: thrombocytopenic purpura and post infection encephalopathy

Congenital Rubella

- 20% of children infected in utero during the first trimester of pregnancy are born with severe congenital abnormalities
  - Remainder have mild defects
  - Common abnormalities are:
    - Neurosensory deafness (cochlear degeneration)
    - Blindness (cataracts, glaucoma, retinopathy)
    - Congenital heart disease (septal defects, pulmonary artery stenosis)
    - Microcephaly and mental retardation
    - Congenital rubella syndrome (bone translucency, retarded growth, hepatospleenomegaly, thrombocytopenic purpura.
- Sometimes missed at birth, 10-20% of children die in the first year and up to 20% develop insulin-dependant diabetes mellitus as young adults

Pathogenesis and immunity

- Rubella virus enters the body by inhalation of droplets
- Multiplies in the upper respiratory tract and migrate to local lymph nodes and systemically via the blood stream
- Naturally acquired immunity to rubella lasts for many years and secondary infection are usually subclinical and boosts the immunity
- Rubella virus slows down cell division
- Maternal IgG (transplacental) and fetal IgM fail to clear the virus during pregnancy and even after birth. The virus replicates as a chronic infection and is shed from body secretions after birth

Diagnosis

- Cell culture (Vero or RK-13 cell lines) from throat swabs

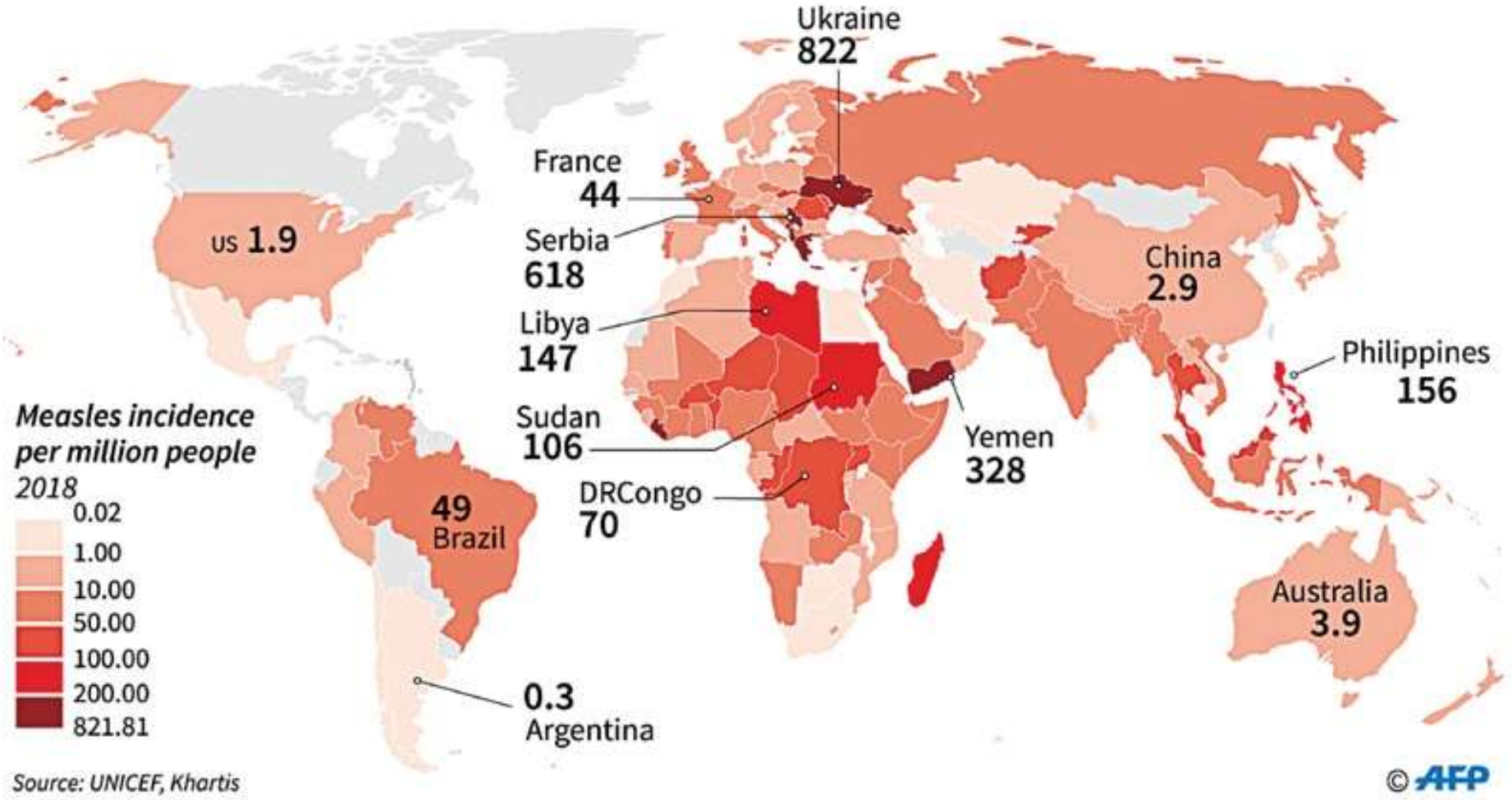
- Serology:
  - Woman with rush in the first trimester of pregnancy
  - Children born with suggestive congenital rubella syndrome
  - Only one serotype
  - Diagnosis for mothers: IgM in serum
  - Diagnosis in infants: IgM (many babies with have maternal IgG acquired in utero; from maternal vaccination or natural infection. IgM does not cross the placenta)

