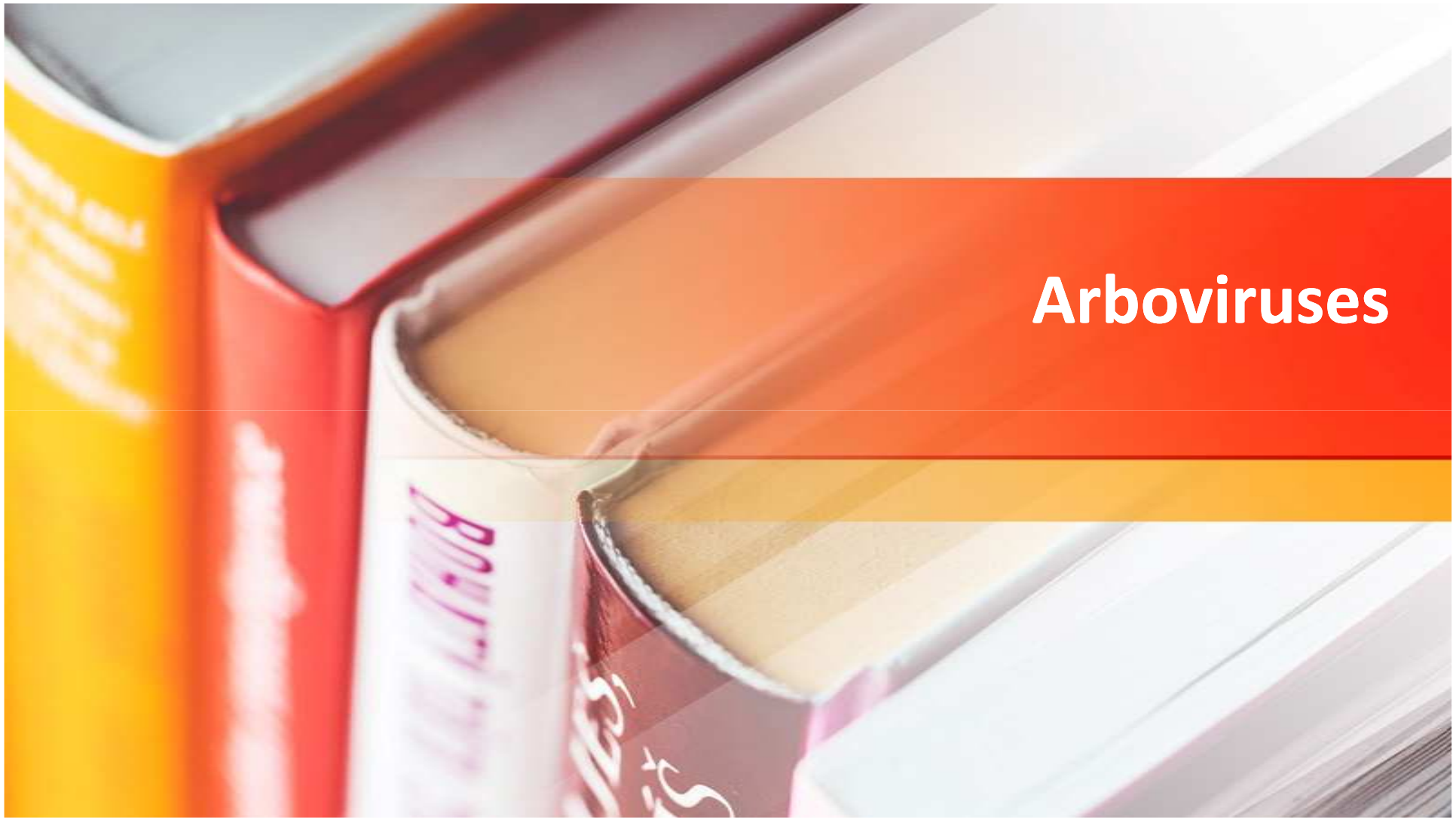


Arboviruses

Learning objectives

ARBOVIRUSES

- **Definition-**Arboviruses (arthropod-borne viruses) are diverse group of RNA viruses that are transmitted by bloodsucking arthropods (insect vectors) from one vertebrate host to another.
- Viruses must multiply inside the insects and establish a lifelong harmless infection in them.
- Viruses that are just mechanically transmitted by insects are not included in this group.

Family -Togaviridae

Virus	Manifestation	Distribution	Vector	Reservoir
Chikungunya virus	Fever & arthritis	Asia, Africa	Aedesaegypti	Monkeys -
O'nyong-nyong virus	Fever & arthritis	Africa	Anopheles	-
Mayaro virus	Fever & arthritis	South America	Aedesaegypti	Monkeys
Ross River virus	Epidemic polyarthritis	Australia	Aedes	Small animals
Sindbis virus	Arthralgia, and rash	Africa, Europe, Australia	Culex	Birds, mammals
Semliki Forest virus	Fever & arthralgia	Africa	Aedes	Birds, rodents
Eastern equine encephalitis virus	Encephalitis	Eastern part of North America	Aedes, Culex	Birds
Western equine encephalitis virus	Encephalitis	Western part of North America	Culex tarsalis, Aedes	Birds
Venezuelan equine encephalitis virus	Encephalitis	South & central America	Aedes, Culex	Horses

