Module 4: Negative strand RNA viruses

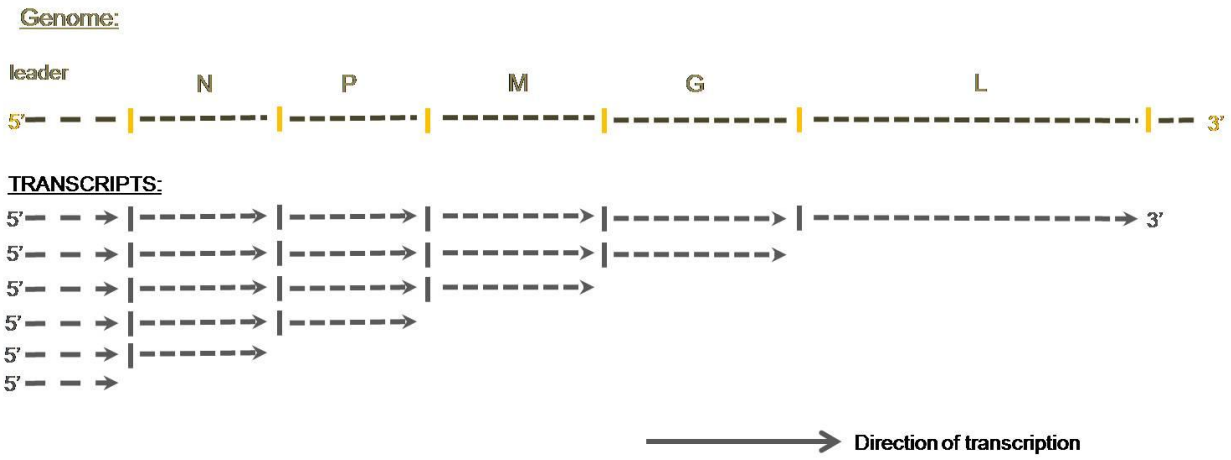
Lecture 23: Negative strand RNA viruses

Negative strand RNA viruses belong to order *Mononegavirales*. The viruses in this group have similar genome organization and replication strategies and are probably diverged from a common ancestor (*Filoviridae*, *Paramyxoviridae*, *Bornaviridae* and *Rhabdoviridae*). They are often associated with emerging infection and havoc to human population (Ebola, Marburg, Nipah and Hendra). Virus contains a negative sense RNA genome which means the polarity of the genome is opposite to that of an mRNA. The negative sense RNA cannot use its genome to synthesize proteins and hence its **RNA is not infectious** (absence of protein synthesis). Because of the above stated property viruses in this group encode their own polymerase (**RNA dependent RNA polymerase [RDRP]**). Another unique property about these viruses is about its transcription, first a leader RNA is synthesized, which is followed by sequential transcription of the genes in the 3' to 5' order to yield individual mRNAs by a stop-start mechanism guided by the conserved gene-start and gene-end signals.

23.1 Genome features

I. Linear non-segmented negative sense RNA genome
II. Organization of genome- 3'-Leader-Virion core- Surface proteins-Polymerase-Trailer 5'.
III. Helical nucleocapsid contains the RNA dependent RNA polymerase.
IV. The leader RNA is neither capped nor polyadenylated and is not functional as mRNA.
V. Replication occurs when the polymerase complex ignores the transcription stop signals at the 3' end of each gene and a full-length positive-sense antigenome is synthesized.
VI. Transcription at the gene-start site is not perfect, which leads to a gradient of mRNA abundance that decreases according to the distance from the 3' end of the genome.

Figure 23.1 Gradient of mRNA abundance from 3' end towards 5' end:



N= Nucleocapsid protein

P= Phosphoprotein

M= Matrix protein

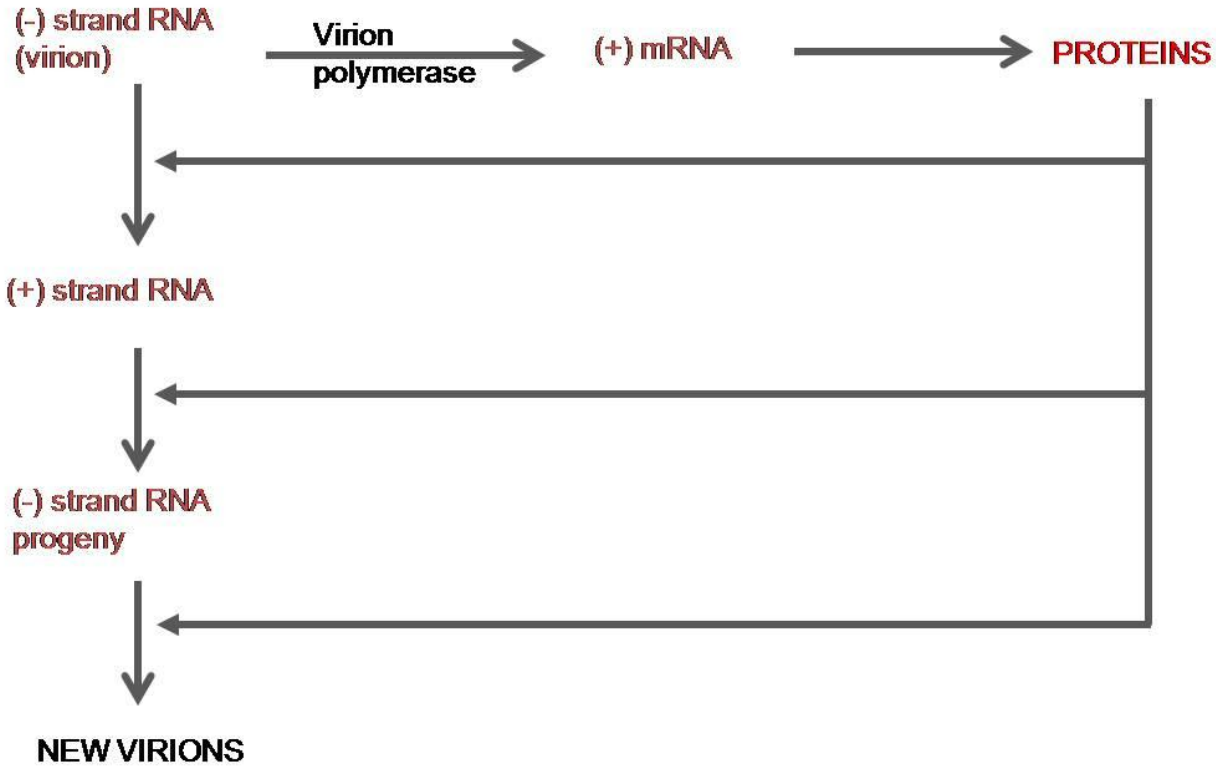
G= Membrane glycoproteins

L= Large polymerase protein

23.2 Genome replication

Virus enters the cell by receptor mediated endocytosis. The initial step after entering inside the cell is to transcribe viral mRNA from genomic RNA with the help of RDRP. The mRNA is further translated to form the viral proteins. The viral replication begins at 3' end and forms a complete positive sense RNA using negative sense genomic template. The viral transcription and replication occurs within a nucleocapsid-polymerase complex that consists of N, P, and L proteins. The switch from transcription to replication occurs when sufficient amount of N protein accumulates in the cytoplasm; it binds to the P protein to form a soluble complex, which is used for replication of the progeny RNA for the genome.