Epidemiology and Control

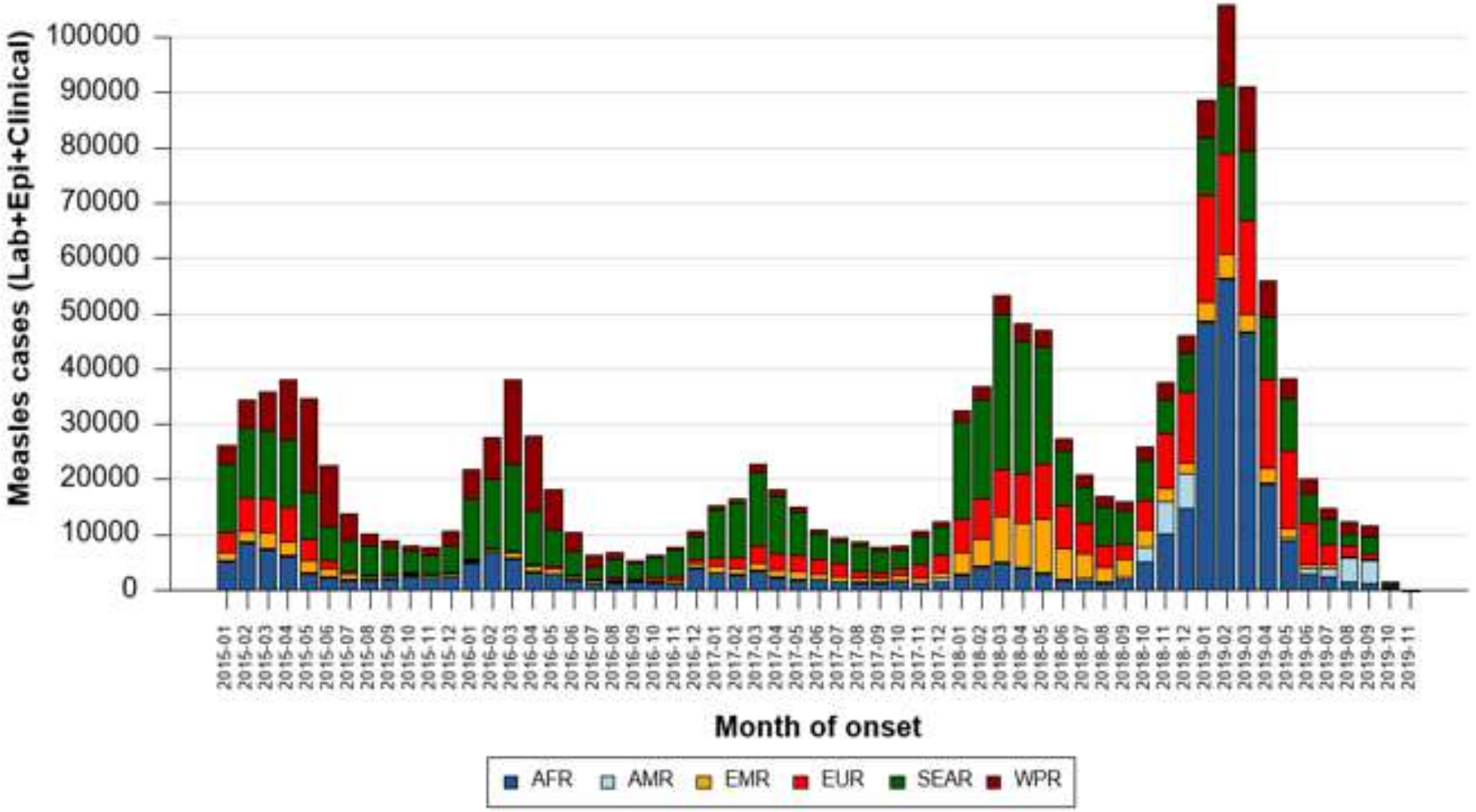
- Highly transmissible via droplets
- Disease is endemic worldwide
- School-age children most susceptible
- Control:
  - Live attenuated rubella vaccine
  - Based in RA27/3 strain grown in human fibroblast line
  - Combined measles-mumps-rubella (MMR) vaccine was introduced in the USA in 1972
  - Immunity up to 90% and for up to 15 years (against disease and viremia)