Family –Flaviviridae

Virus	Manifestation	Distribution	Vector	Reservoir
Japanese B encephalitis virus	Encephalitis	South East Asia	Culex tritaeniorhynchus	Pigs, Birds
St. Louis encephalitis virus	Encephalitis	United States	Culex	Wild birds
West Nile encephalitis virus	Encephalitis	East Africa (Uganda), Algeria, Romania	Culex, Aedes, Anopheles	Birds
Murray Valley encephalitis virus	Encephalitis	America	Culex annulirostris	Birds
Rocio virus	Encephalitis	São Paulo, Brazil	Culex	-
Russian spring-summer encephalitis virus	Encephalitis	Central Europe, Russia	Tick	Rodents, other mammals, birds

General features of arbovirus

Family – Flaviviridae

Virus	Manifestation	Distribution	Vector	Reservoir
Powassan virus	Encephalitis	America	Tick	Rodents
Louping-ill	Encephalitis	Europe	Tick	Sheep
Dengue virus	Hemorrhagic fever	India	Aedes aegypti	?
Yellow fever virus	Hemorrhagic fever	West Africa, Central South America	Aedes aegypti	Monkeys
Kyasanur Forest Disease virus	Hemorrhagic fever	India (Karnataka)	Tick	Monkeys and rats
Omsk Hemorrhagic fever virus	Hemorrhagic fever	Russia	Tick	Small mammals
Zika virus	Fever and arthritis	First occurred in Brazil, then spread to other countries	Aedes aegypti	Monkeys

Family –Bunyaviridae

Virus	Manifestation	Distribution	Vector	Reservoir
California encephalitis virus	Encephalitis	USA	Aedes triseriatus	Rodents
Oropouche virus	Rash and aseptic meningitis	Central and South America	Culicoidesparaensis	Not known
Sandfly fever	fever and myalgia	Southern Europe, North Africa, India	Sandfly	Small mammals
Rift Valley fever virus	fever and myalgia	Africa	Aedes	Sheep, cattle
Crimean Congo hemorrhagic fever virus	Hemorrhagic fever	Africa	Tick	Small mammals
Ganjam virus	fever	India	Tick	Small mammals
Severe fever with thrombocytopenia syndrome virus	Fever, thrombocytopenia	China, Korea	Tick	Sheep, goat, chicken

Family –Reoviridae

Virus	Manifestation	Distribution	Vector	Reservoir
Colorado tick fever virus	Fever, rarely encephalitis	America (mountains)	Tick	Rodents
Orungo virus	Fever	Sub-Saharan Africa	Aedes	-
Kemerovo virus	Fever, meningism	Russia	Tick	-

Family –Rhabdoviridae

Virus	Manifestation	Distribution	Vector	Reservoir
Vesicular stomatitis virus	Oral mucosal vesicles	Indiana	Sandfly	-
Chandipura virus	Encephalitis	India	Sandfly	-

Arboviruses found in India

- Dengue, chikungunya, and Japanese B encephalitis viruses are highly endemic in India

TOGAVIRIDAE- Classification

- Genus Alphavirus-Contains about 30 different mosquito borne viruses out of which about 13 are human pathogens.
- Genus Rubivirus -Contains rubella virus, which is not arthropod transmitted and is not an arbovirus.

Morphology

- Spherical, 50-70 nm in diameter,
- Nucleocapsid
- Capsid contains 42 capsomeres
- Genome: positive-sense, ssRNA
- Enveloped virus- Capsid is surrounded by a lipid envelope that contains two glycoproteins having hemagglutinating activity
- Replication: They replicate in the cytoplasm and release by budding through host cell membranes.
- All togaviruses are *serologically related* to each other.

Togaviridae

- Based on clinical manifestations, the pathogenic members of the genus *Alpha virus* can be categorized in to fever-arthritis group and encephalitic groups.

ALPHAVIRUS (FEVER-ARTHRITIS GROUP)- Chikungunya

- Chikungunya fever is a re-emerging disease characterized by acute fever with severe arthralgia
- ***History-***The name is derived from the *Makonde* word “kungunyala” meaning "that which bends up or gets folded" in reference to the stooped posture which develops as a result of the severe joint pain that occurs during the course of illness.
- ***Human Transmission***- i) *Aedes* mosquito, primarily *Aedesaegypti* which bites during day time, rarely by ii) vertical transmission from mother to fetus , iii) blood transfusion
- ***Transmission cycle-***
- Urban transmission cycle- Human beings serve as reservoir during epidemic periods and the transmission occurs between humans and *Aedesaegypti.*
- Sylvan transmission cycleoccurs usually in African forests involving the wild primates as reservoir (monkeys) and forest species of *Aedes* (e.g. *A.furcifer, A.taylori*) as vectors.

ALPHAVIRUS (FEVER-ARTHRITIS GROUP)- Chikungunya

- ***Clinical Manifestations-***
- Incubation period is about 5days (3–7 days)
- Most common symptoms are fever and severe joint pain (due to arthritis)
- **Arthritis** is polyarticular, migratory and edematous (joint swelling), predominantly affecting the small joints of wrists and ankles.
- Other symptoms include headache, muscle pain, tenosynovitis or skin rashes.
- Symptoms are often confusing with that of dengue. In general, chikungunya is less severe, less acute and hemorrhages are rare compared to dengue (Table 2).
- Most patients recover within a week, except for the joint pain (lasts for months).
- ***High riskgroup***- includes newborns, older adults (≥65 years), and persons with underlying hypertension, diabetes, or heart disease.
-

