Module3: Positive strand RNA virus

Lecture 15: Classification of viruses and nomenclatures

(Part I)

Historically, all the viruses were grouped according to the illness they caused (for eg- hepatitis, encephalitis etc.). It was quite common to name the virus on the disease with which it is associated (foot and mouth disease virus) or the geographical location from which it is isolated (Rift valley fever virus). This kind of nomenclature changed with the advent of molecular biology and more advanced biochemical and biophysical techniques.

The most comprehensive and widely used classification was first given by Dr David Baltimore in which seven groups were proposed based on the genetic contents and replication strategies of the viruses. This classification is focused on the relationship between the viral genome and its mRNA and describes the formation of mRNA by the viruses with either DNA or RNA genome

Figure 15.1. Baltimore classification based on mRNA production by all viruses following infection
Group 1, dsDNA viruses – Replicating through DNA

Group 2, ss DNA viruses- Replicating through DNA

Group 3, ds RNA viruses- Replicating through RNA

Group 4, ssRNA viruses (+) polarity, (sense to mRNAs) - Replicating through RNA

Group 5, ssRNA viruses (-) polarity, (antisense to mRNAs) - Replicating through RNA

Group 6, RNA-retroid genomes (RNA -> DNA -> RNA) - Replicating using reverse transcriptase having dsDNA as an intermediate.

Group 7, DNA-retroid genomes (DNA -> RNA -> DNA) - Replicating using reverse transcriptase having ssRNA as an intermediate.

Current virus classification is based mainly on the morphology, nucleic acid type, host organism it infects, replication mode, and the disease type caused. International committee on taxonomy of viruses (ICTV) was established in 1966 in order to establish a universal system for virus classification. In the eighth report of ICTV which was published in 2005, three orders, 73 families, 9 subfamilies, 287 genera and more than 5000 viruses were approved. It is absolutely impossible to be up to date on the numbers that were approved by the ICTV as everyday new viruses are added to the database. The most current information is available in the ICTV webpage (http://www.ictvonline.org/index.asp).

Family- It is defined as a group of genera with common characteristics. It is written as capitalized, Italicized, and ends in -viridae. Examples- Paramyxoviridae, Poxviridae (poxvirus family).

Subfamily- These are groups of viruses within some large families. They are written as capitalized, Italicized, and end with -virinae. Examples- Paramyxovirinae, Parvovirinae, Alphaherpesvirinae.

Genus- It is defined as a group of virus species sharing common characteristics. They are written as capitalized, Italicized, and end with -virus. Examples- Parovirus, Flavivirus, Coronavirus.

Species- It is defined as a population of strains from one particular source, all of which have a common property that separates them from other strains. While writing the name of the species it is neither capitalized nor italicized. Eg. vaccinia virus, human immunodeficiency virus, influenza A virus.
Some specification not approved by the ICTV:

**Strain** - These are different lines of isolates of the same virus. Eg. Influenza viruses those were isolated from different geographical locations.

**Type** - They show different reactivity towards a positive serum sample, sometime called as serotypes (different antigenic specificity) of the same virus. Eg. Paramyxovirus type 1-9. There may also be subtypes within a particular type.

**Group** - These are divisions often based on nucleotide sequence similarities or origin. HIV group M (Main), N (Neither M or O), or O (Outlier). There may also be subgroups. (also called clade) within a particular group (M group HIV has A-J subgroups).

**Variant** - These are viruses whose phenotype differs from original wild type strain.

**Origins of some viral names**

- Picorna: small having size in the scale of $10^{-12}$ RNA segment
- Birna: two RNA segment
- Toga: wearing a robe
- Rota: Wheel like
- Arbo- Arthropod borne
- Papilloma: infections result in warts
- Adeno: infections of glands
- Hepadna: hepatitis + DNA
- Herpes: produce scaly lesions
- Pox: produce pox lesions
- Corona: crown like

**Satellite viruses and Defective Interfering particles:**

Consider viruses to be a part of ecological habitat where organisms tend to share the relationships with one another: mutualism, commensalism, symbiosis, and parasitism. Viruses also act similarly.

**Satellite viruses** - Viruses with separate genomes that are encapsidated inside viral particles that are produced by a “helper” virus. They also require helper virus replicative machinery to replicate their genomes.