Recent Measles, mumps and rubella Outbreaks

Measles infection rates by country



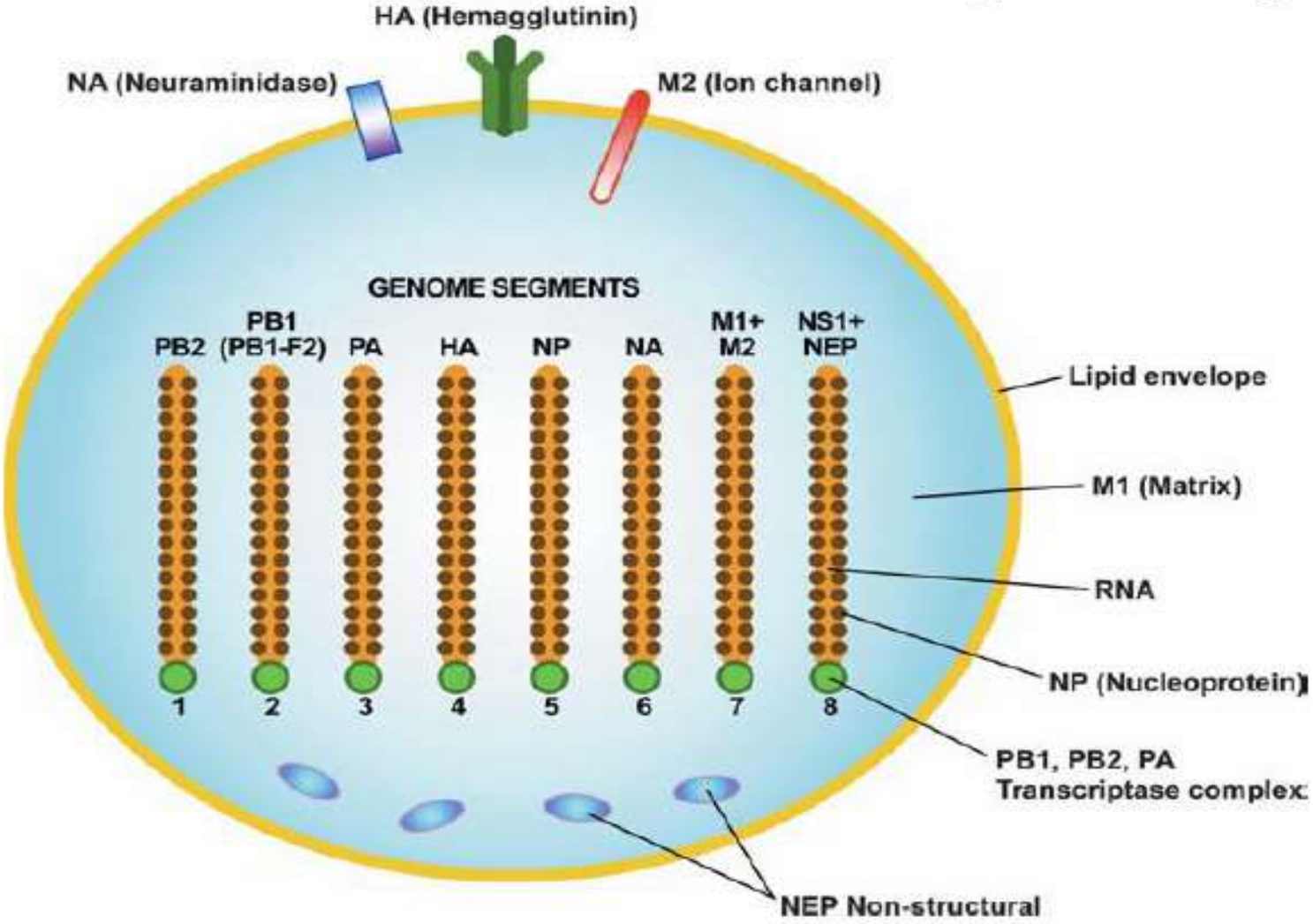
Current global Measles



Orthomyxoviruses

Influenza Viruses

Structure of Influenza Viruses



Influenza Genome

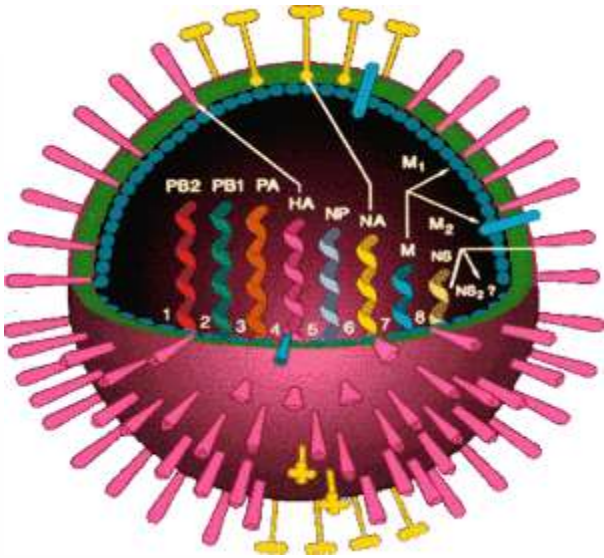
- Consists of s/s (-)sense RNA in 8 segments (7 in Influenza C).
- The structure of the influenza virus genome is known in great detail because of the tremendous amount of genetic investigation (conventional and molecular) which has been done.
- The 5' and 3' terminal sequences of all the genome segments are highly conserved

Influenza Genome

Segment:	Size (nt)	Polypeptide (s)	Function
1	2341	PB2	Transcriptase: cap binding
2	2341	PB1	Transcriptase: elongation
3	2233	PA	Transcriptase: protease activity (?)
4	1778	HA	Haemagglutinin
5	1565	NP	Nucleoprotein: RNA binding; part of transcriptase complex; nuclear/cytoplasmic transport of vRNA
6	1413	NA	Neuraminidase: release of virus
7	1027	M1	Matrix protein: major component of virion
		M2	Integral membrane protein - ion channel
8	890	NS1	Non-structural: nucleus; effects on cellular RNA transport, splicing, translation. Anti-interferon protein.
		NS2	Non-structural: nucleus+cytoplasm, function unknown

Membrane proteins

- Hemagglutinin (HA)
  - Attach to cell surface sialic acid receptors
  - Facilitate entry if the virus into the cell
  - Crucial component of current vaccine
- Neuraminidase (NA)
  - Catalyze the cleavage if glycosidic linkages to sialic acid on host cell and the virion surfaces
  - Inhibition of NA— the most effective antiviral treatment
- M2 protein — small amount in influenza A
  - Ion channel
  - Regulate the internal PH of the virus
  - Blocked by antiviral drug