Manifestations of chikungunya and dengue

Features	Chikungunya	Dengue
Fever	Common	Common
Polyarthritis	Common	None
Tenosynovitis	Common	None
Rashes	Day1-4	Day 3-7
Myalgia	Possible	Common
Leukopenia	None	Common
Thrombocytopenia	None	Common
Retro-orbital pain	Rare	Common
Hypotension	Possible	Common
Minor bleeding	Rare	Common

- ***Epidemiology-***Chikungunya virus was first reported in Africa(Tanzania, 1952), was subsequently introduced into Asia and had caused several outbreaks in various African and Southeast Asian countries (Bangkok and India)
- **India**- Several outbreaks were reported during 1963 -1973;e.g.Kolkata in 1963 and South India in 1964 (Pondicherry -Chennai-Vellore) and Barsi in Maharashtra in 1973
- Since then, it was **clinically quiescent**and no outbreaks were reportedbetween 1973 -2005 from most parts of the world, except for the few sporadic cases, whichoccurred in various places in the world including India (Maharashtra).
- **Re-emergence** (Reunion Outbreak)- In 2005, Chikungunya re-emerged in Reunion Island of Indian Ocean and affected 2,58,000 people (almost one third of country's population)
- **Spread-** Following this, it has been associated with several outbreaks in India and other Southeast Asian and African countries and has also spread to some areas of America and Europe.
- **India (at present)-** Chikungunya is endemic in several states.
- States: Karnataka, Tamil Nadu, Andhra Pradesh and West Bengal have reported higher number of cases.
- In 2014, nearly 14,452 cases were reported, much less than the previous years (95,091 cases in 2008).
- Karnataka accounted for the maximum number of cases in the year 2013 &2014.
- ***Genotypes-*** Chikungunya virus has three genotypes- West African, East African and Asian genotypes.
- Most Indian cases before 1973 were due to Asian genotypes.
- However, Reunion outbreakwas caused due to a mutated strain and is responsible for most of the current outbreaks in India as well as in other parts of the world.
- The genotypes distribution is due to differences in their
- transmission cycles; for example, human cycle in Asia
- and forest cycle in Africa.
- ***Reasonsfor re-emergence-***
- **New mutation** (E1-A226V)-Chikungunya virus underwent an important mutation. Alanine in the 226 position of E1 glycoprotein gene is replaced by valine.
- **New vector** (*Aedesalbopictus*)-This mutation led to a shift of vector preference. Mutated virus was found to be 100 times more infective to *A.albopictus* than to *A.aegypti.*

Table: Case definitions for Surveillance of Chikungunya
Clinical criteria- Acute onset of fever and severe arthralgia / arthritis with or without skin rash and residing or having left an epidemic area 15 days prior to onset of symptoms
Laboratory criteria- At least one of the following : 1. Direct evidence: (i) virus isolation or (ii) RNA by real time PCR. 2. Indirect evidence: (i) IgM Ab in serum in acute or convalescent phase, or (ii) Fourfold rise of IgG Ab titres in serum collected ≥ 3 weeks apart.
Case definitions • Probable case: if clinical criteria are met. • Confirmed case: if both clinical and laboratory criteria are met.
Outbreak criteria: One or more cases in an area where no case was reported before.

- ***Laboratory diagnosis*** of chikungunya is similar to that of other arboviruses as described before.
- **Viral isolation** (in mosquito cell lines)and real time reverse transcriptase **PCR**are best for early diagnosis (0- 7 days).
- **Serum antibody detection**-
- IgM appears after 4 days of infection and lasts for 3 months;IgG appears late (after 2weeks) and lasts for years.
- So, detection of IgM or a fourfold rise inIgG titer is more significant.
- **MAC (IgM Antibody Capture) ELISA (using virus** lysate) is the best format available showing excellent sensitivity (95%) and specificity (98%) with only little cross reactivity with other alphaviruses and dengue.
- In India, MAC ELISA kits are supplied by National Institute of Virology (NIV), Pune
- „ Several other rapid tests (e.g. ICT using envelope antigens) are also available.
- **Molecular method: Reverse-transcriptase PCR has been** developed to detect specific gene (e.g. nsP1, nsP4) in blood

- **Biological markers** likeIL-1β, IL-6 and are increased and RANTES(Regulated on Activation, Normal T Cell Expressed and Secreted) levels are decreasedin chikungunya infection.
- **Hematological finding: Such as leukopoenia with**
- lymphocyte predominance, thrombocytopenia (rare), elevated ESR and C-reactive protein.
- ***Treatment*** isby supportive measures, no specific antiviral drugs are available.
- ***Vaccine***- Recently, few vaccine trials are ongoing.In one of these trial, a live measles vaccine virus (Schwarz strain) is used as a vector; into the genome of which five structural genes from chikungunya virus are incorporated.

ALPHAVIRUS	Features
Sindbis virus	• Chikungunya like illness characterized by arthralgia, and rash. • Transmitted by Culex mosquito. • Sindbis fever is called by different names in northern Europe(Pogosta disease in Finland, Karelian fever in Soviet Union, and Ockelbo disease in Sweden) • In India, though the virus has been isolated, it is not associated with human disease.
Semliki Forest virus	• First isolated from Aedes mosquitoes in the Semliki Forest, Uganda (1942), and since then it is confined to Africa.

