

**GENERAL AND SYSTEMIC VIROLOGY  
(MICRO – 303)**

TOGAVIRIDAE

FLAVIVIRIDAE

AND

BUNYAVIRIDAE

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# **TOGAVIRIDAE** (toga – cloak or mantle)

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## **Classification**

<b>Genus</b>	<b>Virus type</b>	<b>Virus</b>
Alpha virus	Arboviruses	Western Equine Encephalitis (WEE) virus
		Eastern Equine Encephalitis (EEE) virus
		Venezuelan Equine Encephalitis (VEE) virus
Rubivirus	Non-arboviruses	Rubella virus (German measles)

## **General Properties**

### **Shape and Size:**

Spherical shaped, 50 – 70 nm in diameter. Enveloped, capsid presents Icosahedral symmetry, glycoprotein peplomers are arranged (*Cloak*) designated E1, E2 & E3. E2 functions as HA which markedly pH dependant.

**Nucleocapsid:** single stranded RNA is protected by a core protein. Genome is first translated into 4 non-structural proteins (nsP1 - nsP2 – nsP3 – nsP4) and structural proteins (E1 – E2 – E3)

### **Susceptible Hosts:**

Majority of Togaviruses (Alphaviruses) are arthropod borne. Wild mammals or birds act as reservoir and domestic animal species particularly horses and human are usually dead – end hosts.

### **Pathogenicity:**

Some members of the family primarily infect horses under natural conditions and infection leads to fetal encephalitis. Clinically high temperature, 1<sup>st</sup> febrile wave persists for 2 -4 days and is often not observed. 2<sup>nd</sup> wave occurs 2-3 days later with anorexia and encephalitic signs – drowsiness to violent hyper excitability.

Surviving animals rarely recover completely – usually exhibit evidence of permanent cerebral damage.

## **1. ALPHAVIRUS**

### **JAPANESE EQUINE ENCEPHALITIS VIRUS**

#### **Associated disease:**

Disease is endemic in Fareast South East Asia and South India. Mortality is 20%. Clinically fever, encephalitis, sweat profusely, hyper-excitabile and hydrophobic, muscular tremors, incoordination and posterior ataxia develops. (Horses/man)

### **WEST NILE EQUINE ENCEPHALITIS VIRUS**

#### **Associated disease:**

Horses/man: endemic in Europe, Africa, Middle East, India and Pakistan. Clinically, fever, encephalitis, total or partial blindness is a common sequel.

## ALPHAVIRUSES causing ENCEPHALITIS

EEE, WEE, VEE viruses cause destructive disease of horses, but may also cause "serious illness in man. The viruses have spread widely among birds, rodents and animals; normally produce harmless and symptomless infection (reservoir hosts). Virus is secreted in nasal discharge, urine and milk. Direct contact infection can also occur. Mortality in horses is 90%.

**Vectors:** The primary vectors are species of *Culicine* mosquitoes.

**Cultivation:** Lab Animals: Unweaned mice, Hamsters.

Embryonated Eggs: CAM/Yolk sac – cause hemorrhages and death of embryos.

Cell culture: Primary cells, HELA cells – inclusions are not formed.

**Haemagglutination:**

Day old chick, Adult male goose – HA is irreversible (neuraminidase deficient).

**Immunity:** Recovered animals develop solid and permanent immunity.

Inactivated/attenuated vaccines.

## ALPHAVIRUS causing FEVER with RASH / ARTHRALGIA

- ▶ **Chikungunya virus:** Africa and Asia
- ▶ **Ross River virus:** Australia, New guinea, Solomon Islands and western pacific,
- ▶ **Getah virus:** Malaysia, South/East Asia, Japan, Philippines and Australia.

## 2. RUBIVIRUS

### RUBULA VIRUS

- ▶ Virus is associated with "German Measles" - mild febrile illness with rash in children and sometimes in adults.
- ▶ During early stages of pregnancy causes developmental anomalies (defects).

## 3. Other TOGAVIRUS

### LACTIC DEHYDROGENASE (LDH) VIRUS

- Virus infects lab. and wild mice.
- The virus multiplies in macrophage and can alter immune responses.
- The macrophages are killed with the liberation of lactate dehydrogenase enzyme during multiplication of virus.
- Immune associated (Ia) antigens function as receptors for LDH virus.