Influenza Virus

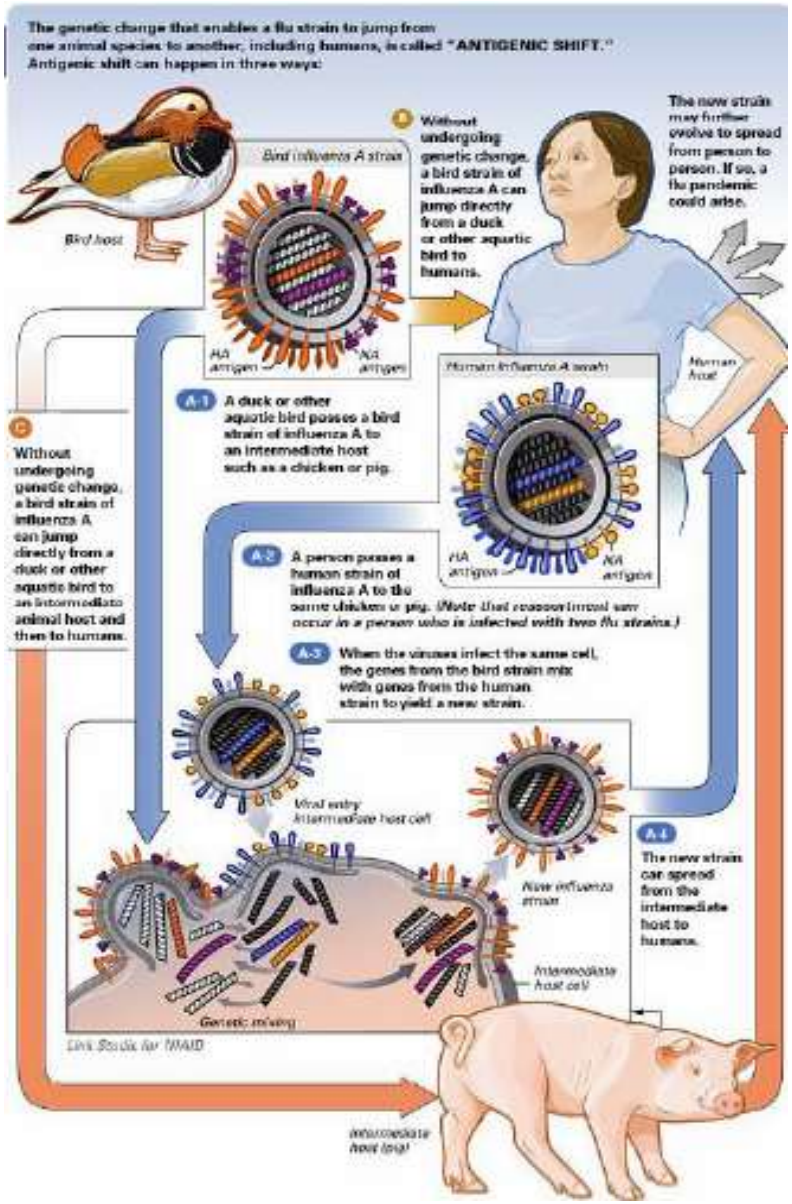
Influenza A

- Clinical and epidemiological importance
- Mutability of virus produces antigenic changes
  - Mutation and whole gene ‘swapping’ (reassortment) between different strains
  - Recombination
    - Results: antigenic drifts and antigenic shifts
- Subtypes based on H and N antigens
  - H antigens: 15
  - N antigens: 9
- Avian subtypes :
  - 2 H subtypes (H5 and H7)
  - 7 N subtypes
- Only H1, H2 and H3 H subtypes and N1 and N2 N subtypes are associated with stable human infection