ALPHAVIRUS	Features
O'nyong-nyong (ONN) virus	ONN virus is closely related to Chikungunya virus both clinically (rashes and arthritis) and antigenically. However, it is transmitted by the *Anopheles* species and infection is confined to Africa.
Mayaro virus	It also causes a chikungunya like illness, endemic in tropical South America. *Aedes aegypti* may be involved in human transmission in an urban setting.
Ross River virus	• **Eidemic polyarthritis** in Australia and New Guinea. • Transmitted by several *Aedes* species such as *Aedesvigilax*, *A.polynesiensis* and *A.aegypti*. • Focal cases were reported in India (Pune,2010)

ALPHAVIRUS (ENCEPHALITIS GROUP)

ALPHAVIRUS	Features
Eastern equine encephalitis	• Causes a rare but severe form of encephalitis (around 5cases/year). • Bird - mosquito cycle is maintained by *Culiseta melanura* but other mosquitoes such as *Aedes vexans* are involved in transmission to mammals.
Western equine encephalitis	• **O**ccurs more frequently (around 20 cases/year), particularly involving infants. • Transmitted by *Culex tarsalis*.
	VEE(Venezuelan equine encephalitis) is confined to South and Central America. It starts with aninfluenza -like illness but can cause serious encephalitis in people with low immunity.

ALPHAVIRUS (ENCEPHALITIS GROUP)

ALPHAVIRUS	Features
Venezuelan equine encephalitis	• Confined to South and Central America. • Start with influenza -like illness but can cause serious encephalitis in people with low immunity. • A larger outbreak of VEE had occurred in Venezuela and Colombia in 1995. • Vector *Culex taenopius*, which has preference for rodents, was replaced by *Aedes taeniorhynchus* due to deforestation. The later is more likely to bite humans and large equines. • VEE has been used as biological weapon.

ALPHAVIRUS (ENCEPHALITIS GROUP)

- **Vaccine-**Inactivated vaccines have been developed for EEE and WEE whereas for VEE, there are both live attenuated(known as TC-83)and inactivated vaccine(known as C-84) available.

- **FLAVIVIRUSES**
- **MOSQUITO TRANSMITTEDFLAVIVIRUSES**
- **ENCEPHALITIS VIRUSES**
- **Japanese B Encephalitis (JE)**
- Japanese B encephalitis is the leading cause of viral encephalitis in Asia, including India.
- ***It was so named*** as the disease was first seen in Japan (1871) as "Summer encephalitis epidemics" (however, it is now uncommon in Japan)andto distinguish it from encephalitis A (encephalitis lethargica /von Economo disease) which was endemic during that time.
- ***Vector*** :*Culex* mosquito
- *C. tritaeniorhynchus*is the major vector worldwide including India.
- *C. vishnui* is the next common vector found in India.
- ***Transmission cycle***- JE virus infects several extra human hosts, e.g. animals and birds. Two transmission cycles are predominant.
- Pigs→ *Culex*→ Pigs
- Ardeid birds→ *Culex*→ Ardeid birds

- ***Animal hosts-***
- Pigs have been incriminated as the major vertebrate host for JE. JE virus multiplies exponentially in pigs without causing any manifestation. Pigs are considered as the **amplifier host** for JE.
- Cattle and buffaloes may also be infected with JE virus; although they arenotthe natural host. They may act as ***mosquito attractants.***
- Horses are probably the only animal to be symptomatic and show encephalitis
- Humans are considered as dead end; there is no man→ mosquito →man cycle (unlike in dengue)
- ***Bird hosts***-Ardeid (wading) birds such as herons, cattle egrets, and ducks can also be involved in the natural cycle of JE virus.
- ***Geographical distribution***- Currently, JE is endemic in Southeast Asian region.
- It is increasingly reported from India, Nepal, Pakistan, Thailand, Vietnam and Malaysia.
- Because of immunization, its incidence has been declining from Japan and Korea.
- It is estimated that nearly 50,000 cases occur every year globally with 10,000 deaths.

- **In India: JE has been reported since 1955**
- JE is endemic in 15 states; Uttar Pradesh (Gorakhpur district) accounted for the largest burden
- Between 2010–17; total 10,710 number cases (average 1,340 cases/year) and 1,782 deaths (average 222 deaths/year) have been reported
- In 2017, nearly 2,040 cases of JE were reported from India with 230 deaths. Maximum cases reported from UP followed by Assam, Manipur, West Bengal, Tamil Nadu, Tripura, Bihar, and Odisha.

- ***Clinical Manifestations-***JE is the most common cause of epidemic encephalitis.
- **Incubation period** is not exactly known, probably varies from 5-15days
- **Subclinical infection is common**- JE typically shows *iceberg phenomena*.Cases are much less compared to subclinical/in-apparent infection with a ratio of 1:300-1000.
- Even during an epidemic the number of cases are just 1-2 per village
- **Clinical course** of the disease can be divided into three stages
- *Prodromal stage* is a febrile illness; the onset of which may be either abrupt (1-6 hours), acute (6-24 hours) or more commonly subacute (2-5 days)
- *Acute encephalitis stage*- Symptoms include convulsions, behavioral changes,meningeal signs or paralysis.
- *Late stage and sequelae*- It is the convalescent stage in which the patient may be recovered fully or retain some neurological deficits permanently. Case fatality ratio is about 20-40%.

Laboratory Diagnosis

- IgM Capture Antibody (MAC) ELISA supplied by NIV, Pune has been the recommended method for diagnosis of JE.
- It is a two-step sandwich ELISA, uses JERA (JE recombinant antigen) to detect JE-specific IgM antibody in serum
- Reverse-transcriptase PCR has also been developed to detect JE virus specific envelope (E) gene in blood.