Figure 23.2 Replication strategy in negative sense RNA viruses:

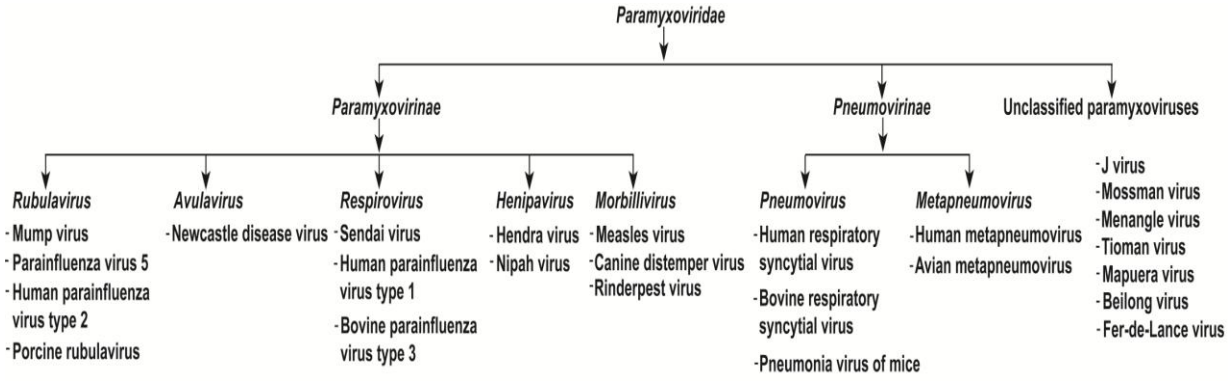


Lecture 24: Paramyxoviruses

Paramyxoviruses are included under the family *Paramyxoviridae*, order *Mononegavirales* along with *Rhabdoviridae, Flioviridae,* and *Bornaviridae*. All the viruses in this group are enveloped and contain surface glycoproteins over it. The genome of the virus is single stranded RNA of negative polarity. Many life threatening diseases are caused by members of the family *Paramyxoviridae*. The impact of the disease caused by these viruses has been reduced dramatically through the use of vaccine.

24.1 Classification of Paramyxoviruses:

The family is divided into two subfamilies which are further divided into different genera.



24.2 Virion properties

Paramyxoviruses are pleomorphic and about 150- 350 nm in diameter. Virions are enveloped and covered by surface glycoproteins. The genome consists of ssRNA of negative polarity and 13-19 kb in size. The RNA at 3' end is not polyadenylated and 5' end of the RNA is not capped. With the exception of family *Pneumovirinae,* the genome size of the viruses are the even multiples (also called as rule of six) of six. The viral N protein binds effectively with the six nucleotide of the genomic RNA for its effective replication.

24.3 General Concepts

I. The Paramyxoviruses are the leading cause of respiratory disease in children; general illnesses include croup and inflammation of respiratory tract.

II. Paramyxoviruses share similar features; they contain a bilayer envelope containing spikes, have a helical symmetry and contain a negative stranded ssRNA genome. An RNA-dependent RNA polymerase (RDRP) is carried by the virus particle in order to perform the replication of the RNA genome.

III. Replication of the virus takes place in the cytoplasm and are released by the budding process.

IV. Virus antigens are confined in the lipid envelopes (spikes) and within the nucleocapsid core.

V. The viruses have a wide variety of host range that includes humans and primates.

VI. Viruses produce syncytia upon infection to susceptible cells by fusion and later cell lysis.

24.4 Different Paramyxoviruses:

24.4.1 Parainfluenza:

Nearly 25-35% of acute respiratory infections in infants and children are caused by this group of viruses. Disease starts with mild flu like symptoms which may progress to life-threatening (Croup, bronchiolitis and pneumonia) condition in the untreated cases. Parainfluenza viruses are the most common cause of **croup.** The viruses were divided into 4 distinct serotypes (numbered 1-4). These serotypes usually produce local inflammation in the upper and lower respiratory tract causing denudation of the ciliated epithelium (nose and throat). The virus generally sheds over 5-12 days following infection. Serotype 1 and 2 are attributed for the severe forms of the disease in young children.

24.4.2 Mumps:

This is another common disease of children. Acute infection of Mumps virus produces inflammation of salivary glands leading to its enlargement. Only one serotype is available for this group of virus. The common target tissues include glandular and nervous tissue. The virus enters through the pharynx or conjunctiva, systemic infection of the virus can cause viremia. Secondary dissemination of the virus occurs to salivary glands, gonads, pancreas, and central nervous system after their multiplication in the lymphoid tissues. Incubation period of the disease may vary between 18-24 days while in many cases it is asymptomatic. The most characteristic feature of the disease is painful swelling of the parotid glands. Sometimes disease may lead to deafness and severe inflammation in the male reproductive system.

24.4.3 Measles:

It is also an acute disease of infants and children. The virus commonly causes a rash over the body with a high fever, occasionally conjunctivitis and pneumonia. In severe form of the disease virus may cause inflammation and pathological condition in the brain. Like Mumps virus only one serotype exists for Measles virus. Measles is also a systemic infection spread by dissemination of the virus through blood. Acute disease affects the lymphatic and respiratory systems while persistence of the virus in children leads to subacute sclerosing *panencephalitis*. Virus enters the body via the oropharyngeal route, multiplies locally within the lymphatic system, and spreads to the mucosal surface of respiratory, gastrointestinal and central nervous system. Clinically, respiratory symptoms and fever are evident during the early stages which on later changes to a rash during the eruptive phase. Rash over the body and head are sometimes called as **Koplik's spots**. They are ulcerated mucosal lesions characterized by necrosis and infiltration of neutrophils, and are the pathognomonic (hallmark and unique to measles) signs of the measles. Secondary infection by bacteria may sometimes complicate the situation and even turn worse in untreated condition.