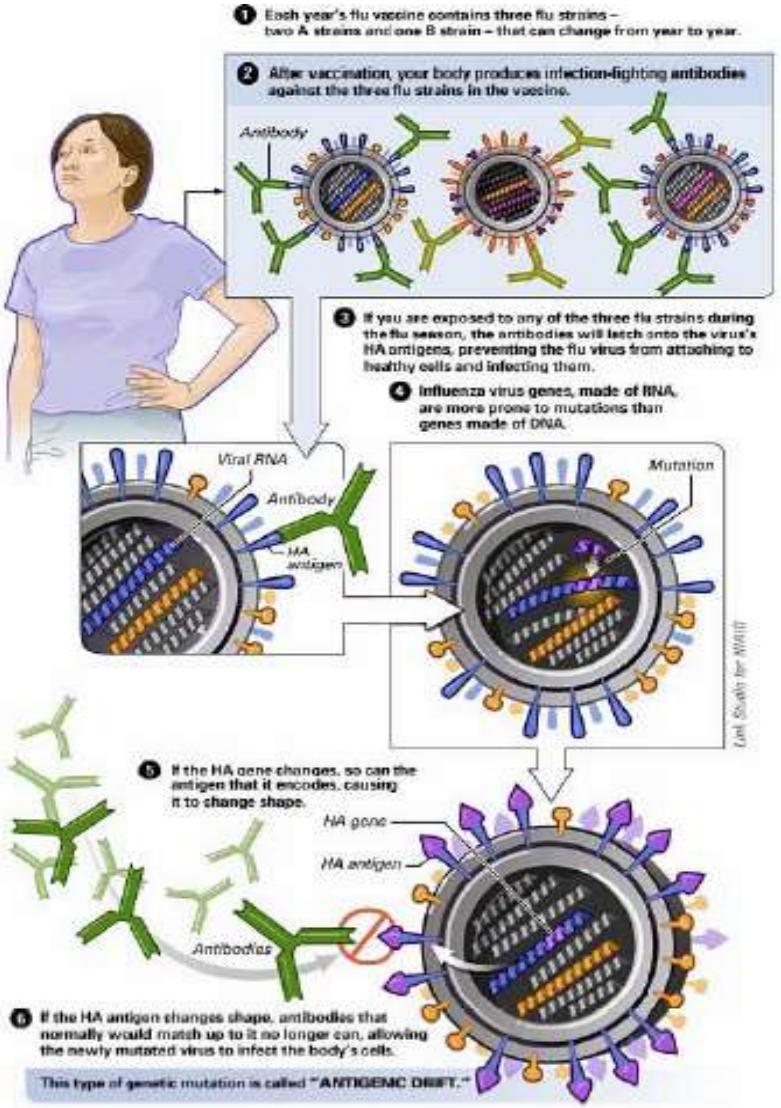
Influenza A

- Antigenic drift
  - Subtle changes in H, N or non structural genes
  - Cause by point mutations
  - Occurs every few years
  - Allow viral maintenance in a population
  - Responsible for seasonal outbreaks

- Antigenic shift
  - Major antigenic changes
    - Due to reassortment or whole gene ‘swapping’
    - New subtype may develop mutations too
  - Correlates with epidemics and pandemics
    - Little or no immunity



Antigenic Shift



Antigenic Drift

Influenza Viruses in Birds, Pigs and Humans

Characteristics of Influenza Viruses

- Three major groups based on ribonuclear antigens
  - A: Most studied, Human, swine, avian, equine, marine mammals
  - B: Humans only
  - C: Humans, swine
- Subtype A
  - Greatest virulence and epidemic spread
- Specificity
  - Receptors
    - α-2-6 in humans
    - α-2-3 in birds
    - α-2-6, α-2-3 in pigs

Human influenza A virus

- Only H1, H2 and H3 H subtypes and N1 and N2 N subtypes are associated with stable human infection

- Circulating strains
  - H1N1, H3N2, H1N2
  - Antigenically and genetically distinct from swine counterparts
  - H3N2-intorduced in 1968 (Hong Kong Pandemic)
    - Preceded H2N2
    - Reassortment between avian and human influenza A

- Transmission
  - Person to person via respiratory route by aerosols

Avian Influenza Viruses among Birds

- Contact with infected birds
  - contaminated nasal, respiratory, or fecal material
  - usually fecal-oral transmission
- Indirect spread
  - virus-contaminated water and fomites
    - Virus suspensions in water have been shown to retain infectivity for more than 100 days at 17° C
  - In contrast to influenza virus infections in mammals (humans, swine, and horses) - primarily transmission by aerosols
- Transmission from wild birds to domestic poultry
  - greatest where domestic birds roam freely, share a water supply with wild birds, or use a water or food supply that might become contaminated by droppings from infected wild bird carriers
- 2002-2005 SE Asia, 150 million birds infected (H5N1)

Highly Pathogenic Avian Influenza A Viruses

- 'fowl plague'
- low pathogenic avian influenza virus, LPAIV (mild) > highly susceptible poultry species > a series of mutation (several cycles of infection) > highly pathogenic avian influenza viruses, HPAIV (overwhelming systemic and rapidly fatal disease)
- HPAI in poultry is characterized by a sudden onset, severe illness of a short duration, and a mortality approaching virtually 100 % in vulnerable species.
- H5 and H7

Why avian viruses at present rarely infect and spread between humans

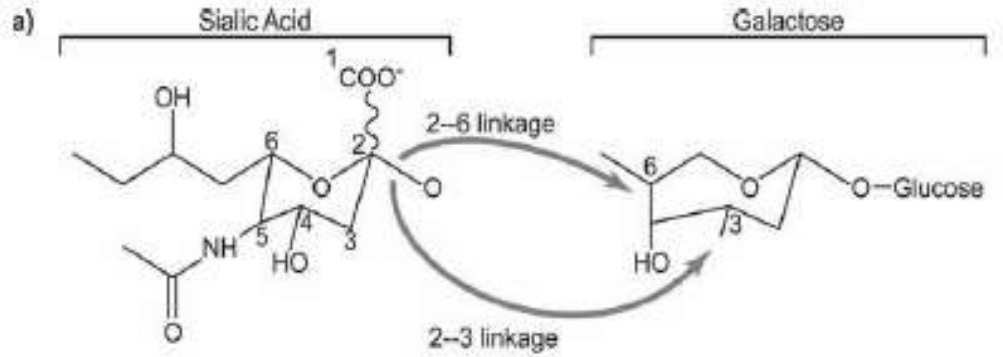
- Avian and human flu viruses seem to target different regions of a patient's respiratory tract.
  - SA α 2,6Gal dominant on epithelial cells in human nasal mucosa, with SA α 2,3Gal occasionally detected
    - Paranasal sinuses, pharynx, trachea, bronchi
  - SA α 2,3Gal found in lower respiratory tract
    - non-ciliated cuboidal bronchiolar cells at the junction between the respiratory bronchiole and alveolus
    - Alveolar wall
- Human-derived viruses
  - preferentially recognize SA α 2,6Gal
  - bind extensively to epithelial cells in the bronchi
  - Bind to a lesser degree to alveolar cells
- Avian viruses
  - preferentially recognize SA α 2,3Gal
  - bind extensively to alveolar cells but less widely to bronchial epithelial cells
  - More difficult to transmit via the upper respiratory tract
- A/Hong Kong/213/03 H5N1 (human isolate)
  - recognizes both SA α 2,3Gal and SA α 2,6Gal
  - binds extensively to both bronchial and alveolar cells