**Defective Interfering particles (DI particles)** - Their genomes are derived from a helper virus. They are deletion mutants which have lost their ability to encode proteins, but retain their ability to replicate with the help of a replication machinery of other helper
virus. They called defective interfering particles because they are defective in their ability to produce proteins, and tend to interfere with the replication of helper virus by competing with the resources.
Lecture 16: Classification of viruses and nomenclatures
(Part II)

16.1 Principles of virus nomenclature

Followings are the principles of virus nomenclature set by ICTV

a) It aims to provide stability, avoid confusion, and to avoid creation of unnecessary names.

b) It is independent of any other biological nomenclature.

c) The basic unit of classification is TAXON.

d) A taxon should be officially approved by the ICTV committee.

The universal virus nomenclature should follow a hierarchical level of Order, Family, Subfamily, Genus, and Species.

Table 16.1 Current status of virus taxonomy:

<table>
<thead>
<tr>
<th>Order</th>
<th>Numbers of Families</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caudovirales</td>
<td>3</td>
</tr>
<tr>
<td>Herpesvirales</td>
<td>3</td>
</tr>
<tr>
<td>Mononegavirales</td>
<td>4</td>
</tr>
<tr>
<td>Nidovirales</td>
<td>3</td>
</tr>
<tr>
<td>Picornavirales</td>
<td>5</td>
</tr>
<tr>
<td>Tymovirales</td>
<td>4</td>
</tr>
<tr>
<td>Not assigned any order</td>
<td>72</td>
</tr>
</tbody>
</table>
Table 16.2 Classification of viruses based on the type of nucleic acid:

<table>
<thead>
<tr>
<th>dsDNA viruses</th>
<th>ssDNA viruses</th>
<th>dsRNA viruses</th>
<th>ssRNA (+) viruses</th>
<th>ssRNA (-) viruses</th>
<th>RNA and DNA (RT) viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poxviridae</td>
<td>Circoviridae</td>
<td>Reoviridae</td>
<td>Picornaviridae</td>
<td>Bornaviridae</td>
<td>Retroviridae (RNA)</td>
</tr>
<tr>
<td>Asfaviridae</td>
<td>Anellovirus</td>
<td>Birnaviridae</td>
<td>Caliciviridae</td>
<td>Rhabdoviridae</td>
<td>Hepadnaviridae</td>
</tr>
<tr>
<td>Iridoviridae</td>
<td>Paroviridae</td>
<td></td>
<td>Hepevirus</td>
<td>Filoviridae</td>
<td>(DNA)</td>
</tr>
<tr>
<td>Herpesviridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoviridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyomaviridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillomavirida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 16.1 Classification of order Mononegavirales:
Lecture 17: Positive strand RNA viruses

The viruses which contain positive strand RNA genome act directly as mRNA upon infection to a host cell. Most viruses in this category have icosahedral symmetry and vary approximately 50-150 nm in diameter. The members contain positive sense RNA genome containing either lipid envelope (Togaviridae, Flaviviridae, and Coronaviridae) or devoid of envelope (Picornaviridae). They are important because they can cause serious life-threatening diseases including hemorrhagic fever and encephalitis (dengue, yellow fever). Members of genus Alphavirus (Sindbis virus, Semiliki Forest virus, and Equine encephalitis) are transmitted to animal by mosquito bite. Among all Alphaviruses sindbis virus is well studied and understood.
### Table 17.1 Examples of positive strand RNA viruses:

