

Presented By:

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# Aedes – Borne Viruses

Lab Diagnosis

Dengue, Chikungunya and Zika

# ArboViruses

The **arthropod-borne viruses** (arboviruses) and **rodent-borne viruses** represent ecologic groupings of viruses with complex transmission cycles involving arthropods or rodents. These viruses are classified in several virus families.

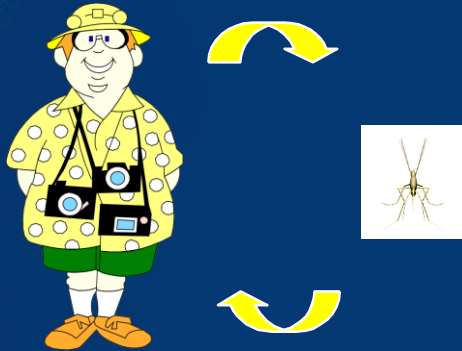
Family	Characterization	Arthropod hosts	example
Flaviviridae	Pos, ss-RNA	Aedes, Culex <i>Ixodidae</i>	Dangue, Yellow fever, Zika
Togaviridae	Pos, ss-RNA	Aedes, Culicine	Chikungunya, EEE, WEE, VEE
Arenaviridae	ambi-sense ss-RNA	Rodent	LCM
Reoviridae	ds-RNA	Culicidae, <i>Ixodidae</i> <i>Ixodidae</i>	Bluetongue, CTFV
Bunyaviridae	ambi-sense ss-RNA	Culicidae	California E, La Crosse E.
		<i>Ixodidae</i> , Argasidae	CCHF
		Phlebotominae Culicidae, <i>Ixodidae</i>	Sand fly fever, RVF
		Rodent	Hantaan

# Arboviruses in Iran

- ❖ Crimean-Congo Haemorrhagic Fever (CCHF)
- ❖ West Nile Virus (WNV)
- ❖ Rift Valley Fever (RVF)
- ❖ Dengue2 Virus (D2)
- ❖ Yellow Fever (YF)
- ❖ Chikungunia Virus (CHK)
- ❖ Sandfly Fever
- ❖ Kyasanur forest disease virus (KFDV)

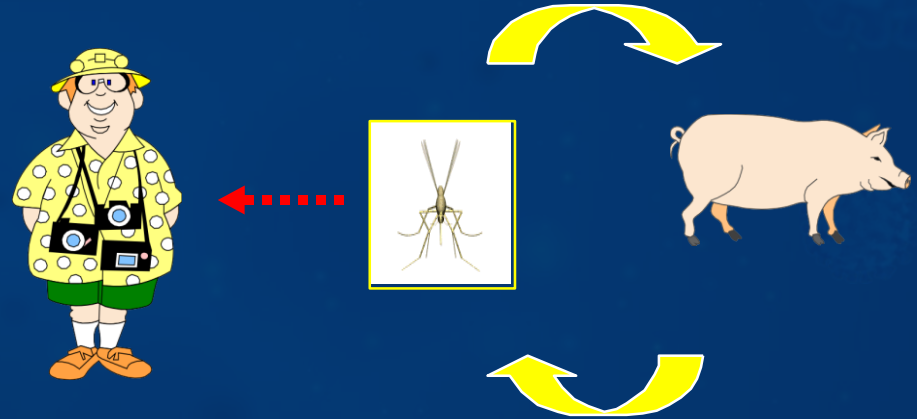
# Transmission Cycles

## Man-Arthropod-man Cycle Urban Cycle



e.g. dengue, urban yellow fever.

## Animal-Arthropod-Man Cycle Jungle Cycle



e.g. Japanese encephalitis, EEE, WEE,  
jungle yellow fever.

# Vectors & Reservoirs

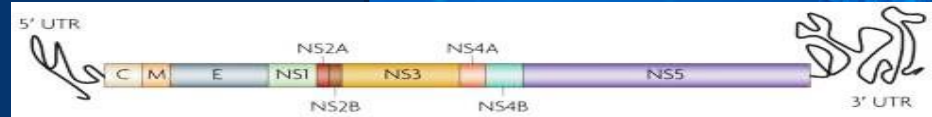
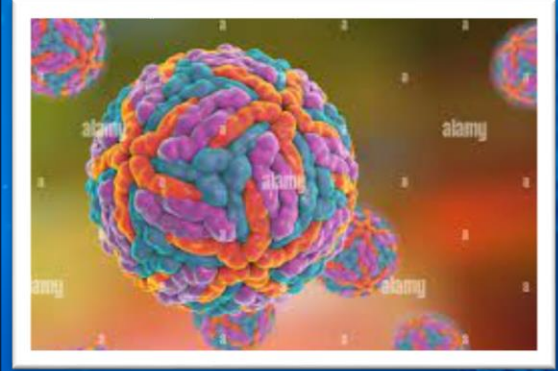
<ul style="list-style-type: none"><li>• <b>Mosquitoes</b> Japanese encephalitis, dengue, yellow fever, St. Louis encephalitis, EEE, WEE, VEE , Rift valley fever etc.</li></ul>	<ul style="list-style-type: none"><li>• <b>Birds</b> Japanese encephalitis, St Louis encephalitis, EEE, WEE</li></ul>
<ul style="list-style-type: none"><li>• <b>Ticks</b> Crimean-Congo haemorrhagic fever, various tick-borne encephalitides etc.</li></ul>	<ul style="list-style-type: none"><li>• <b>Pigs</b> Japanese encephalitis, Yellow Fever</li></ul>
<ul style="list-style-type: none"><li>• <b>Sandflies</b> Sandfly fever.</li></ul>	<ul style="list-style-type: none"><li>• <b>Rodents</b> VEE, Russian Spring-Summer encephalitis</li></ul>