Vaccine Prophylaxis

- **1)Live attenuated SA 14-14-2 vaccine-**
- It is prepared from SA 14-14-2 strain
- It is cell line derived; primary hamster kidney cells are commonly used.
- Single dose is given subcutaneously, followed by booster dose after 1 year.
- It is manufactured in China, but now licensed in India.
- Under Universal Immunization Programme, it is given to children (1-15years) targeting 83 endemic districts of four states –UP, Karnataka, West Bengal and Assam.
- Schedule: Two doses; 1st at 9 completed month- 12 months of age and 2nd at 16 ▪ 4 months
- Administered: 05 mL/dose, subcutaneously at left upper arm.

- **2)Inactivated vaccine** (Nakayama strain and Beijing strain)-
- It is a mouse brain derived formalin inactivated vaccine.
- It is prepared in Central Research Institute, Kasauli (India).

- **Inactivated vaccine (Beijing P3 strain): It is a cell line** derived vaccine
- Combined vaccine: A genetically engineered JE vaccine that combines the attenuated SA14-14-2 strain and yellow fever vaccine strain 17D (YF 17D) virus as a vector for genes encoding the protective antigenic determinants, has been tested in several clinical trials.

Other Mosquito-borne Encephalitis Flaviviruses

Other Flaviviruses	Features
West Nile virus	• First described in West Nile region of Uganda (Africa). • Transmitted among wild birds by Culex mosquitoes in Africa, Middle East, Europe, Asia and recently in America. • Febrile illness (fever,myalgia with rashes on the trunk) • Occasionally it can also cause severe encephalitis.
Murray Valley encephalitis viruses	• Endemic in northern Australia and Papua New Guinea. • Major mosquito vector is Culex annulirostris

Other Mosquito-borne Encephalitis Flaviviruses

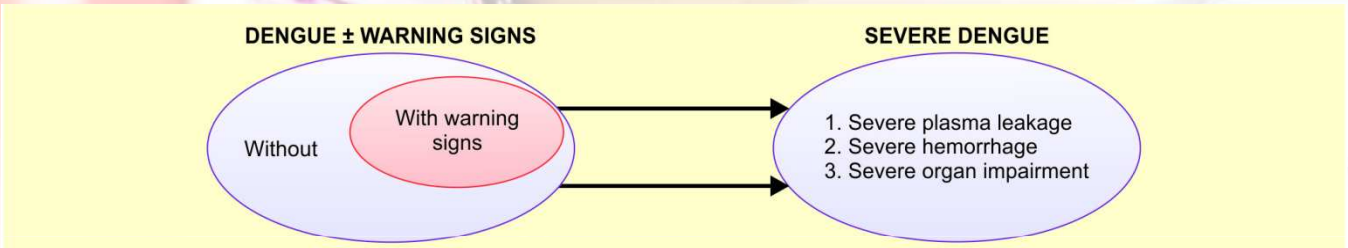
Other Flaviviruses	Features
St. Louis encephalitis viruses	• Transmitted by mosquito (Mansonia pseudotitillans). • Related to Japanese B encephalitis virus. • It has caused several outbreaks of encephalitis, mainly affecting the United States.
Rocio encephalitis viruses	It was first observed in São Paulo State, Brazil, in 1975. It had caused several epidemics of meningoencephalitis in coastal communities in southern São Paulo, Brazil, during 1975. Transmission is believed to be by Culex.

- **HEMORRHAGIC GROUP OF FLAVIVIRUSES**
- **Dengue viruses**
- Dengue virus is the most common arbovirus found in India.
- ***Serotypes-***Dengue has four serotypes (DEN-1, to DEN-4). Recently, the fifth serotype (DEN-5) was discovered in 2013 from Bangkok.
- ***Vector*** *-Aedesaegypti*is the principal vectorfollowed by*Aedesalbopictus.*They bite during the day time.
- *A.aegypti* is a nervous feeder (so, it bites repeatedly to more than one person to complete a blood meal) and resides in domestic places, hence is the most efficient vector.
- *Aedesalbopictus*is found in peripheral urban areas, it is an aggressive and concordant feeder i.e. can complete its blood meal in one go; hence is less efficient in transmission.
- *Aedes* becomes infective only by feeding on viremic patients (generally from a day before to the end of the febrile period)
- Extrinsic incubation period of 8-10days is needed before *Aedes* becomes infective
- Once infected, it remains infective for life
- *Aedes*can pass the dengue virus to itsoffspringsby transovarial transmission
- Transmission cycle- Man and *Aedes* are the principal reservoirs. Transmission cycle does not involve other animals.
- ***Pathogenesis-****Primary dengue infection* occurs when a person is infected with dengue virus for the first time with any one serotype. Months to years later, a more severe form of dengue illness may appear (called *secondary dengue infection*)due to infection with another secondserotype which is different from the first serotype causing primary infection.

Antibody response against dengue virus

- -
- Infection with dengue virus induces the production of both neutralizing and non-neutralizing antibodies.
- The **neutralizing antibodies** are protective in nature. Such antibodies are produced against the infective serotype (which last lifelong) as well as against other serotypes(which last for some time). Hence, protection to infective serotype stays lifelong but cross protection to other serotypes diminishes over few months.
- The **non-neutralizing antibodies**are heterotypic in nature; i.e they are produced against other serotypes but not against the infective serotype.
- Suchantibodies produced following the first serotype infection, can bind to a second serotype; but instead of neutralizing the second serotype , it protectsit from the host immune system by inhibiting the bystander B cell activation against the second serotype.
- **ADE-**The above phenomena is called as antibody dependent enhancement (ADE) which explains the severity of secondary dengue infection.
- Among all the serotypes combinations, ADE is remarkably observed when serotype 1 infection is followed by serotype 2, which also claims to be the most severe form of dengue infection.