Pigs

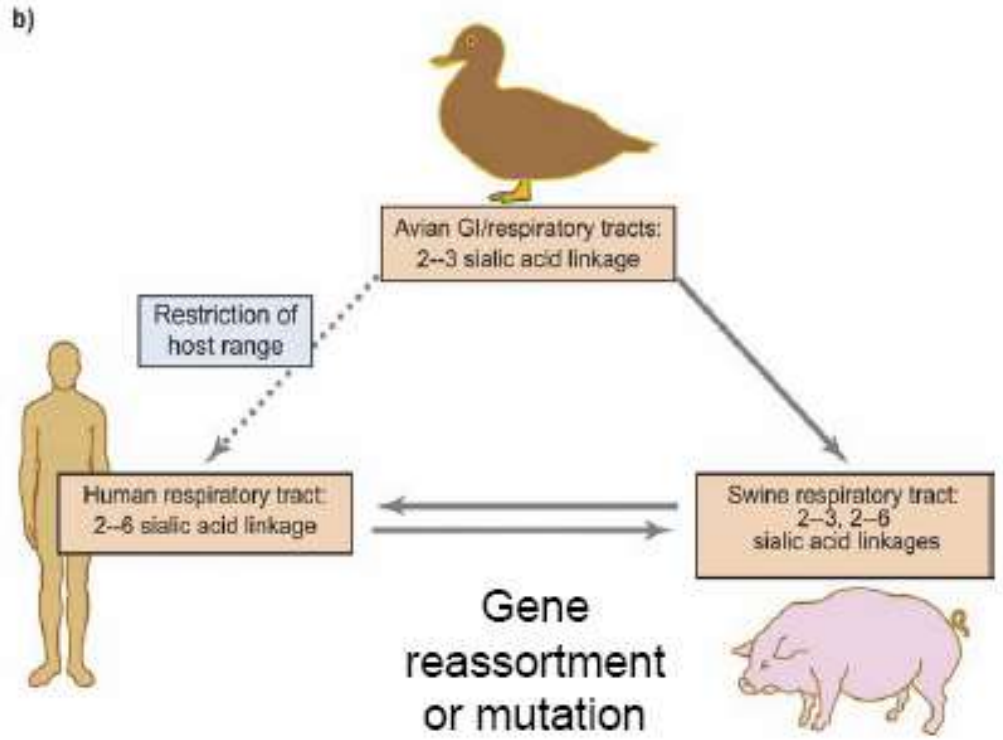
- Respiratory epithelial cells in the pig contain both 2,3- and 2,6 linkages, susceptible to both human and avian influenza viruses .
- a potential source of new pandemic strains
  - mixed infection of avian and human strains, potentially resulting in new reassortant viruses.
  - purely avian strains can adapt to human receptor recognition.

H1N1, H3N2, H1N2

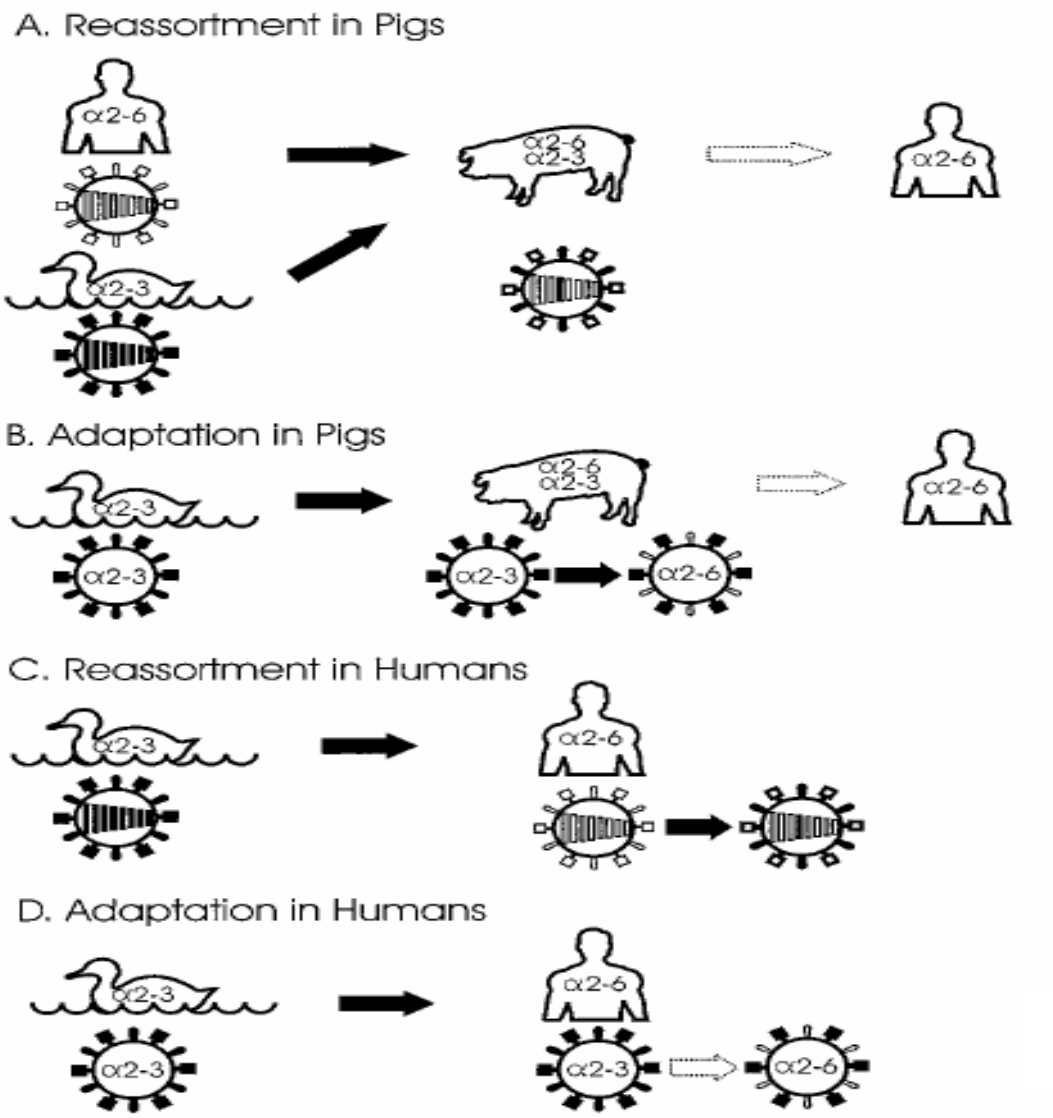
Sialic acid residues can be covalently attached to galactose residues of integral glycoproteins and glycolipids via either 2–3 or 2–6 α linkages.

