Dengue (DEN)
Clinical description

**Dengue fever**
Clinical features vary according to the age of the patients. Infants and young children may have non-specific febrile illness with rash. Older children and adults may have either a mild febrile syndrome or the classical disease. The classical dengue is characterized by an acute febrile illness of 2-7 days duration with 2 or more of the following: headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, leucopaenia, maculopapular rash.

**DHF** is characterized by 4 necessary criteria:
1. Fever or recent history of acute fever
2. Hemorrhagic manifestations based on at least one of the following: positive tourniquet test, petechiae/purpura, haematemesis/melena, other overt bleeding.
3. Low platelet count (100,000/mm\(^3\) or less).
4. Objective evidence of “leaky capillaries:”
   - Elevated hematocrit (20% or more over baseline).
   - Low albumen.
   - Pleural or other effusions.

**Diagnosis**
The following diagnostic systems are developed and standardized for regular use at the institute.

- **Serological tests**
  - Haemagglutination inhibition (HI)
  - Complement fixation (CF)
  - Neutralization (N)
  - MAC-ELISA.

- **Virus isolation and detection**
  - Infant mice.
  - Tissue culture.
  - Mosquito inoculation.
  - Immunohistochemistry.
  - IFA.
  - Antigen capture ELISA.

**Grades of DHF**

- **Grade I:** Fever and nonspecific constitutional symptoms; positive tourniquet test is only hemorrhagic manifestation.
- **Grade II:** Grade I manifestations + spontaneous bleeding.
- **Grade III:** Signs of circulatory failure (rapid/weak pulse, narrow pulse pressure, hypotension, cold/clammy skin). Frank shock is direct evidence of circulatory failure.
- **Grade IV:** Profound shock.

**Dengue Shock Syndrome (DSS)**
Clinically, DHF grade III and IV represent DSS. (Source: WHO)
Laboratory criteria for confirmation of dengue infection

- Isolation of virus from serum, plasma, leucocytes and autopsy samples.
- Presence of IgM antibodies or demonstration of 4-fold or greater increase in IgG titres in convalescent serum samples.
- Detection of antigen by Immunohistochemistry or Immunofluorescence or antigen capture ELISA.
- Detection of viral genome sequences by PCR.

Genotyping

Genotyping, based on envelope gene (1500bp) sequencing of DEN2 isolates from the 1960s and the 1990s, showed that the earlier isolates belonged to the American genotype while the later isolates belong to the Asian genotype. The recent reports implicate the Asian genotype with the occurrence of DHF/DSS.

Localization of proteins

Polyclonal antibodies against DEN virus detected antigen exclusively in the cytoplasm with stronger staining in the perinuclear region. Dually stained DEN2 virus infected cells with reagents specific for the virus and cellular organelles showed co-localised DEN antigen with Golgi apparatus indicating that the viral proteins matured through the Golgi.

Phylogenetic tree based on 427 bp partial NS1 gene sequences of dengue-1 & 2 strains

| DEN virus antigens in cytoplasm | Golgi bodies (red) | DEN virus antigens co-localized with golgi bodies (yellow) | Phylogenetic tree based on 427 bp partial NS1 gene sequences of dengue-1 & 2 strains |

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Vectors

*Aedes aegypti* is the principal vector both in rural and urban areas. Repeated isolations have been obtained from this species from many parts of the country. DEN virus has also been isolated from *Ae. albopictus* on 4 occasions - Asansol, (1974); Bankura (1997) in West Bengal; Pandharpur, Solapur district, Maharashtra (2002) and from Kerala (2001). However, the role of this species as vector needs further investigations.

Transmission

Dengue viruses are transmitted to humans by bite of infected *Aedes* mosquitoes. They acquire the infection either from viraemic persons or from transovarially infected parent mosquitoes. Once infected, the mosquito can transmit the virus for the rest of its life. The virus circulates in the blood of humans for 2-7 days.

Transmission potential

*Ae. aegypti* and *Ae. albopictus* are capable of transmitting virus to susceptible hosts in laboratory conditions. Transovarial transmission (TOT) has also been demonstrated. The virus was also obtained from field collected immature stages of mosquitoes, confirming occurrence of this phenomenon in nature.

Aedes aegypti

*The principal vector*

Immunofluorescence in mosquito head squash

Vector biology and ecology

Detailed studies undertaken by NIV on different aspects of vector biology and ecology have contributed significantly. Salient findings are as follows:

Expanded geographical distribution

Distribution of *Ae. aegypti* has increased considerably. Many urban and rural areas, negative earlier, have become positive and *Ae. aegypti* has become a well-established mosquito species in many parts of the country.

Vector-related risk factors

Increased communication due to new road and rail networks has facilitated repeated introduction of the vector and virus to new areas. Unreliable, inadequate and interrupted water supplies lead to water storage in domestic containers. This provides opportunity for establishment of vectors in the area.

A tyre dump: Ideal breeding habitat for *Ae. Aegypti*

Increasing usage of containers like metal and plastic drums facilitates prolific breeding of *Ae. Aegypti*.

Bus depots and discarded tyre dumps have been identified as important risk factors for unusual population build-up of vectors. Movement of tyres between depots and between urban and semi-urban centers provides ideal transport mechanism for eggs from one place to another.

Regular vector control program is not practised in any part of the country. This has further compounded the situation by providing perpetual breeding sources.

Extensive population build-up in rural areas is caused due to heavy breeding of mosquitoes in selected key containers like cement tanks, unused earthen pots, coconut-shells, etc.

Studies at NIV demonstrated that treatment of potential breeding containers with Abate at a concentration of about 1ppm stops breeding for a period of 8-12 weeks. Government of Maharashtra has included this in their control program strategies.

Immunization

Several vaccines are at different levels of experimentation worldwide. They are not likely to be available at least in the next 5 years for public use. It is believed that long-term solution lies in effective and suitable vaccine program.

There is an urgent need to determine the disease burden, through surveillance for effective intervention and management of cases.

Pathogenesis of dengue, host and virus factors should be properly determined.

Vector genetics

Virus-specific receptors are recognized in mid-gut of female mosquitoes. There are susceptible and refractory strains of mosquitoes; and these characteristics are apparently governed genetically.

Prevention and control

Vector control is still a method of choice for containment of outbreaks. However, the method has limitations. Control of larvae can be achieved by reducing breeding containers.