24.4.4 Respiratory Syncytial virus:

Respiratory Syncytial virus is one of the leading causes of bronchiolitis and pneumonia in infants under one year of age. The viruses produce a characteristic syncytia formation in the respiratory epithelium cells; hence the name is given as respiratory syncytial virus. The virus starts its infection in the upper or lower respiratory tract infecting ciliated epithelium. Spread of the virus proceeds by cell fusion. Severe form of the disease may cause bronchiolitis, pneumonia, or croup in infants.

* Croup is sometimes called as barking cough and is characterized by swelling around the vocal cords. It usually associated with the inflammation of larynx, trachea, and bronchioles.

Lecture 25: Orthomyxoviruses

The name originates from the Greek word "ortho" which means **correct** while "myxo" stand for **mucus**. The word essentially stands for virus that infects epithelial cells in the right way, exactly opposite to that of paramyxoviruses. The family *Orthomyxoviridae* contains viruses of single stranded segmented RNA genome (6-8 segments). Out of all, influenza viruses are the most important members of this family which includes influenza virus A, B, and C. The name originates during 18th century from a disease that was thought to "**influence"** by stars. The pathogenic viruses are included in the genus influenza virus A, whereas other two genera (B and C) circulate constantly in human subjects. Several devastating pandemics caused because of influenza virus in past include famous Spanish Flu (1918), Asian Flu (1956), and Hong Kong Flu (1967) which killed millions of people.

25.1 Distinct Characters

I. The viruses cause an acute respiratory disease with prominent systemic symptoms with its major manifestation on the respiratory system.

II. Influenza virus type A is responsible for periodic epidemics worldwide; while virus types A and B cause regional epidemics during the cold weather.

III. **Antigenic drift** (minor changes in the viral surface proteins) and **Antigenic Shift** (major changes in the viral genome due to rearrangement of the virus segments or reassortments) are responsible for both epidemics and pandemics of influenza viruses.

Table 25.1 Differences between influenza virus types:

	Influenza virus type A	Influenza virus type B	Influenza virus type C
Animal Reservoir	Present	Absent	Absent
Spread	Pandemic/ Epidemic	Epidemic	Sporadic
Severity	High	Moderate	Mild
Variations	Antigenic shift/drift	Antigenic drift	Antigenic drift

25.2 Virus Classification

The family *Orthomyxoviridae* contains the genera Influenza virus A, Influenza virus B, Influenza virus C, Thogotovirus, and Isavirus.

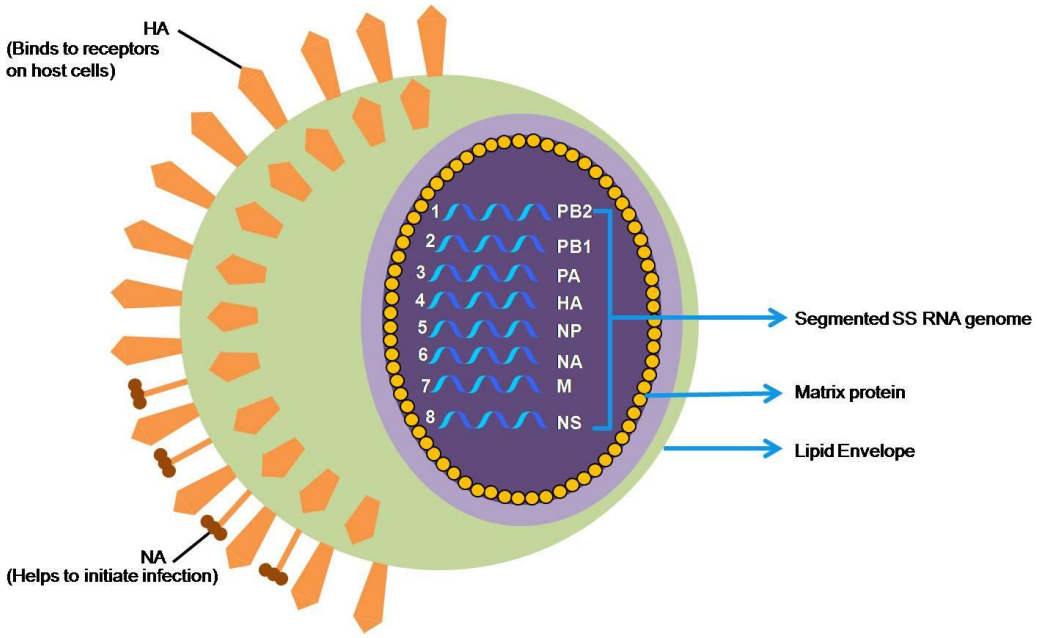
Table 25.2 Different members of family *Orthomyxoviridae*:

Genus	Virus type
Influenzavirus A	Influenza A virus
Influenzavirus B	Influenza B virus
Influenzavirus C	Influenza C virus
Thogotovirus	Thogotovirus
	Dhori virus
	Batken virus
Isavirus	Infectious salmon anemia virus

25.3 Virion properties

Orthomyxovirus virions are pleomorphic in shape and around 80–120 nm in diameter. The nucleocapsids are helical in symmetry and contains eight (influenza virus A, B, and isavirus), seven (influenza virus C), or six (Thogotovirus) RNA segments. Genome is single stranded negative sense RNA of 10 – 14Kb in size. The viruses also contain surface glycoproteins over the lipid envelope. The two envelope glycoproteins are hemagglutinin protein (H) and neuraminidase protein (N). There are **15** different subtypes of "H" and **9** different subtypes of "N". This provides a total of **135** (15 x 9) possible combinations. Hemagglutinin is the most important protein of the virus and determinant of its virulence while neuraminidase helps in the budding and release of the progeny virions. Both hemagglutinin and neuraminidase frequently undergo genetic modifications decreasing the effectiveness of the host immune response. Influenza virus "C" lacks the "N" protein. Envelope is lined by Matrix protein "M1" and an ion channel matrix protein "M2". Three proteins namely PB1, PB2 and PA form the viral RNA polymerase complex which is associated with genomic RNA and nucleoprotein.