<table>
<thead>
<tr>
<th>Family</th>
<th>Virus example(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal viruses</td>
<td><em>Flaviviridae</em></td>
</tr>
<tr>
<td></td>
<td>West Nile virus</td>
</tr>
<tr>
<td></td>
<td>Yellow fever virus</td>
</tr>
<tr>
<td></td>
<td>Dengue virus</td>
</tr>
<tr>
<td></td>
<td>bovine viral diarrhea virus</td>
</tr>
<tr>
<td></td>
<td>classical swine fever</td>
</tr>
<tr>
<td></td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td></td>
<td><em>Coronaviridae</em></td>
</tr>
<tr>
<td></td>
<td>Severe acute respiratory syndrome (SARS) virus</td>
</tr>
<tr>
<td></td>
<td>Avian infectious bronchitis virus (IBV)</td>
</tr>
<tr>
<td></td>
<td><em>Togaviridae</em></td>
</tr>
<tr>
<td></td>
<td>Rubella virus</td>
</tr>
<tr>
<td></td>
<td>Chikungunya virus</td>
</tr>
<tr>
<td></td>
<td>Semliki Forest virus</td>
</tr>
<tr>
<td></td>
<td>Sindbis virus</td>
</tr>
<tr>
<td></td>
<td>Eastern equine encephalitis virus</td>
</tr>
<tr>
<td></td>
<td>Western equine encephalitis virus</td>
</tr>
<tr>
<td></td>
<td>Venezuelan equine encephalitis virus</td>
</tr>
<tr>
<td></td>
<td>Ross River virus</td>
</tr>
<tr>
<td></td>
<td><em>Picornaviridae</em></td>
</tr>
<tr>
<td></td>
<td>Foot and mouth disease virus</td>
</tr>
<tr>
<td></td>
<td>Human rhinovirus</td>
</tr>
<tr>
<td></td>
<td>Encephalomyocarditis virus</td>
</tr>
<tr>
<td></td>
<td>Polio virus</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A virus</td>
</tr>
<tr>
<td>Plant viruses</td>
<td><em>Potyviridae</em></td>
</tr>
<tr>
<td></td>
<td>Potato virus Y</td>
</tr>
<tr>
<td></td>
<td><em>Flexiviridae</em></td>
</tr>
<tr>
<td></td>
<td>Potato virus X</td>
</tr>
<tr>
<td></td>
<td><em>Comoviridae</em></td>
</tr>
<tr>
<td></td>
<td>Cowpea mosaic virus</td>
</tr>
</tbody>
</table>

### 17.1 Virion properties

Virions are spherical in shape and are having uniform appearance. The diameter of virus varies between 70-100 nm. The enveloped virion particle encircles the icosahedral capsid. Envelope contains spikes of viral glycoprotein which are major antigenic determinants of the virus. The spike glycoproteins are highly variable among strains and also between different serotypes.
17.2 Structure of positive strand RNA genome

They are positive sense, single-stranded RNA, vary between 9-12 kb in size, with the exception of coronaviruses. Terminal 5’ end of the genome is capped while 3’ end is polyadenylated. Viruses with single stranded RNA genome do not require secondary or tertiary fold in their capsid to accommodate its genome. That means they are highly organized and tightly packed. Generally a dimer of coat protein interacts with the 3’ end of the RNA, which is essential for the virus replication. This interaction is also needed for the packaging of the genomic RNA inside the virion. The viral genomic RNA is arranged in the icosahedral capsid in various ways in order to neutralize the negative charge of the nucleic acids.

17.3 Replication of positive strand RNA

The positive strand RNA virus transfers its genome directly to the ribosome and starts translation for the synthesis of viral proteins. Infectious cycle begins with the entry of virus into the cell through endocytosis. The genomic RNA uncoats after getting into the cytoplasm of the infected cells. The RNA is then translated into the viral polyprotein precursors which are later cleaved by proteolysis to form the structural and non structural viral proteins. The structural proteins are involved in the maturation and assembly of the virion while nonstructural proteins act as RNA replicating enzyme for genomic RNA synthesis. Some of the viruses in this class form the subgenomic RNA during replication process (Coronavirus, Caliciviruses, and Togaviruses).
Figure 17.1 Schematic representations of togavirus and rubella virus genome:

**Togaviridae genome**

5'cap

<table>
<thead>
<tr>
<th>M</th>
<th>H</th>
<th>Pro</th>
<th>Un</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>NsP1</td>
<td>NsP2</td>
<td>NsP3</td>
<td>NsP4</td>
<td></td>
</tr>
</tbody>
</table>

 polyA 3'

**Rubella virus genome**

5'cap

<table>
<thead>
<tr>
<th>M</th>
<th>Un</th>
<th>Pro</th>
<th>H</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>E2</td>
<td>E1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 polyA 3'

M - Methyl transferase  
H - Helicase  
Pro - Protease  
Un - Unknown  
R - Replicase  
C - Capsid protein  
E - Envelope protein  
NsP - Nonstructural protein
Lecture 18: Picornaviruses

Family *Picornaviridae* contains viruses that infect many species of animal as well as humans. Poliovirus was the first virus that was propagated in the cell culture and purified using plaque assay. Most of the picornaviruses grow in a variety of cell lines making them a useful tool to understand the biology of positive strand RNA viruses.