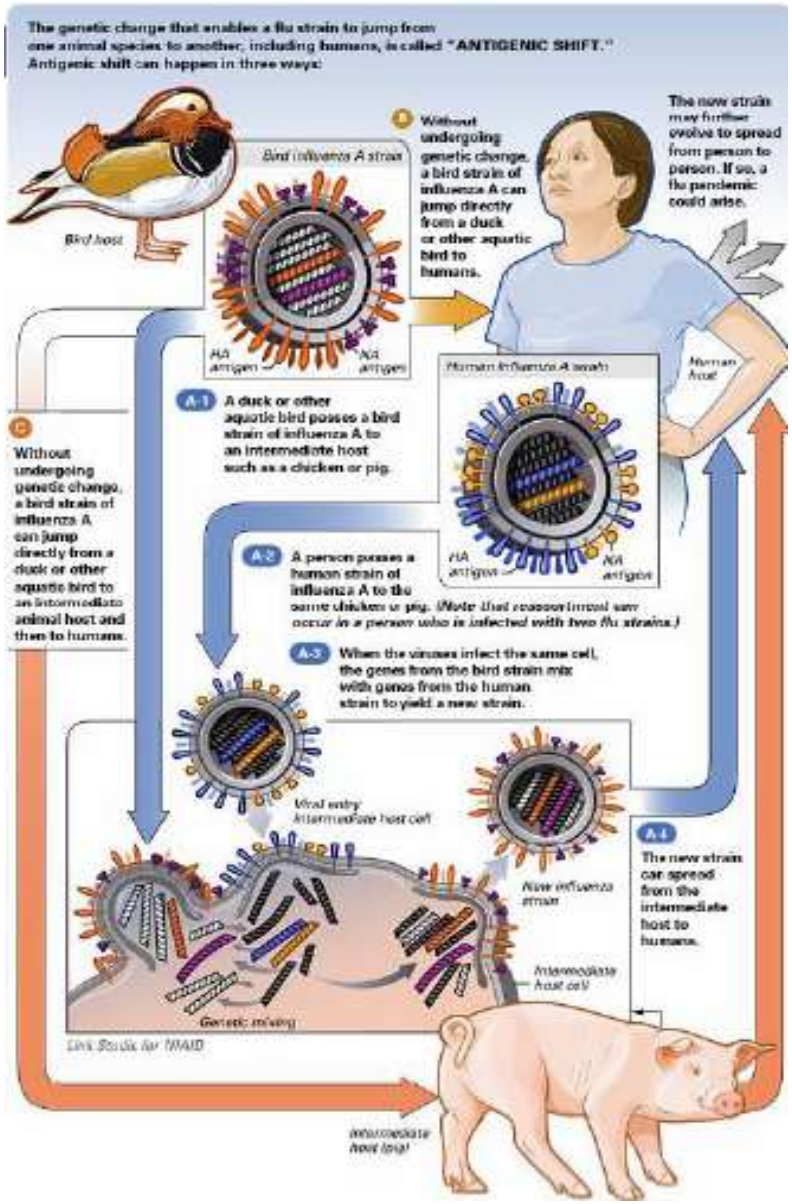


The avian, human, and swine upper respiratory tract epitheliae preferentially express 2–3 linkages, 2–6 linkages, and both 2–3 and 2–6 linkages respectively