## FLAVIVIRIDAE

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### Classification

The family contains 3 genera as follows;

1. Filavirus
2. Pestivirus
3. Hepatitis C virus

Genera	Vector	Virus Name
Flavivirus	Mosquitoes	Japanese encephalitis V. (infects human, horse, swine)
		Murray vallen encephalitis virus
		St. Louise encephalitis virus

Flavivirus	Mosquitoes	Yellow fever virus (infects human)
		<i>Dengue fever virus</i> (infects human) (also known as hemorrhagic fever virus)
		West Nile fever virus (infects sheep, goats, cattle, human and wild rodents)
		Israel turkey meningoencephalitis virus
	Ticks	Longing ill virus (infects human, sheep, , goats, horses)
		Powassan virus
		Tick borne encephalitis virus
Pestivirus (Non-arboviruses)	Bovine viral diarrhea (BVD) virus	
	Swine fever (Hog cholera) virus	
	Border disease virus of lambs	
Hepatitis C virus: (Non-arbovirus) +ve sense ssRNA. The virus has multiple genotypes which make vaccine development difficult. So, polyvalent vaccines may be protective. Virus is difficult to grow in cell culture. It can be detected by specific techniques such as PCR.		

## **Morphological Features**

- Spherical shaped enveloped particles with single stranded, positive sense, linear RNA.
- Capsid has icosahedral symmetry. The lipid bilayer envelope of viruses possesses glycoprotein spikes.

## **1. Flavivirus**

### **General Properties**

- ▶ Virions are slightly smaller than alphavirus, Virus contains a positive strand RNA genome and an envelope.
- ▶ Most of the flaviviruses are serologically related and antibodies to one virus may neutralize another virus.
- ▶ The RNA does not have a poly-adenylated sequence and the virus lacks a visible capsid structure in the virion.
- ▶ The attachment and penetration by the flaviviruses occur in the same way as described for alphavirus. But the flaviviruses can also attach to the Fc receptors on mΦ, monocytes and other cells when the virus is coated with antibodies.
- ▶ Thus the antibody actually enhances the infectivity of these viruses by providing new receptors for the virus and by promoting viral uptake into these target cells.
- ▶ The major differences between *alphaviruses* and *flaviviruses* are in their organization of genome and mechanism of protein synthesis.
- ▶ The alphaviruses bud from the plasma membrane to release itself whereas flaviviruses acquire envelope by budding into intracellular vesicles rather than at the cell surface. The flaviviruses release by exocytosis or cell lysis mechanism. This route is less efficient and the flavivirus may remain cell-associated.

### **Epidemiology** (of *Arboviruses* → *Togaviruses* + *Flaviviruses*)

Enveloped viruses must stay wet, can be inactivated by drying, soap and detergents. Virus can infect mammals, birds, reptiles and insects. Asymptomatic or non-specific encephalitis, haemorrhagic fever, arthritis may be seen.

### **Transmission**

- ▶ Specific arthropods for each virus
- ▶ Peoples who enter ecological zone of arthropods are at risk.

- ▶ Humans are usually dead – end host as they can not spread virus back to vectors.
- ▶ Birds and small mammals are reservoir hosts.
- ▶ Arboviral disease occurs during summer and rainy months.

### Pathogenesis

- Arboviruses can cause lytic or persistent infections of both vertebrate and invertebrate hosts.
  - Infection of invertebrates is usually persistent with continued virus production.
- Female mosquitoes acquire alpha- and flaviviruses by taking a blood meal from a viraemic vertebrate host.
- This virus then infects the epithelial cells of the midgut of mosquitoes, spread through the basal lamina of the mid gut to the salivary glands via circulation. The salivary glands then release virus into saliva.
- On biting a host, the female mosquitoes regurgitates virus-containing saliva into the victims' blood stream → blood plasma → target cells such as endothelial cells of the capillaries, monocytes and mΦ.
- These viruses may cause mild systemic disease, encephalitis, arthrogenic disease and hemorrhagic disease
- After replication in monocytes - mΦ system, a secondary viraemia can produce sufficient virus to infect target organs such as the brain, liver, skin and vasculature – resulting in severe life threatening conditions, such as encephalitis, yellow fever (hepatitis), hemorrhagic fever (Dengue hemorrhagic fever) and dengue shock syndrome.

Primary target cells of the flavivirus are monocyte and macrophages. These cells express Fc receptors for antibody and release cytokines upon challenge.

Flavivirus infection is enhanced 200 – 1000 fold by non-neutralizing antiviral antibody that promotes binding to the virus to the Fc receptor and its uptake into the cells.

### Immunity

Immunity may enhance uptake of flaviviruses into mΦ and other cells that express Fc receptors.