Figure 25.1 Schematic representation of an influenza virus:

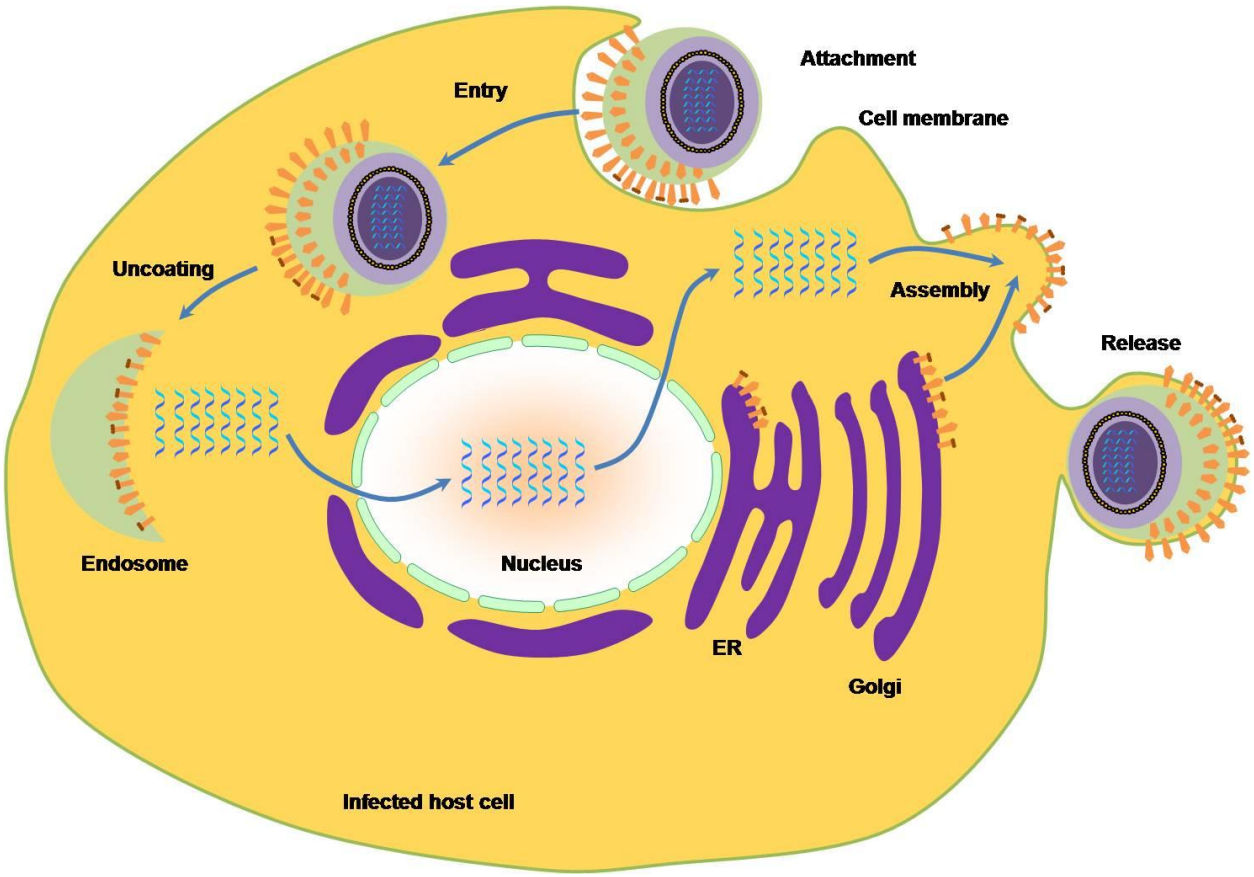


25.4 Virus Replication

Influenza virus enters the cell after binding to sialic acid receptor present on the cell membrane. Different cells of the body contain different sialic acid receptor types which largely determine the host range of these viruses. The respiratory tract epithelium of humans contains α 2-6 linkage sialic acid receptor while birds contain α 2-3 sialic acid receptor. The types of sialic acid receptor in the epithelium determines the tropism of human as well as avian influenza viruses. A single amino acid change in the hemagglutinin protein [E (Glutamic acid) 190D (Aspartic acid)] changed the binding efficiency of influenza virus from α2,3 to α2,6 which caused the outbreak of 1918 influenza virus (Spanish flu). Virus enters the cell by receptor mediated endocytosis and uncoats under the low pH condition of endosome. RNA synthesis of influenza virus takes place in the nucleus of the cell. Nucleoprotein of influenza virus contains nuclear localization signals (NLS) that help in transportation of ribonucleoprotein complex into the nucleus. Negative sense RNA genome of influenza viruses serve as a template for the synthesis of positive sense RNA. Positive sense replicative intermediate RNA acts as a template for progeny RNA genome. Interestingly, viral endonuclease activity of PB2

protein cleaves the 5' cap and 10-13 nucleotides from a cellular mRNA in order to transcribe the viral RNA. The phenomenon is called as **cap snatching**. All orthomyxoviruses undergo **splicing** phenomena to produce two proteins from one gene such as influenza virus A uses gene segment 7 to produce M1 and M2 protein. Similarly 8^{th} segment of influenza virus produces NS1 and NS2 protein after undergoing splicing. In certain influenza viruses, frame shift mutation leads to formation of PB1-F2 protein. Viral protein synthesis occurs in the cytoplasm and its maturation takes place in endoplasmic reticulum and Golgi apparatus. Viral nucleoproteins are required for replication of genomic RNA which then enters the nucleus along with polymerase protein for transcription. Progeny virus is released by budding through the plasma membrane.

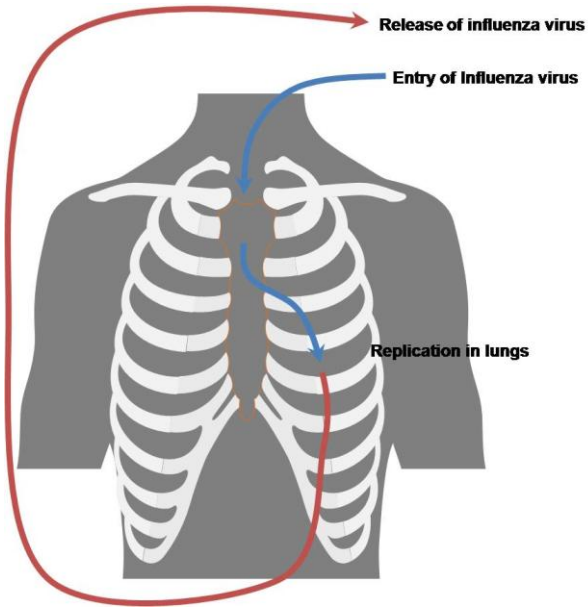
Figure 25.2 Schematic representation of an influenza virus replication cycle:



Lecture 26: Influenza pathogenesis and bird flu

When influenza virus (commonly referred as flu) enters the host via aerosol it replicates in the epithelial cells of upper and lower respiratory tract. The infection causes destruction of the ciliated cells along the lining of the respiratory tract that leads to inflammation and formation of exudates. The signs include tracheitis, bronchitis, and pneumonitis. Sometime collapse of adjacent air sacs and emphysema is also evident. Microscopically, there is complete denudation of the epithelial lining and infiltration of inflammatory cells.