Clinical Classifications



CRITERIA FOR DENGUE ± WARNING SIGNS

Probable dengue
Live in/travel to dengue endemic area
Fever and two of the following criteria:
- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Leukopenia
- Any warning sign

Laboratory-confirmed dengue
(important when no sign of plasma leakage)

Warning signs*
- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

*Requiring strict observation and medical intervention

CRITERIA FOR SEVERE DENGUE

1. Severe plasma leakage
leading to:
- Shock (Dengue shock syndrome)
- Fluid accumulation with respiratory distress

2. Severe bleeding
As evaluated by clinician (causing hemodynamic instability and may require blood transfusion)

3. Severe organ involvement
- Liver: AST or ALT ≥1000 IU/mL
- CNS: Impaired consciousness
- Heart and other organs

- ***Factors determining the outcome-***
- *Infecting serotype*- Type 2 is apparently more dangerous than serotypes
- *Sequence of infection*- Serotype 1 followed by serotype 2 seems to be more dangerous and can develop into DHF and DSS
- *Age*- Though all age groups are affected equally, children < 12 years are more prone to develop DHF and DSS

- ***Global Scenario-*** Dengue is endemic in >100 countries with 2.5 billion people at risk.
- Tropical countries of Southeast Asia and Western pacific are at highest risk.
- About 50 million of dengue cases occur every year worldwide, out of which 5lakh cases (mostly children) proceed to DHF.

Situation in India

- Disease is prevalent throughout India in most of the **urban cities/towns affecting almost 31 states/Union territories.**
- Between 2010–17, >6 Lakh cases with >1560 deaths have been reported from India. Maximum cases have been reported (in descending order) from West Bengal, Tamil Nadu, Punjab, Kerala, Delhi, Karnataka, and Maharashtra
- In 2017, nearly 1,57,220 cases were reported; maximum of cases were reported from Tamil Nadu followed by West Bengal
- All four dengue serotypes have been isolated from India.
- Serotype prevalence varies between seasons and places, but DEN-1 and DEN-2 are widespread. DEN-5 has not been reported yet.

- ***Laboratory diagnosis***- The outline of laboratory diagnosis of dengue is similar to that of other arboviruses as described earlier.
- **NS1 antigen detection-**ELISA and immunochromatography based rapid cards are available for detecting NS1 antigen in serum.They gained recent popularity because of the early detection of the infection.
- NS1 antigen becomes detectable from day-1 of fever and remains positive up to 18 days.
- Highly specific- It differentiates between flaviviruses. It can also be specific to different dengue serotypes.
- **Antibody detection (Fig 2)-**
- ***Primary infection***- Antibody response is slow and of low titer.IgM appears first after 5 days of fever and disappears within 90 days.IgG is detectable at low titer in 14-21days ofillness, and slowly increases.
- ***Secondary infection***- IgG antibody titers rise rapidly.IgGis often cross reactive with many flaviviruses and may give false positive result after recent infection or vaccination with yellow fever virus or JE. In contrast, IgM titer is significantly low and may be undetectable.
- ***Past infection-*** Low levels of IgG remain detectable for over 60 years and, in the absence of symptoms, is a useful indicator of past infection.

- **MAC-ELISA (IgM antibody capture ELISA): This is the** recommended serological testing in India. Kits are supplied by NIV, Pune
- Principle: It is a double sandwich ELISA; which captures human IgM antibodies on a microtiter plate using anti-human-IgM antibody followed by the addition of dengue virus four serotypes specific envelope protein antigens (this step makes the test specific) . There is a signal enhancement due to use of avidin-biotin complex (ABC) which makes the test more sensitive
- Cross-reactivity with other flaviviruses is a limitation of this test.

- IgG specific ELISA format is also available separately
- Rapid tests such as dipstick assays are also available

- ***Other antibody detection assays*** used previously are-
- HAI (Hemagglutination inhibition test)
- CFT (Complement fixation test)
- Neutralization tests such as plaque reduction test, neutralization and micro neutralization tests

- **Rapid diagnostic tests (RDT) for dengue**
- Rapid diagnostic tests (e.g. ICT) for dengue IgM antibodies
- or NS1 antigen are available, but have poor sensitivity and
- specificity. Government of India had passed an order in 2016,
- that a positive RDT for dengue NS1 or IgM should be considered
- as probable diagnosis; must be confirmed by ELISA.

- **Virus isolation**
- Dengue virus can be detected in blood from –1 to +5 days
- of onset of symptoms. Virus isolation can be done by
- inoculation into mosquito cell line or in mouse.