If non-neutralizing antibody is expressed, virus uptake is enhanced. Inflammation resulting from the CMI (cell mediated immunity) can destroy tissues. Hypersensitive reactions as DTH, immunocomplexes, activation of complement system. These may weaken vasculature → rupture → hemorrhage.

## 2. PESTIVIRUS

### **BOVINE VIRAL DIARRHEA (BVD) VIRUS**

#### Associated disease:

Virus causes infection of cattle, sheep, goats, mule, and dears. BVD is a mild disease with high morbidity but low mortality. Viral infection during pregnancy can result into abortion, teratogenic effects and the birth of persistently infected animals.

Mucosal disease (MD) is always fetal but less contagious; frequently show severe clinical signs and lesions.

Cultivation: *Embryonated Eggs:* certain serotypes grow via CAM/Yolk sac – pocks formation

*Cell Culture:* Embryonic bovine cell culture *CPE:* will be pyknotic nuclei, cytoplasmic vaculation, shrinkage, cell death in 2 – 5 days.

#### Haemagglutination:

Absent but reports suggest certain serotypes agglutinate monkey, pigs, sheep and chick RBCs.

Immunity: Recovered animals from natural infection usually confer solid immunity.

## SWINE FEVER VIRUS

### Associated disease:

Virus causes highly contagious disease of swine – *Hog Cholera*; characterized by sudden onset of high fever, hemorrhages of the internal organs, conjunctivitis, and incoordination of gait, vomiting, constipation later diarrhea, hemorrhages on the abdomen, skin ears and snouts, bronchitis, encephalomyelitis.  
Mortality is extremely high.

Cultivation: *Cell Culture:* Porcine primary & establish cells. No CPE,  
Enhanced CPE with NDV (End Test)  
*Embryonated Egg:* only adapted strains.

### Haemagglutination:

None

Immunity: Recovered animals experience permanent immunity. SFV has antigenic component in common with mucosal disease virus of cattle (can be investigated as immunizing agent).

## BORDER DISEASE VIRUS OF LAMBS

### Associated disease:

Virus causes “*Hairy Shaker disease*” – a contagious condition characterized by excessive hairiness of the birth – coat, poor growth and nervous abnormalities.  
This condition is described in UK, Australia and USA.

Experimental I/P or S/C inoculation of ewes (at 85<sup>th</sup> day of gestation) reproduce the disease in lambs. Antibodies reactive with the virus of Border disease and bovine viral diarrhea have been reported in the mothers of 2 microcephalic babies suggesting that a virus in this group may infect man.

## BUNYAVIRIDAE

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### Classification

There are five genera in the Bunyaviridae which are grouped based on the following aspects;

- i) Number and sizes of virion proteins
- ii) Lengths of L, M, and S strands of the genome
- iii) Their transcription pattern

Genera	Vector	Members
Bunyavirus	Mosquitoes	Bunyamera virus Akaban fever virus La Crosse virus (causes Encephalitis in man)
Nairovirus	Ticks	<i>Crimean – Congo virus</i> <i>Nairobi sheep disease virus</i> Dera Ghazi Khan Virus (Sheep, goat, cattle → causes Haemo-fever)
Phlebovirus	Mosquitoes, Sand flies, Gnats, Ticks	Phlebotomous virus Rift valley fever virus (Sheep, goat, cattle → causes Haemo-fever)

Unkunvirus	Ticks	Unkuniemi Virus
Hentavirus	Non-arboviruses, transmitted by direct contact and inhalation of rodent feces.	Hantaan virus

## General Properties

**Shape and Size:** Virions are spherical, 90 – 120 nm in diameter. Surface projections (8-10 nm) protrude through the lipid bilayer. *Envelope* proteins (G1 – G2) functions as attachment and the HA provides the basis for serological groupings within genera.

**Nucleocapsid:** The family possesses 3 molecules of single stranded RNA (-ve sense) which are contained in 3 circular nucleocapsid strands and are surrounded by the viral envelope.

- ☑ The *small (S) RNA* codes for N protein and a non-structural protein.
- ☑ The *medium (M) RNA* codes for envelope glycoprotein (G1 – G2) and a second non-structural protein (NSm). G1 is viral attachment protein.
- ☑ The *large (L) RNA* presumed to code for L protein which may be the virion – associated transcriptase (RNA dependant RNA polymerase)

There are 3 major virion proteins; 2 envelope (G1 – G2), and one Nucleocapsid protein (N) and a minor large protein (L) and at least 2 non-structural proteins (NS). There is no matrix protein in Bunyaviruses.

Unlike other negative sense RNA viruses, Bunyaviruses also have segmented genome due to which genetic reassortment can occur when two different viruses infect the same cell.