Figure 26.1 Mode of entry and exit of influenza virus in humans:



26.1 Molecular determinants

The hemagglutinin (HA) protein of influenza virus is a key in determining the virulence and pathogenicity of influenza virus. HA protein of influenza virus has to cleave in order to start the infectious cycle. The highly pathogenic influenza virus has many basic amino acid (**arginine**) residues at the cleavage site of the HA protein, while the less pathogenic viruses contains less basic amino acid residues at the cleavage site. The virus containing less basic amino acids are restricted to multiply in certain selected tissues as the protease required for the cleavage site is present only in certain specific tissues. In humans and

birds respiratory and gastrointestinal tract contains the proteases to cleave the HA protein. In contrast, the viruses containing more basic amino acid residues at the cleavage site increases the range of cells capable of producing infectious virions. This property of the virus is important in determining its tissue tropism.

Non structural (NS1) protein in influenza virus is another key factor in determining the virulence of the viral strains. NS1 protein binds to dsRNA and blocks the interferon activation (refer lecture 11 and12). The influenza virus containing mutation in the NS1 proteins are attenuated as the mutated form of the protein does not bind with the dsRNA which causes increased level of interferon in the infected cells.

Polymerase protein PB2 is another important component that determines the virulence of influenza viruses. PB2 helps in RNA transcription and replication of the virus. The PB2 containing mutation in certain amino acid sequences may increase or decrease the virulence of the virus. Another protein such as PB1 is also suggested as a determinant of influenza virus virulence.

26.2 Clinical features

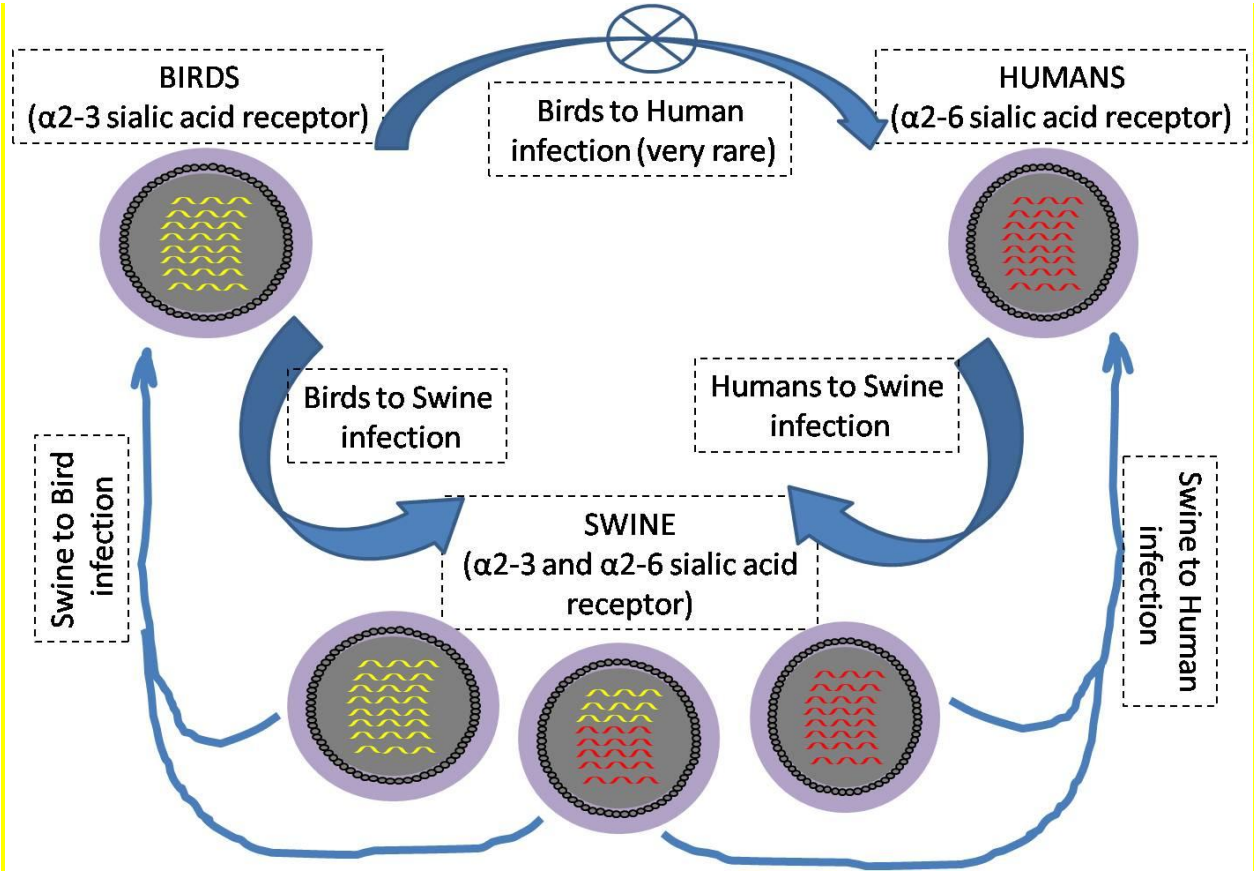
The incubation period of the virus in human is from 2 to 4 days, may extend up to 8 days based on the immune status of the patient. Initial symptoms of the disease include fever and lower respiratory tract illness. Diarrhea, abdominal pain, headache, and vomiting are other common symptoms observed during the early phase of infection. The later phase of the disease shows the symptoms of more advanced respiratory distress such as acute respiratory distress syndrome (ARDS). Multi-organ failure involving kidney and heart are also associated during the later part of the disease.

26.3 Influenza virus reassortment

Reassortment is another very interesting phenomenon and occurs in viruses having segmented genome. Reassortment stands for mixing of viral genetic information in order to evolve them as a new species having potential to cause a greater pandemic. If a cell or animal is infected with two different influenza viruses, the RNA segments of both viruses

are copied in the nucleus. When new virus particles are assembled at the cell membrane, each of the 8 RNA segments may originate from any one of them or a mixture of two infecting viruses. The progeny virion that contains RNA segments from both the parents is called as reassortant. This process is illustrated in the diagram below for involution of swine influenza virus.