# Dengue Virus

DENV belongs to the flavivirus genus within the *Flaviviridae* family.

positive-sense, ss RNA virus, three structural proteins – the capsid (C), membrane (M) and envelope (E) glycoproteins –non-structural proteins (**NS1**, NS2A, NS2B, NS3, NS4A, NS4B and NS5)



approximately 390 million dengue infections occur each year, particularly in India, Indonesia, Brazil, China and Africa

the true incidence and impact of DENV is higher than currently reported

# Dengue Virus

- ❖ Dengue virus is the most common arbovirus in the world
- ❖ **four serotypes**, - recently, the fifth serotype (DEN-5) was discovered in 2013 from Bangkok
- ❖ **Vector** – **Aedes aegypti** is the principal vector followed by *Aedes albopictus*. 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
- ❖ *Aedes* becomes **infective** only by feeding on viremic patients (generally from a day before to the end of the febrile period)



# Dengue Virus

- ❖ **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 **it's offspring** by 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 second serotype 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** – protective - against the infective serotype (which last lifelong)

The **non-neutralizing antibodies** are heterotypic in nature; i.e they are produced against other serotypes but not against the infective serotype

Such antibodies produced following the first serotype infection, can bind to a second serotype; but instead of neutralizing the second serotype, it protects it 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.