## 1. BUNYAVIRUS

### AKABAN FEVER VIRUS

(Virus is named after a village in Japan)

#### Associated disease:

Akaban fever is associated with congenital defects in calves, lambs and kids (in Japan and Australia) cases of congenitally acquired encephalomyelitis, hydrocephaly, abortions, and still births. Infected pregnant animals do not show clinical signs of disease. Transmission is by Mosquitoes (*Aedes* and *Culex* spp.)

**Isolation:** Virus can be isolated from the tissue including Placentome.

**Cultivation:** *Cell Culture:* VERO cell lines, BHK – 21 cells.

CPE is plaque formation within 4 days.

*Lab Animals:* 1 -2 days old mice, l/cerebrally show nervous signs within 10 days.

#### Haemagglutination:

RBCs of Goose and day old chick

**Immunity:** Animals are immune to re-infection.

There is no vaccine. Control measures are directed against mosquitoes.

## 2. NAIROVIRUS

### CRIMEAN – CONGO VIRUS

#### Associated disease:

Causes acute haemorrhagic tick borne fever, affecting man (Crimean region, USSR, Congo Africa, Hazara – Pakistan) comprises the Crimean – Congo haemorrhagic fever serogroups and are capable of causing severe and fetal disease in man. Crimean – Congo haemorrhagic fever occurs widely in Europe, African and Asia.

Infection has also occurred in hospitals in Dubai, Pakistan, South Africa.

### NAIROBI SHEEP DISEASE VIRUS

**Associated disease:** Nairobi sheep disease and Ganjum and Dugbe viruses make up a separate serogroup. These tick born viruses are primarily responsible for infections in animals but all three are capable of infecting man.

Cause an acute infectious disease of sheep and goats, characterized by high fever followed by severe hemorrhagic gastroenteritis. Other signs include painful respiration, nephritis, and pregnant mortality.

Ewes may abort and Mortality is 90 %.

**Cultivation:** *Cell culture:* Primary cells of sheep and goats. BHK – 21

*CPE:* basophilic Intracytoplasmic inclusions which surrounds the nucleus.

*Lab animals:* Unweaned mice, I/C or I/P → encephalitis and death may occur.

**Immunity:** Recovered animals possess a solid long lasting immunity.

### **DERA GHAZI KHAN VIRUS**

The virus form a serogroup-principal agent isolated from ticks infesting birds and small animals

### **3. PHLEBOVIRUS** (Phlebo – *Phlebotomous* flies that carry this virus)

#### **RIFT VALLEY FEVER VIRUS**

**Associated disease:**

RVF virus was regarded as a disease of domestic animals in South Africa (Sahara) but human infections were rare and were usually confine to herdmen.

In 1977, epizootic occurred in Egypt and there were several thousands cases of human disease resulting in about 600 deaths. Infected camel or transported sheep introduced virus into Egypt from Sudan. The principal vector was *Culex* spp. of Mosquitoes.

RFV disease of sheep and goats and cattle causing abortions and heavy mortality in pregnant and new born animals. The disease is characterized by fever, anorexia, vomiting, mucopurulent discharge from nose, hemorrhagic diarrhea. In man, signs are sudden fever, pain in the extremities and joints, tenderness of the abdominal and gastrointestinal distress.

**Cultivation:** Embryonated egg: CAM with out pocks.

*Lab animals:* Mice, I/P and die with hepatitis within 3 days.

*Cell Culture:* Primary cell culture from chicken, hamsters and lambs. BHK – 21

CPE : multiple acidophilic Intranuclear inclusions (known as “*Daubney – Hudson Garnham* bodies).

**Haemagglutination:**

RBCs of day old chick at 25 C and pH = 6.5

RBCs of mouse, guinea pigs, Human group A RBCs

**Immunity:** In recovered animals, antibodies persist for many years.

### **4. UUKUVIRUS**

#### **UUKUNIEMI VIRUS**

Uukuniemi virus - a tick born virus isolated in Finland.

### **5. HANTAVIRUS**

#### **HANTA VIRUS**

(Hantaan River in N/S Korea)

Korean haemorrhagic fever or Haemorrhagic fever with renal syndrome.

### **UNCLASSIFIED BUNYAVIRUSES**

***Bangui Virus:*** Febrile illness with a rash.

***Bhanja Virus:*** Isolated from cattle, goat ticks in India. Causes neurological disease in man

***Wanowrie Virus:*** Isolated from sheep ticks – 2 days fever with abdominal pain and vomiting.