Figure 26.2 Reassortment of avian and human influenza virus in swine:



26.4 Bird Flu

Bird flu is also known as avian influenza (flu infection in birds). Historically the disease was known as **fowl plague**. The virus caused havoc in the human population when a bird infecting virus mutated to infect humans. The first case was reported in Hong Kong in 1997 and that was known as H5N1 (avian influenza A virus). Human cases of H5N1 were reported in many parts of the world. Major risk group includes farmers and others who are in contact with poultry, visiting the countries affected with bird flu, and people consuming undercooked poultry meat from infected birds.

26.4.1 Symptoms

In human subjects symptom include sore throat, nasal discharge, headache, fever, diarrhea, breathing difficulty, and cough. If birds survive following infection, they show complete loss of egg laying capacity, respiratory distress, diarrhea and nervous signs (tremors and paralysis).

26.4.2 Pathogenesis

The virus cause damages in the respiratory epithelium and alveoli. The alveolar spaces are filled with fibrinous exudates and inflammatory cells. Vascular congestions and proliferation of fibroblast is also evident in many cases. Varied degree of hepatic damage is also seen in many cases. In birds picture is quite different than humans, as virus replicates in the intestine also. Infected birds show the signs of viremia and necrosis of different visceral organs, and often succumb to death following few days of infection.

26.4.3 Treatments

Antiviral drugs such as oseltamivir (Tamiflu) or Zanamivir (Relenza) reduces the severity of infection if taken within 48 hrs after the onset of symptoms. In later stages of infection patient may need respirator for breathing.

Lecture 27: Rhabdoviruses

Rhabdo in Greek means 'rod-shaped'. The first description of rabies dates from the 23rd century BC in Mesopotamia. In Latin word "*rabere"* means to rage (In sanskrit word *Rabhas* means to do violence). During early 1881, French chemist **Louis Pasteur** and his assistant, **Emile Roux**, begin their research on rabies and successfully vaccinated **Joseph Meister who was bitten by a rabid dog**. Rabies is a zoonotic viral disease most often transmitted after the bite of rabies infected animal. Large cases of the rabies are reported each year from different parts of the world. The members of the family *Rhabdoviridae* belong to order *Mononegavirales* and contain six genera. The Rhabdoviruses contain negative strand RNA genome ranging from 11 to 15 Kb in size. Those Rhabdoviruses that infect plants are rod shaped with rounded ends eg Potato yellow dwarf virus while those infecting animals are bullet shaped eg Rabies virus (Lyssa virus).

Rhabdoviridae is divided into six genera

1) Novirhabdovirus
2) Vesiculovirus
3) Lyssa virus
4) Ephemerovirus

↕ Animal viruses

1) Cytorhabdovirus
2) Nucleorhabdovirus

↕ Plant viruses

Virus	Virus type	Host species
Vesiculovirus	Vesicular stomatitis Indiana virus	Vertebrates
Lyssavirus	Rabies virus	Vertebrates
Ephemerovirus	Bovine ephemeral fever virus	Vertebrates
Novirhabdovirus	Infectious haematopoetic necrosis virus	Vertebrates
Cytorhabdovirus	Lettuce necrotic yellows virus	Plant
Nucleorhabdovirus	Potato yellow dwarf virus	Plant

27.1 Virion properties

Rhabdoviruses contain a linear, single stranded, negative sense RNA genome. Virions are 45- 100 nm in diameter and 100- 430 nm long. The virion has a cylindrical nucleocapsid surrounded by an envelope with large glycoprotein spikes. The virions are sensitive to heat and UV rays but stable towards the changes in pH. The virus is bullet shaped which is due to its lipid envelope. The genome is 11.9 kb in size which encodes for 5 genes in the following order.

3' –N-P-M-G-L- 5'

N- Nucleocapsid protein

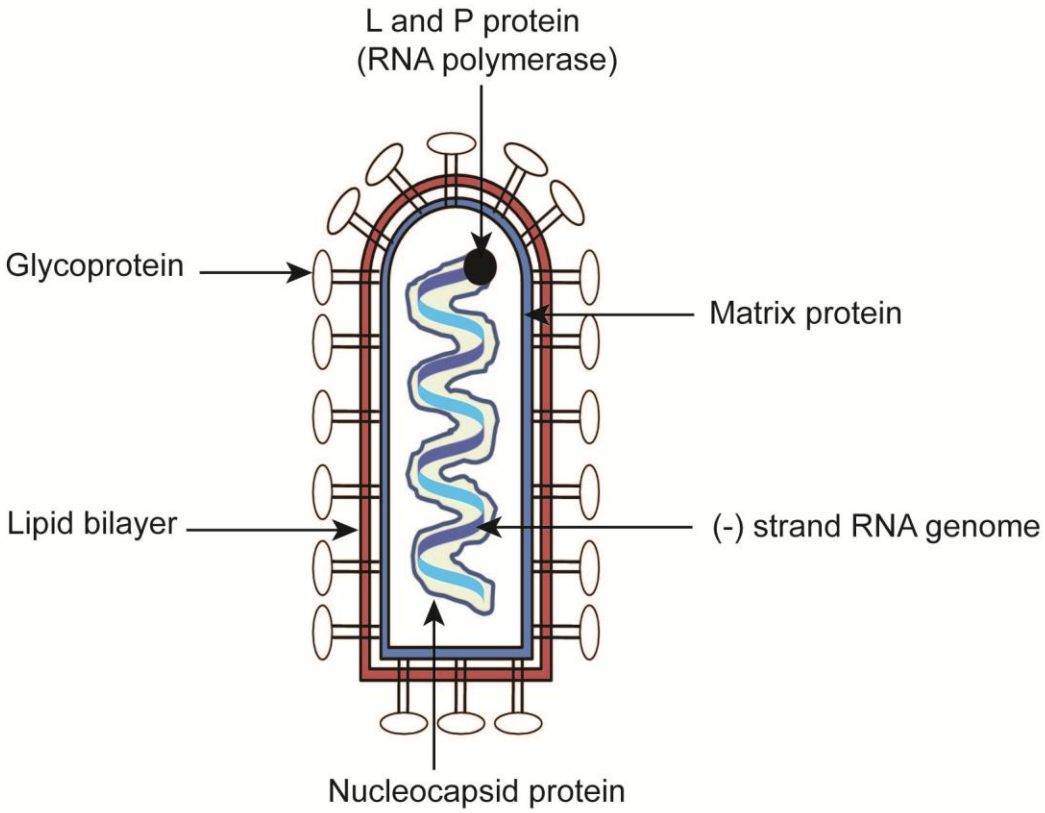
P- Phosphoprotein- cofactor of the viral polymerase

M- Inner virion protein/ helps in budding of the virion.