**18.1 Important picornaviruses**

**18.1.1 Poliovirus**

In general poliovirus cause mild disease condition in the oro-pharyngeal cavity and gut epithelium. The disease becomes serious when virus migrates to central nervous system and cause systemic viremia. Poliovirus is a disease associated with poor hygienic and sanitary conditions. Poliovirus infection to central nervous system can cause encephalitis and paralysis of limbs and respiratory muscles. Polio vaccine is very effective in controlling the outcome of the disease. Polio has been eradicated from most of the countries.

**18.1.2 Hepatitis A virus**

Hepatitis A virus is a problem of young children in the developing countries where hygienic condition is not good. Infection in the childrens is often mild and once infected the immunity lasts for life long. The infection to adults can cause severe jaundice which may prove sometimes fatal also.

**18.1.3 Coxsackievirus**

The first coxsackievirus was isolated from mice showing symptoms of inflammation in skeleton muscles. The virus can cause inflammation of central nervous system and heart muscles with rashes over the body.
18.1.4 Foot and mouth disease virus

Foot and mouth disease (FMD) virus is a highly contagious disease of cloven footed animals. The disease has a major impact on the trade and economy of the countries dependent on agriculture and animal products. The disease causes severe drop in milk production of cattle. Symptoms of the disease include lesions in hoof and mucosal surface of oral cavity. The condition is febrile and animal may die if not treated. Vaccines are available against FMD for cattle, sheep, goats and pigs.

18.1.5 Rhinovirus

Rhinovirus is very common cause of upper respiratory tract infection in humans. Most of the children get infected with rhinovirus by the age of 3 years. Most of the rhinovirus can survive and replicate in the lower temperature (33⁰C) of respiratory epithelium.

18.2 Picornavirus Virion

Picornaviruses are small RNA viruses having diameter of 25-30 nm. The virus has icosahedral symmetry and capsids are made up of four proteins (VP1-4). Some of the picornaviruses contain CANYONS over the vertices of the icosahedron which act as virus attachment site on those viruses (Poliovirus and rhinovirus). Many serotypes have been evolved because of the variation in the capsid proteins which creates a challenge to make a vaccine against the picornaviruses. The genome is composed of ssRNA of 7-8 kb in size. The 5’ end of the RNA is covalently attached with a protein called as VPg (genome linked viral protein) while its 3’ end is polyadenylated. Interestingly, 5’ end of the genome contains many secondary structures called as internal ribosome entry site (IRES).

18.3 Picornavirus replication

Some picornaviruses enter cell through a receptor called as CD155 (Poliovirus receptor). CD155 is a member of immunoglobulin super-family and are widely expressed in many cell types. Many Enteroviruses enter the cell after binding to CD55 or decay accelerating
factor, a member of complement system. Once inside the cell the viral encoded VPg gets detached from the 5’ end of the RNA. The viral RNA acts as an mRNA and binds to the ribosome with the help of IRES present at the 5’ end of the genome. Virus encodes a single polyprotein which is cleaved by virus coded proteases into single structural and nonstructural proteins. The polyprotein is first cleaved into P1, P2, and P3. P1 get cleaved into VP0, VP1 and VP3 and myristylated at its N terminus. VP0 further cleaved into VP2 and VP4, other cleavage products include 2C (ATPase), 3B (VPg) and 3D (RNA polymerase). Virus replication takes place in the replication complexes with the help of RNA polymerase. Usually five copies each of VP0, VP3 and VP1 assembles to form a procapsid which encapsidates the viral genome. Lysis of the infected cells releases the progeny virions.

Figure 18.1 Schematic representation of picornaviruses genome:
18.4 Recombination in Picornavirus

The process of recombination is mostly relevant in cases of segmented viral genome. When a cell is infected simultaneously with two or more strains, the progeny virion may contain the genome derived from both the strains. A recombinant virus is one which contains part of its genome from one virus and remaining from the other. Polio virus vaccine contains the mixture of all three strains responsible for the disease in oral formulation.
Lecture 19: Flaviviruses- West Nile virus

The family *Flaviviridae* contains around 70 pathogens of both human as well as animals. These 70 members are distributed among three genera, namely *Flavivirus*, *Pestivirus*, and *Hepacivirus*. Out of these 70 members, 30 were arthropod borne human pathogens. Yellow fever virus is the prototype of the genus flavivirus which was the major cause of human illness during the 18th and 19th centuries. Members such as West Nile virus, dengue virus, and Japanese encephalitis viruses are now considered as most important human pathogens. Genus *Pestivirus* contains many important pathogens of veterinary importance such as hog cholera (classical swine fever), bovine viral diarrhoea, and border disease. Genus *Hepacivirus* contains hepatitis C virus, an important human pathogen which causes viral hepatitis.