Molecular Method

- ™ Detection of specific genes of viral RNA (3'-UTR region) by real time RT-PCR: It is the most sensitive (80–90%) and specific assay (95%), can be used for detection of serotypes and quantification of viral load in blood.
- Viral RNA can be detected in blood from –1 to +5 days of onset of symptoms.
- A negative PCR result is interpreted as "indeterminate"; which has to be sent for serological confirmation after the 5th day of illness
- **Genotype detection: Each serotypes of dengue virus** comprises of several genotypes which can be detected
- by molecular typing.
- A total of 13 genotypes have been detected so far; three for DENV-1, two for DENV-2, four each for DENV-3 and 4 serotypes respectively.
- Dengue virus keep undergoing genetic alterations leading to introduction of new genotypes and also a shift between the existing genotype lineages within a serotype; which may attribute to rapid increase in the clinical severity (DHF and DSS) in many parts of the world. Hence, there is need for close molecular monitoring of dengue virus.

- ***Treatment-*** There is no specific antiviral therapy. Treatment is symptomatic & supportive such as-
- Replacement of plasma losses
- Correction of electrolyte and metabolic disturbances
- Platelet transfusion if needed

Prevention

- **Vaccine:**
- Mosquito control measures.

Dengue vaccine

- After several trails, a dengue vaccine has been licensed for human use since 2015.
- It is a Chimeric Yellow Fever-Dengue, Live-Attenuated, Tetravalent Dengue Vaccine (CYD-TDV); commercially available as dengvaxia (developed by Sanofi Pasteur).
- It uses live attenuated yellow fever (YF) 17D virus as vaccine vector in which the target genes of all four dengue serotypes are integrated by recombinant technique.
- Age: It is indicated for 9-45 year of age.
- Schedule – 3 injections of 0.5 mL administered subcutaneously at 6 month intervals
- It is available as lyophilized form; reconstituted with normal saline.
- Contraindications: (i) allergic reactions to vaccine; (ii) immunodeficient Individuals (e.g. HIV) (iii) Pregnant and breast feeding women
- Efficacy against hospitalized dengue illness was found around 80 %.
- WHO recommends this vaccine to start in high burden countries (seroprevalence >70%).
- Currently, The vaccine is approved in Mexico, Philippines, Brazil, Indonesia, Thailand and Singapore. In India, it is not available yet.

Zika virus

- ssRNA virus, belongs to family Flaviviridae.
- Monkeys are the reservoirs.
- Place of discovery (1947), Zika forest in Uganda.
- ***Transmission:***
  - Mosquito transmitted virus
  - Mosquito borne—*Aedes aegypti*, *Aedes albopictus*
  - Mother-to-child : Common in first trimester
  - Sexual transmission: Transmission has been observed from (i) asymptomatic males to their female partners. Longer shedding of Zika virus in semen has been reported, (ii) Symptomatic females to their male partners.

Epidemiology

- The first outbreak was reported in 2007 in Yap Islands (49 confirmed and 59 probable cases).
- *Aedes hensilli* was the predominant Mosquito..
- **Recent Outbreak (2015-2016):**
  - It began in April 2015 in Brazil and then subsequently spread to other countries in South America, Central America, the Caribbean, Europe USA and Australia.
  - In February 2016, the WHO declared the Zika virus outbreak a public health emergency of international concern.
- **Situation in India:** 3 confirmed cases (Gujarat in 2017); first report from India. As the vector is prevalent, India has a higher risk of getting affected by ZIKV in near future.

Clinical Manifestations

- Incubation period - few days to 1 week.
- Asymptomatic: symptomatic ratio is 5:1.
- Zika fever- Symptomatic people develop minor illness such as fever and a rash, conjunctivitis.
- Congenital transmission leads to development of fetal anomaly such as microcephaly.
- Few cases of Guillain-Barré syndrome have been reported in patients with ZIKV disease.

Lab Diagnosis

- **Reverse transcriptase PCR** (RT-PCR) - investigation of choice.
  - Detect ZIKV RNA in blood and urine up to 5 and 7 days of onset of symptoms respectively.
  - Multiplex real time RT-PCR – available
  - Targets - non-structural 5 (NS5) region of ZIKV, non-structural protein 4 (nsP4) from CHIKV and 3′ untranslated region (3′UTR) of DENV 1–4.
- **IgM antibody detection** (ELISA): It appears in blood after 1 week of symptoms and remain positive up to several months. It cross reacts with dengue antibodies.
- **Plaque-reduction neutralization test** is more specific serological (antibody detection) test; but it is cumbersome, not widely used.

Treatment Zika virus

- No effective treatment
- Vaccine – not available
- Only symptomatic treatment available such as fluid replacement and analgesic such as acetaminophen.

ZIKV Vaccine

- **ZIKV DNA vaccine (VRC 705):**
  - Most advanced trail is the one conducted by Vaccine Research centre (VRC) under NIAID, USA.
  - Genetically engineered plasmid encoding Zika virus protein.
  - Entered phase-2 vaccine trail in 2017 and is expected to get completed by 2019.
- **Killed ZIKV vaccine**: Another trail (phase-I) is been evaluated by Bharat Biotech's Hyderabad, India. It uses an African strain of ZIKV.

General preventive measures

- Mosquito control measures
- Infected patients should prevent mosquito bites for the first week of illness
- **Sex/ pregnancy restriction:** CDC has recommended for considering condom use or abstinence for at least 6 months for males and 8 weeks for females after travel to endemic area or developing symptoms or ZIKV diagnosis.
- **During outbreak time** (2015-16):
  - Affected countries such as Brazil, and others have advised women to postpone getting pregnant until more was known about the risks.
  - Travel of pregnant women from other countries (including India) to ZIKV affected countries have been restricted.