G- Glycoprotein that assists in making virion spikes

L- Large protein that represents RNA dependent RNA polymerase and helps in transcription and replication.

Figure 27.1 Schematic representation of rhabdovirus virion:

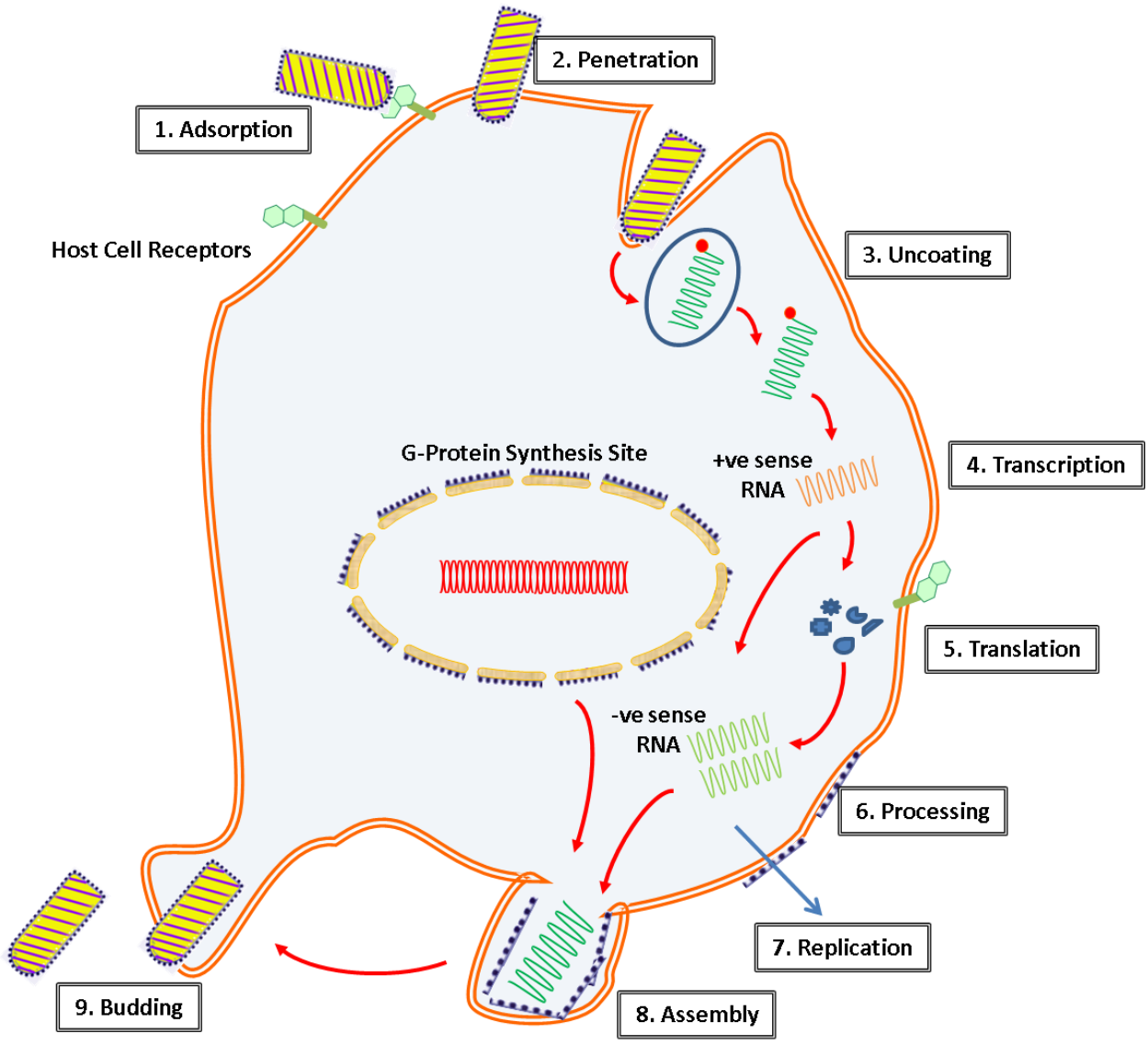


27.2 Virus Replication

In animals, virus replication takes place in the cytoplasm while in plants it may occur in nucleus. The virus entry into the host cell occurs by receptor mediated endocytosis followed by pH dependent fusion of virus envelope with endosomal membrane. As a result of fusion, the nucleocapsid is released into the cytoplasm. The first step of replication involves mRNA transcription from genomic RNA using RNA dependent RNA polymerase. For successful replication a large amount of nucleoprotein (N) and phosphoprotein (P) should be expressed. Switching of transcription to positive sense antigenome occurs after a threshold amount of N and P, which are then further used as a template for synthesis of negative stranded genomic RNA. There is a single promoter site at the 3' end of the viral genome where the polymerase attaches to the genomic RNA template and moves along the viral RNA. While moving it hits with start – stop signals at both the ends of the viral genes. Due to this only a small fraction undergoes continuous

transcription process and hence this phenomenon is also known as **attenuated transcription**. Consequently more mRNA is produced towards the genes that are located at the 3' end and hence producing a gradient of mRNA in the order of N>P>M>G>L. As a result of the mRNA gradient, large amount of structural protein such as nucleocapsid protein is produced as compared to L protein.

Figure 27.2 Schematic representation of rhabdovirus replication cycle:



27.3 Important Rhabdoviruses

27.3.1 Vesicular stomatitis virus

Vesicle means blister and stomatitis means inflammation of oral mucous membrane. Vesicular stomatitis virus (VSV) is a disease of various animal species including cattle, horse, sheep and pig. Animals develop the lesions in the feet and mouth similar to that of foot and mouth disease. The VSV can replicate in a variety of cell lines. Most of our understanding regarding the rhabdovirus replication and transcription came from the study of VSV.

27.3.2 Bovine Ephemeral fever virus

It is an arthropod transmitted disease of cattle and buffalo characterized by biphasic or polyphasic fever, depression, diarrhea, and loss of appetite. The disease is also called as **3-day stiff-sickness**. The virus causes inflammation and injury to the inner lining of the endothelial blood vessels.