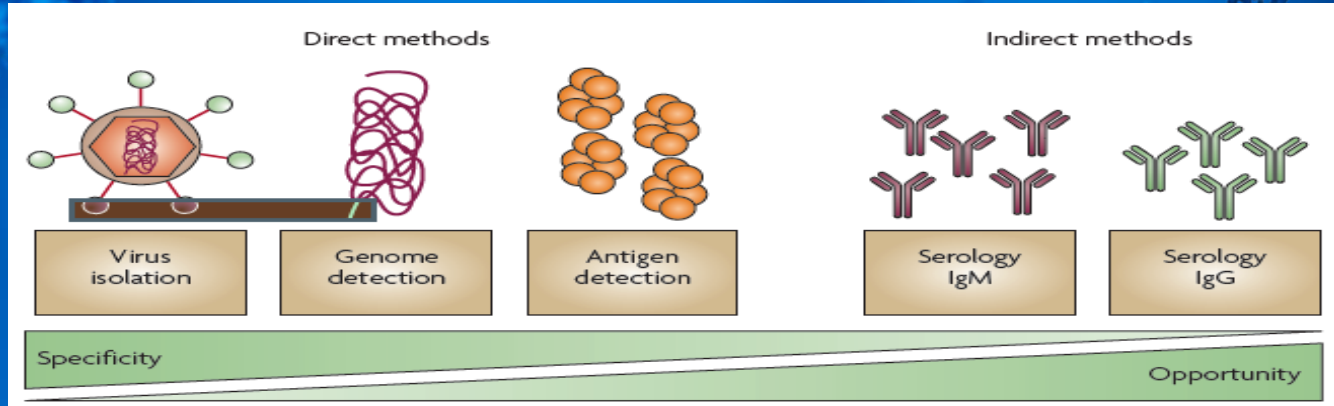
# Factors determining the outcome

- ❖ ***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

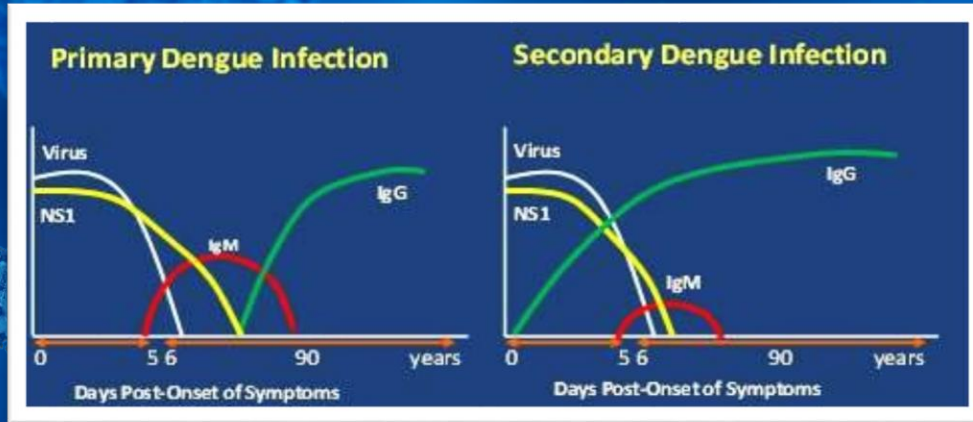
- ❖ Dengue is endemic in >100 countries with 2.5 billion people at risk
- ❖ Tropical countries of Southeast Asia and Western pacific are at highest risk
- ❖ Each year, up to 400 million people get infected with dengue. Approximately 100 million people get sick from infection, and 40,000 die from severe dengue (CDC)

## Diagnosis of Dengue Infection



- ❖ There are several diagnostic options for the diagnosis of DENV fever. These include assays that detect the virus directly (**virus isolation**) or its components (**viral RNA or antigen**) and serological assays that detect specific dengue antibodies, immunoglobulin M (**IgM**), immunoglobulin G (**IgG**) and immunoglobulin A (IgA). The choice of assay will depend both on the **timing** of sample collection and the **purpose** of the testing .





### Diagnostic strategy

from **D0** to **D5**: **PCR** only  
 from **D5** to **D7** inclusive: **PCR** and serology  
 after **D7**: serology only

Viraemia is generally detectable for about **4 – 5 days** after the onset of fever and correlates with fever duration. In a **primary DENV infection**, the anti-DENV virus **IgG** develops relatively **slowly**, with **low** titers **8 -10 days** after the onset of fever and persists for **10 months to life**, while the anti-DENV **IgM** is typically detected about **5 days** after the onset of fever and persists for approximately **3 – 8 months**.

In a **secondary DENV infection**, however, DENV **IgG** develops **rapidly**, with high titers soon after the onset of fever, while IgM can be undetectable .

**NS1 Ag detection** rapid cards are available for detecting NS1 antigen in serum.

NS1 detectable from **day-1 of fever** and remains positive up to **18 days**



## Treatment

There is no specific antiviral therapy. Treatment is symptomatic & supportive

## Vaccine

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

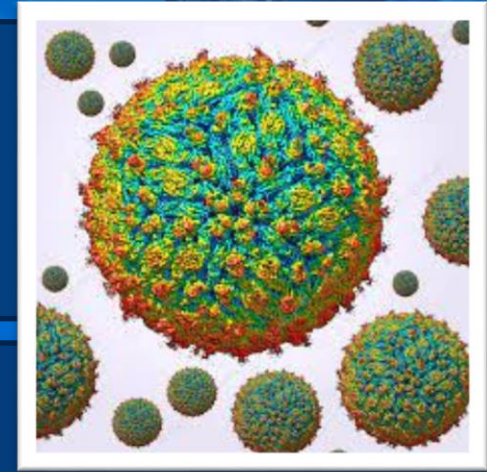
Schedule – 3 injections of 0.5 mL administered subcutaneously at 6 month intervals

# 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 asymptomatic males to their female partners.

# Epidemiology

The first outbreak was reported in 2007 in **Yap Islands** (49 confirmed and 59 probable cases )

## **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**

## 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

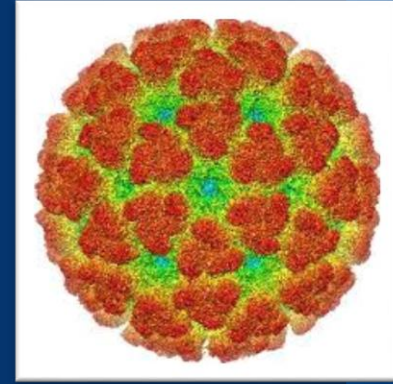
**Plaque-reduction neutralization test** is more specific serological (antibody detection) test; but it is cumbersome, not widely used.

# Chikungunya

❖ CHIKV; family *Togaviridae* ss RNA virus with a positive-sense

❖ **Human Transmission**

- I. primarily *Aedes aegy*
- 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 *Aedes aegypti*.
- ❖ **Sylvan** transmission cycle occurs usually in African forests involving the wild primates as reservoir (**monkeys**) and **forest species of Aedes** (e.g. *A.furcifer*, *A.taylori*) as vectors.