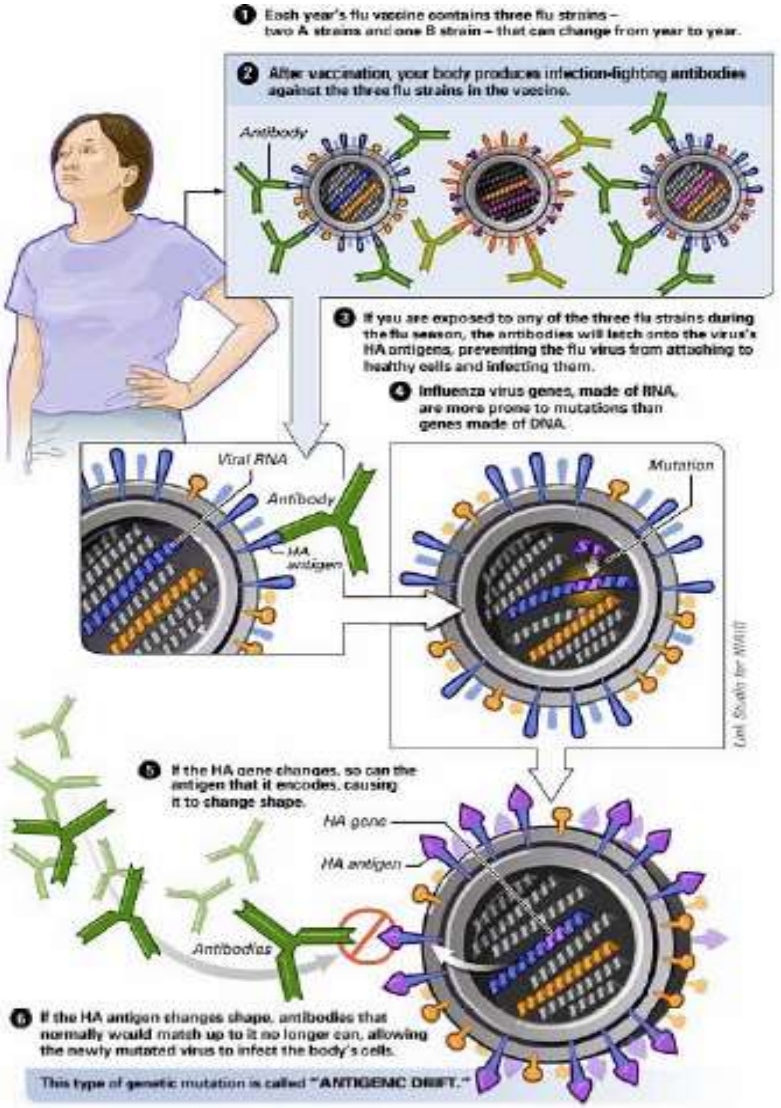


Molecular basis for generation in pigs of Influenza A with pandemic potential





Antigenic Shift



Antigenic Drift

Influenza Pandemics

- New influenza A virus strain spreads rapidly throughout the world

- Three to four times each century
  - We are due?
  - Any time of the year
  - Excess morbidity and mortality

CHARACTERISTICS OF SEASONAL INFLUENZA EPIDEMICS AND THE THREE INFLUENZA PANDEMICS OF THE TWENTIETH CENTURY

	Influenza virus type	Estimated rates of symptomatic infection (%)	Estimated total excess deaths worldwide	Case fatality rates (%)	Mortality pattern
Seasonal influenza epidemics	Various A & B viruses	5–20*	Up to ½ million annually*	~0.001*	Excess mortality rates are 50–200 times greater in persons ≥65 years old compared with those <65 years. However, in the first 2–7 years following a pandemic, mortality rates in persons ≥65 years old may only be 2–30 times greater than those <65 years. The majority of excess deaths (more than 85%) occur among the elderly and those with high-risk medical conditions.
Spanish influenza pandemic 1918–1919	A/H1N1	20–40	>40 million	>2.5	Excess mortality rates were 3 times greater in persons <65 years old compared with persons ≥65 years old. 99% of excess deaths occurred in people <65 years. In Australia, 60% of excess deaths occurred in healthy persons 20–45 years.
Asian influenza pandemic 1957–1958	A/H2N2	10–60	2 million	0.01–0.05	Excess mortality rates were 18 times greater in persons ≥65 years compared with those <65 years. 36% of excess deaths were in persons <65 years old.
Hong Kong influenza pandemic 1968–1969	A/H3N2	25–30	1 million	0.01–0.05	Excess mortality rates were 13 times greater in persons ≥65 years compared with those <65 years. 41% of excess deaths were in persons <65 years old.

* Mortality due to seasonal influenza epidemics varies greatly depending on the predominant type/subtype of virus circulating and, for influenza A, the time since the subtype evolved in the human population.

Data sources: Mandell et al[1]; Taubenberger and Morens[2]; Department of Health and Ageing[11]; World Health Organization[4, 12, 13]; and Simonsen et al.[14]

Clarifications

- Seasonal flu
  - Influenza viruses circulating in humans
    - Maintained by antigenic drift

Avian flu ≠ Pandemic flu

- Avian flu
  - Bird flu
    - Occurs in aquatic birds (waterfowl)
    - Drifts and shifts cause transmission and pathogenesis in other species

- Pandemic flu
  - New influenza A virus strain spreads rapidly throughout the world
  - Caused by shifts
    - Transmission and pathogenesis
    - Limited immunity

Pandemic Threat of 2005

- Avian H5N1 epidemics in poultry
  - Ability to cross species barrier
  - Level of concern
- Pandemic virus
  - Emergence of a new virus subtype in which population has no or little immunity
  - Ability to replicate and cause serious illness in humans
  - Efficient transmission from human to human
    - H5N1 only meets the first two criteria
- Predictions
  - It cannot be predicted whether H5N1 will meet third criterion
  - Historical data: no pandemic preceded by pathogenic avian epidemic
  - Genetic alterations in H5N1 need to occur
    - Risk and timing cannot be predicted

H1N1

- Swine influenza
- Became a pandemic in 2009.
- Despite H1N1 becoming pandemic it has a much lower virulence than N5N1 and caused fewer deaths

End

Questions