27.3.3 Infectious hematopoietic necrosis virus

It is a disease of salmonid fishes. The virus is associated with significant economic losses to the countries producing salmon in large quantity. Infection is characterized by darkened body colour, pale gills, distension of abdomen due to accumulation of fluid, and hemorrhages around fins.

Lecture 28: Rabies

WORLD RABIES DAY is being celebrated every year on **SEPTEMBER 28**.

Rabies is a dreaded disease and is of zoonotic importance. Disease is generally caused by the bite of a rabid dog/ animal. Although rabies in humans is 100% preventable by appropriate medical care, more than 55,000 people in Africa and Asia, die from rabies every year. Children are often at greatest risk as they are more likely to be bitten by dogs.

28.1 Clinical features and epidemiology

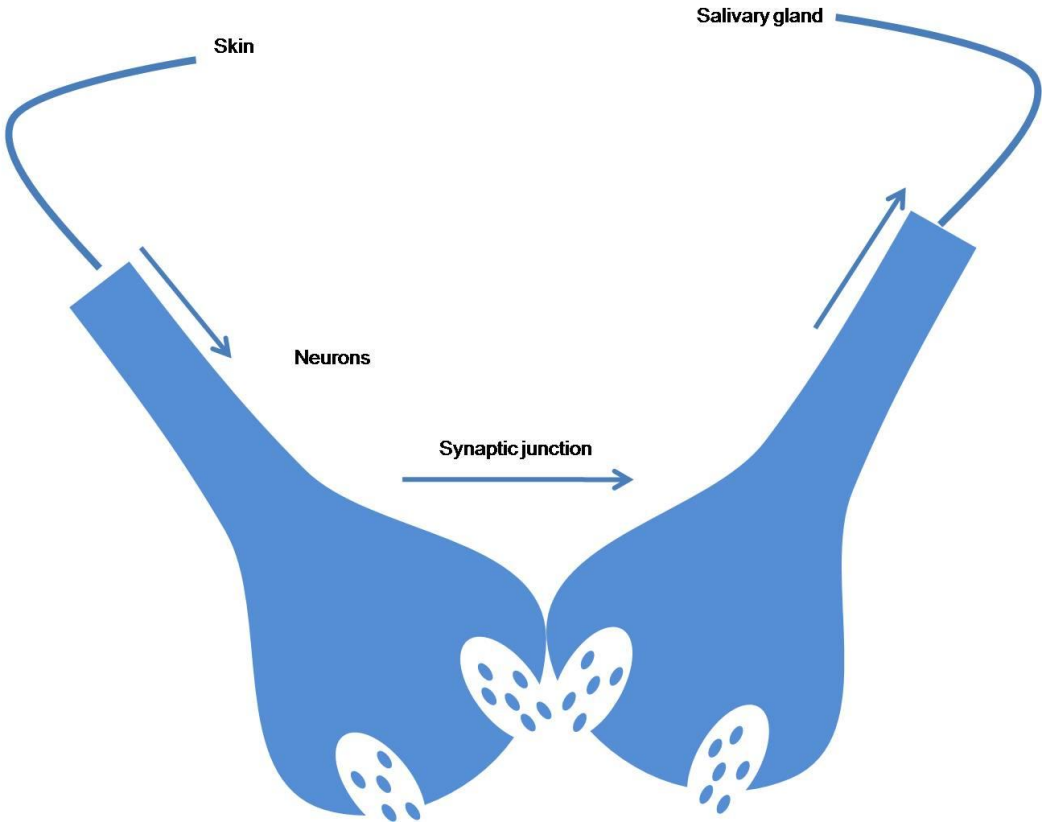
Rabies is a zoonotic disease and it may infect any warm blooded animal including humans. Virus is usually present in the saliva of the infected animal. The disease usually occurs in two clinical forms, furious and dumb (paralytic). The furious form is characterized by insomnia, confusion, agitation and often leading to delirium. It is also called as hydrophobia as the infected person cannot gulp water because of pharyngeal paralysis. Sometime profuse salivation and encephalitis are also evident. Progressive encephalitis leads to dumb form of the disease. Terminal stages include convulsive seizures, coma and respiratory arrest.

28.2 Pathogenesis and Pathology

In rabies the prognosis of the disease is mainly decided by the location and severity of the site of bite and the species of animals involved. Rabies has three stages. The first stage is characterized by behavioral changes also known as **prodromal** stage. In the second stage called as **excitative** stage the animal exhibits disease in furious forms and in the third stage the animal manifests the **paralytic** form of the disease. After entry the virus first affects the peripheral nervous system and replicates in the brain. Dumb and paralytic forms of the disease appear as it progresses towards the central nervous system. In the nervous system the virus is formed by budding in various membranes and glands. Neurons accumulate ribonucleoprotein as intracytoplasmic inclusion bodies often termed as **Negri bodies** and are pathognomonic for rabies. Salivary glands help the virus to bud

on plasma membrane and release it in very high concentrations through the saliva. Death usually occurs due to respiratory arrest.

Figure 28.1 progression of rabies virus through neurons in body:



28.3 Diagnosis

Any patient having a history of animal bite should be suspected for rabies. Differential diagnosis with West Nile virus, herpesvirus, enterovirus is required. History and clinical symptoms like encephalitis, salivation, etc provide concrete support for rabies. Besides this, **Negri** bodies in the neurons of affected animals can be visualized using Seller's stain. RT-PCR assay can be conducted to test for the presence of viral RNA in the brain of suspected animal. RT-PCR of saliva can also be carried out. Immuno-histochemical staining can also be done from frozen sections of brain tissue. Fluorescent antibody test can also be done for the detection of viral antigens from the tissue samples.

28.4 Immunity, prevention and control

Rabies is a highly immunogenic disease and many vaccines are available for the same. In early stages of infection, infectious rabies virus is susceptible to antibody mediated neutralization and this efficacy has been proved in exposed humans of the classical Pasteurian post- exposure vaccination and it provides even better results when administered along with hyper-immune globulin.

Blood brain barrier shows promising results when its permeability is increased artificially as it helps in viral clearance and does not allow most immune cells across. Thus altering the permeability of blood brain barrier to contribute to viral clearance can also be exploited in case of Rabies.

Besides this, prophylactic measures should be taken like scheduled vaccination, washing the wounds with soap for 5 to 10 times while treating the infected cases and regular spaying of the animals can prevent the disease.