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

Chikungunya virus has **three genotypes**- West African, East African and Asian genotypes

The most **common symptoms** of infection are **fever** and **joint pain**. Other symptoms may include headache, muscle pain, joint swelling, or rash

There is currently **no vaccine** to prevent or **medicine** to treat chikungunya



# LAB DIAGNOSIS

**Virus isolation** in mosquito cell lines

**Serum antibody detection:**

**IgM** appears after **4 days** of infection and lasts for 3 months. **IgG** appears late (after 2 weeks) and lasts for years. So, detection of IgM or a fourfold rise in IgG 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.

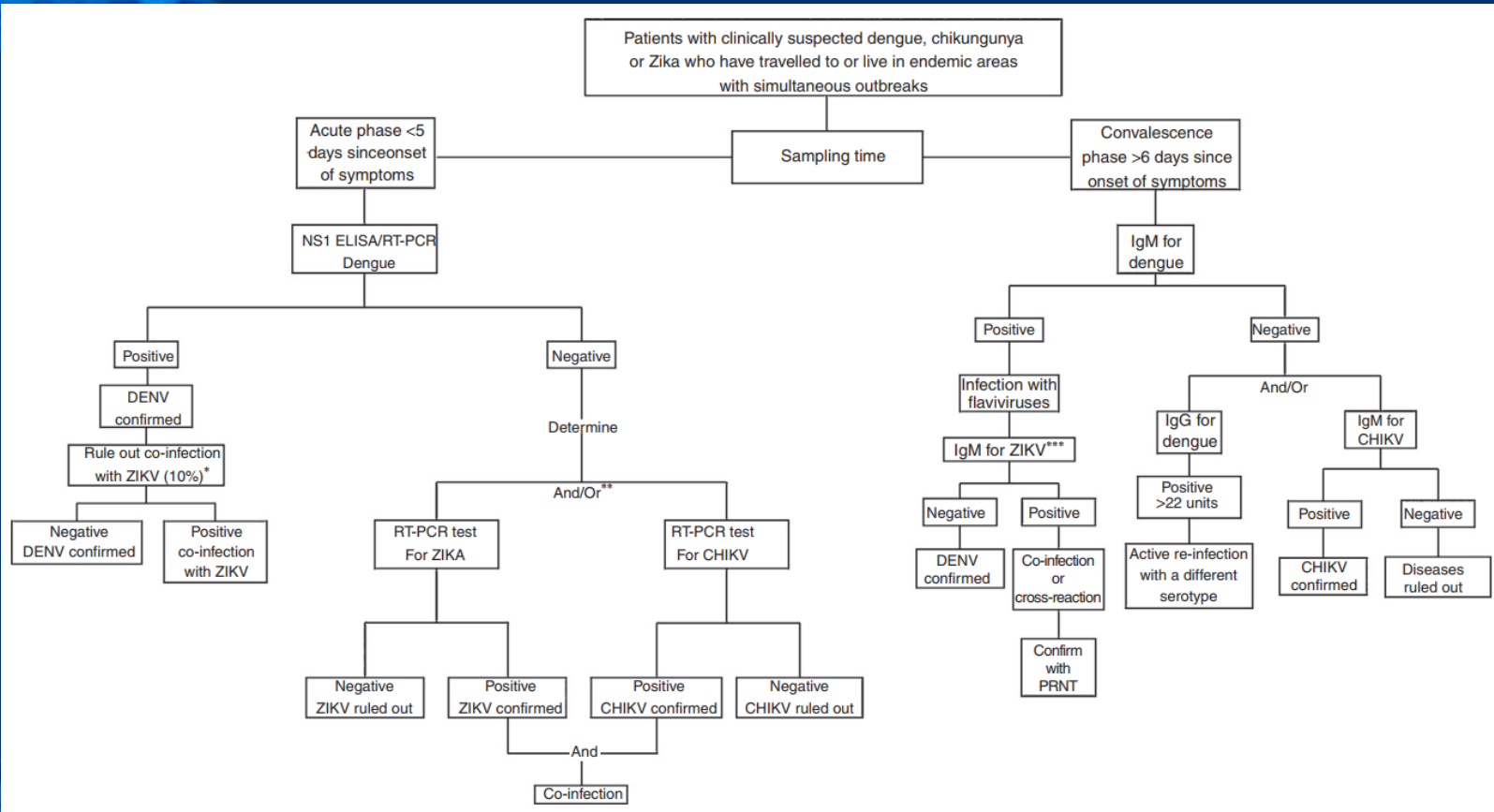
**Molecular method:** Reverse-transcriptase PCR has been developed to detect specific gene (e.g. nsP1, nsP4) in blood - best for early diagnosis (0- 7 days)

**Treatment** is by 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.

# Differential diagnosis, dengue, chikungunya, zika





# Thanks!

**Any questions?**

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