19.1. Properties of Flaviviruses

Virions are spherical and 40-60nm in diameter. They contain a lipid derived envelope with spikes of glycoprotein embedded on it. The genome consists of a positive sense single-stranded RNA of approximately 9.6 to 12.3 kbp. 5’ cap is present only in the members of genus *Flavivirus*. The viral genome codes for both structural (3-5 in numbers) as well as non-structural (7-8 in numbers) proteins. The viruses are easily inactivated by common disinfectants and heat. Flaviviruses infect a variety of cells including Vero (African green monkey), BHK-21 (baby hamster kidney) and chicken embryo fibroblasts. Infection of flavivirus is lethal to new born mice. Viruses enter the cells through receptor mediated endocytosis and replication of the viral genome takes place in the cytoplasm. Replication involves synthesis of negative sense RNA from the positive sense genomic RNA, which then serves as a template for mRNA synthesis. Translation of viral mRNA leads to formation of a single polyprotein which is then cleaved into structural and non structural proteins. The maturation of the virion takes place in endoplasmic reticulum and are released following lysis of the infected cells.
West Nile Virus

West Nile virus was first identified in 1937 in Africa as a cause of mild febrile infection. Later on it was identified as a causative agent of fatal encephalitis in humans and horses. The virus is transmitted to the human being by the bite of Culex mosquitoes, while birds serve as a reservoir host. The animal and humans are considered as an incidental host when virus gets transmitted following insect bite harboring the virus in large quantity.

Figure 19.1 West Nile Virus transmission cycle

Ecology of West Nile virus transmission:

Weather plays an important role in the transmission of West Nile virus to humans and animals. Change in temperature and dryness is an ideal condition for the breeding of mosquitoes. Also during the rainy season the behavioral patterns of birds, mosquitoes, and humans changes. These lead to a hypothesis of having high incidence rate of West Nile virus during initial precipitation and dryness in the air. The role of wind velocity and pressure difference has also been suggested for the outcomes of West Nile virus incidences, but their actual impact is an open area of discussion.
Clinical Features:

Birds often contain virus and do not show any disease symptoms while humans and horses are dead-end hosts. Clinical manifestation of the disease is only evident in case of horses and humans. Infected animals show neurological signs, depression, muscle weakness, and fever. Death occurs in 40% of infected cases based on the immune status of the animal and the strain of virus. Milder form of disease in humans shows fever, abdominal pain, diarrhea and restlessness. These symptoms last for a week. More severe form that can be life threatening is called as West Nile encephalitis.
Pathogenesis:
Infection of West Nile virus causes high-titer viremia, necrosis, hemorrhage and inflammation in many vital organs. These include heart, brain, liver, kidney, intestine, and nervous system. Lesions are evident on brain and spinal cord following West Nile virus infection.

Diagnosis:
Diagnosis of this disease is done mainly on the basis of serology for virus specific immunoglobulin in the serum of infected patient. Traditional techniques such as virus neutralization assay, in–situ hybridization, and immuno-histochemistry are regularly used to diagnose the disease. Modern molecular biological techniques such as reverse transcriptase polymerase chain reaction (RT-PCR) are also used to detect the virus infection from tissue samples.

Immunity, Prevention and Control:
Animal previously infected with West Nile virus are resistant to reinfection and many vaccines are available in the market against West Nile disease. The possible way to control the disease is by controlling mosquito population.
Lecture 20: Flaviviruses- Dengue virus

20.1 Introduction

Dengue virus infection is a leading cause of illness and death in many tropical countries. It becomes the most important mosquito transmitted disease in many parts of the world. Nearly 100 million people are infected annually while millions are under risk. Dengue fever can be caused by 4 different serotypes of the dengue virus. Infection by one virus does not protect against the other virus because of different serological reactivity among viral serotypes. Dengue virus is transmitted among people by the bite of mosquitoes *Aedes aegypti* and *Aedes albopictus*. They are approximately 5mm in size and bites during early morning or late afternoon. Only female mosquitoes can transmit the virus not the males. In some part of the world dengue is endemic, which means the disease occurs every year when the mosquito population is at its peak. The virus first originated from African monkeys, which was suggested as natural reservoir for dengue virus.