Yellow fever virus

- Acute, febrile illness.
- ***Geographical distribution***- Endemic in West Africa and Central South America. Not found in the rest of the World including India.
- ***Typing***- *Seven genotypes and one serotype.*
- ***Vector-*** *Aedes aegypti* or the tiger mosquito.
- ***Transmission cycle*****:**
  - Jungle cycle - Between monkeys and forest mosquitoes. Humans can only get infection occasionally during their forest visits.
  - Urban cycle - Occurs between humans and urban mosquitoes (*Aedes aegypti*)

Yellow fever virus

- ***India***- Not invaded, but potential of developing because the vector, *A.aegypti*, is widely distributed here, and India has the tropical climatic condition similar to Africa. Various reasons have been hypothesized to explain the absence of yellow fever in India-
- **Measures in airport:**Govt. of India has laid down strict guidelines for vigilance and quarantine of the travelers in the international airports.
- Unprotected (i.e. unvaccinated) travelers coming from endemic zone to India will be kept in quarantine for 6 days
- Breteau index or the *Aedesaegypti* index should be less than one,surrounding 400mt of anairport.
- (Breteau index - No. of containers showing breeding of *Aedesaegypti* larvae / No. of houses surveyed ×1000)

Yellow fever virus

- India has all the potential of developing yellow fever in future because the vector *A.aegypti*, is widely distributed here, and India has the tropical climatic condition similar to Africa. Various reasons have been hypothesized to explain the absence of yellow fever in India:
  - **Measures in airport:** Govt. of India has laid down strict guidelines for vigilance and quarantine of the travelers in the international airports.
  - **Cross reacting dengue antibody** provides protection against yellow fever. However, yellow fever immunization does not protect from dengue
- ***Period of communicability:***
  - Man- Patients are infective to mosquito during the first 3-4days of illness
  - *Aedes*- After an extrinsic incubation period of 8-10day, the mosquito becomes infective andonce infected, remains infective for life.

Clinical manifestations

- Incubation period is about- ***3-6 days***
- **Febrile illness**
- **Severe cases –**
  - Hemorrhagic manifestations
  - Platelet dysfunction,
  - Features of liver involvement (hepatitis) are-
  - Mid-zonal necrosis and presence of councilman bodies
  - Intranuclear inclusions may be seen inside the hepatocytes called as ***Torres*** bodies.
  - Appearance of jaundice
  - Renal dysfunction
  - Encephalitis occurs very rarely.
  - Mortality rate is high (>20%), especially among children & elderly.

Epidemiology

- It is estimated that about 200,000 cases of yellow fever with 30,000 deaths occur annually and majority of outbreaks (~90%) occur in Africa.
- Epidemics usually occur in humid and semi-humid savanna area adjoining a rain forest.
- Infection: case ratio during epidemic ranges from 20:1 to 2:1.
- All age groups are susceptible.

Laboratory diagnosis

- **Serology: IgM ELISA can be done after 3 days of onset of** symptoms.
- **Molecular method: RT-PCR detecting specific viral** RNA (NS5 region) in blood is more confirmatory than serology

Yellow fever 17D vaccine

- Live attenuated vaccine
- Prepared from allantoic cavity of chick embryo
- In India- It is prepared in Central Research Institute(CRI), Kasauli
- Dosage- Single dose, given subcutaneously
- Vaccine is effective within 7 days of administration, which lasts for 35 years.
- Validity of yellow fever vaccine certificate: Certificate is issued after 10 days of vaccination and renewed (i.e. reimmunization) every 10 years

Yellow fever 17D vaccine CONT.

- Contraindication of yellow fever vaccine include-
  - Children <9m, (<6m- during epidemic),
  - Pregnancy(except during outbreak),
  - HIV infected people
  - People with allergy to egg

ENCEPHALITIS GROUP

- Tick-borne Encephalitis (TBE) Viruses
- Powassan Encephalitis Viruses
- Louping-ill Virus

HEMORRHAGIC FEVER GROUP
Kyasanur Forest Disease (KFD) Virus

- Vector: Hard ticks (*Haemaphysalis spinigera)*
- Hosts: Monkeys, rodents and squirrels are common hosts
- Seasonality: KFD is increasingly reported in dry months (January-June) which coincides with human activity in forest

Clinical Manifestation in Humans

- Incubation period varies from 3–8 days
  - First stage (hemorrhagic fever)
  - Second stage in the form of meningoencephalitis

Laboratory Diagnosis

- Diagnosis is made by virus isolation from blood or by IgM antibody detection by ELISA.
- Recently, nested RT-PCR and real time RT-PCR have been developed detecting viral RNA (NS-5 non-coding region) in serum samples and can provide early, rapid and accurate diagnosis of the infection

VACCINE

- **Killed KFD Vaccine -** formalin-inactivated chick embryo vaccine - developed for KFD in the Haffkine institute, Mumbai.
- **Schedule: Two-doses at interval of 2 months, followed** by booster doses at 6–9 months and then every 5 years
- **Target area: KFD vaccine is recommended in endemic** areas of Karnataka (villages within 5 km of endemic foci).

GENUS BUNYAVIRUS

- California Encephalitis Virus Complex
- Oropouche Virus
-

GENUS PHLEBOVIRUS

- sandfly Fever
- Rift Valley Fever Virus
- Severe Fever with Thrombocytopenia syndrome (sFTs) Virus

GENUS NAIROVIRUS

- Crimean Congo Hemorrhagic Fever Virus
- Ganjam Virus

GENUS COLTIVIRUS

- COLORADO TICK FEVER VIRUS