20.2 Transmission and spread

*Virus enters the human body through the* mosquito saliva and localizes to various target organs including lymph nodes and liver and starts replication. The virus is then released from target organs and reaches to other lymphatic tissues through blood circulation. Finally virus starts circulating in blood following its release from the lymphatic tissues.

In general the cycle begins with a dengue-infected person. When an uninfected female *Aedes* bites to dengue-infected person during his viremic stage, the virus gets transmitted into the gut of the mosquito. The virus replicates during certain period of time within the mosquito which is called as extrinsic incubation period. The infected mosquito then bites to a susceptible person and transmits the virus, as well as to every other person for its entire life. The virus replicates in the second person and start producing symptoms, this is called as intrinsic incubation period (Inside human body).
20.3 Clinical Features

The dengue virus infection causes fever, headache, joint pain, muscle pain, vomiting, and sometime hemorrhages. Patients may suffer from severe depression after acute form of the disease. Sequential infection with different serotypes of dengue virus put patient into greater risk of developing dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). DSS is the severe form of DHF. DSS is characterized by week and rapid pulse, low blood pressure, and altered mental status.
20.4 Control

Disease can be controlled by eliminating the female *Aedes* mosquito population. Educating the people to carry out vector control program in their homes and neighborhoods can also help to some extent.
Lecture 21: Coronaviruses

Family *Coronaviridae* contains two genera, *coronavirus* and *torovirus*. Coronaviruses contain pathogens that mainly infect mammals and birds causing respiratory, gastroenteritis, reproductive and generalized infections. The toroviruses are so far reported in horses and cattle and are associated with diarrhea.

### 21.1 Important members of genus coronavirus

1) **Transmissible gastro-enteritis virus**
   
   It is a highly contagious disease of swine. Clinical signs of this disease include vomiting, diarrhea, weight loss and dehydration. Disease is more severe in case of young piglets. The virus infects and destroys the enterocytes and intestinal villi resulting in loss of mucosal surface. The measure pathology is restricted to gastrointestinal tract.

2) **Feline enteric coronavirus**
   
   It is systemic and fatal disease of cats. Clinical signs include fever, anorexia (Loss of appetite), and weight loss. Sometimes ocular and neurological signs are also visible. Virus replicates mostly in monocyte and macrophages.

3) **Avian Infectious bronchitis virus**
   
   It is the respiratory disease of poultry characterized by cough, gasping, respiratory rales and dyspnea. Clinical signs include visceral gout, nephritis and cheesy exudate in the trachea. Birds may develop secondary bacterial infection.

4) **Mouse hepatitis virus**
   
   Mouse hepatitis virus is a causative agent of enteric infection in infant mice. It is a highly contagious virus of mice and mortality may approach to 100% in virulent outbreak cases. Symptoms include dehydration and rapid weight loss in infants. Lesions of the disease are visible mainly in small intestine and proximal part of large intestine.

### 21.2 Virion characteristics

Coronaviruses are enveloped, approximately 80-250 nm in diameter, and are pleomorphic in shape. The viruses contain large club shaped spike protrusion from the surface of the icosahedral internal core. The core contains helical nucleocapsids which is linear single stranded RNA of 25-31 kb in size. The 5’ end of the genome is capped and 3’ of the genome is polyadenylated similar to that of picornaviruses. The major structural protein of the coronavirus includes nucleoprotein (N), spike protein (S), membrane protein (M), envelope protein (E), and hemagglutinin esterase protein (HE). The S protein is a major antigenic determinant of the virus and contains the conformational epitopes towards their
N terminal. Host immune responses are directed mainly against N and S proteins which are the main targets for the vaccine designing strategy.

**Figure 21.1 Schematic representation of mouse hepatitis virus:**

21.3 Virus replication

Coronaviruses are reported to utilize many receptors for their entry inside the cells. Severe acute respiratory syndrome (SARS) coronavirus uses angiotensin-converting enzyme 2 (ACE-2), whereas mice hepatitis virus uses carcinoembryonic antigen related cell adhesion molecule 1 (CEACAM-1) while others use sialic acid as a receptor.

Viral RNA serves as an mRNA for the synthesis of RNA dependent RNA polymerase (RDRP). Two large open reading frames are translated as a single polyprotein which are later cleaved into separate viral protein. The RDRP then forms the full length complementary genome which serves as the template for the formation of subgenomic RNA. All the mRNAs are then translated into various viral proteins. Many of the viral proteins undergo posttranslational modifications in endoplasmic reticulum and golgi body and therefore the progeny virions buds out from these location and not from cell membrane.
Figure 21.2 Schematic representation of replication scheme in mouse hepatitis virus:
Figure 21.3 Overall view of coronavirus infectious cycle:

Entry and Uncoating

Genomic RNA used as message to produce viral RNA-dependent RNA polymerase (RDRP)

RDRP uses genomic RNA to synthesize full-length (-) strand.

3' \rightarrow \text{RDRP uses (-) strand to produce large amounts of a small 60-70 bases leader RNA from its 3' end.}

3' \leftarrow \text{Leader primes the synthesis of mRNAs by binding to complementary regions on the (-) strand using RDRP.}

mRNAs are translated by host ribosomes into viral proteins. The leader bound the 3' end of the minus strand makes mRNA for RDRP which is also genomic RNA.
Lecture 22: Severe acute respiratory syndrome (SARS):
Pathogenesis

22.1 Causative agent

Severe acute respiratory syndrome (SARS) is a highly infectious viral disease caused by members of family Coronavirusidae. The first case was recognized in 2003 with signs of atypical pneumonia. SARS is caused by a novel coronavirus (CoV) which can infect a wide range of animal species. Many SARS-CoVs has been found in bats suggesting it to be the reservoir of the virus. The first outbreak of SARS was notified in Southern China where the bats and humans lived in the close proximity with each other. It mainly affects the adult human beings. Transmission is through direct contact with infected patient or through infected body fluids. Incubation period varies from 5 days to 2 weeks. Symptoms include cough, shortness of breath, and difficulty in breathing with mild fever.

22.2 Virion properties

The viral genome of SARS-CoV contains ssRNA of 29.7 kb in size, one of the largest among RNA viruses group. The virion is spherical in shape around 80-100 nm in diameter. Various projections from the surface of the virion protrude to form a crown like structure (hence named coronavirus). 5’ end of the virus encodes for an open reading frame which is translated into a large polyprotein that is cleaved by viral proteases to form many non-structural proteins. The non structural proteins include RNA dependent RNA polymerase, ATPase and helicase. The non structural proteins helps in replicating viral genome as well as viral transcripts and subgenomic mRNA’s which are used to synthesize viral proteins. The viral membrane protein includes spike protein, and a membrane protein which completes its maturation in endoplasmic reticulum and golgi apparatus. The spike (S) protein present on the surface of the virion is the major antigenic determinant of the virus. Spike protein is responsible for tissue tropism as well as gradient of pathogenesis of SARS-CoV. The virus infects the cells of the respiratory epithelium by binding to its receptor, angiotensin-converting enzyme 2 (ACE-2).
22.3 Pathophysiology

Respiratory and gastrointestinal tract are the only organs reported to support SARS-CoV replication. The virus invades the respiratory epithelium and cause lytic effect on the infected cells. The effects are more pronounced in the pulmonary tissues where wide spread alveolar damages are visible. Although the morbidity and mortality is higher among old age group (65 years), intensive care treatment and mechanical ventilation can improve the life expectancy of the patients.

Figure 22.1 Clinical picture of SARS:
22.4 Diagnosis

Diagnosis is based on

I. PCR for identifying SARS-CoV genome sequence.
II. Enzyme-linked immunosorbent assay (ELISA) from serum samples collected from patient.
III. *Immunofluorescence* test (IFT) from serum samples collected from patient.
IV. Virus isolation from infected tissue samples using cell culture techniques.

22.5 Treatment

Normal treatment is mostly symptomatic and includes followings:-

I. Antibiotics to treat secondary bacterial infections
II. Antiviral drugs
III. Corticosteroids to reduce pathology of lungs
IV. Ventilators and artificial breathing

22.6 Prevention and control

a) Reduce the contact with SARS infected individuals
b) Hand washing and personal hygiene
c) Covering mouth and nose while sneezing to avoid droplet infection
d) Avoid sharing food and drinks from an infected person
e) Disinfection of the working space and surroundings.