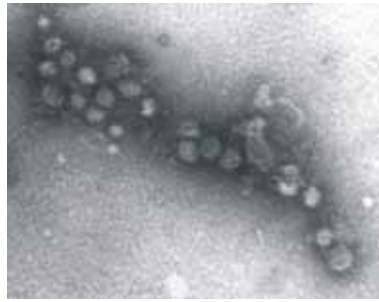




Dengue (DEN)

Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF)

Dengue fever is an important disease in the tropics and subtropics. Based on clinical description, the disease is believed to be in existence for over two centuries.

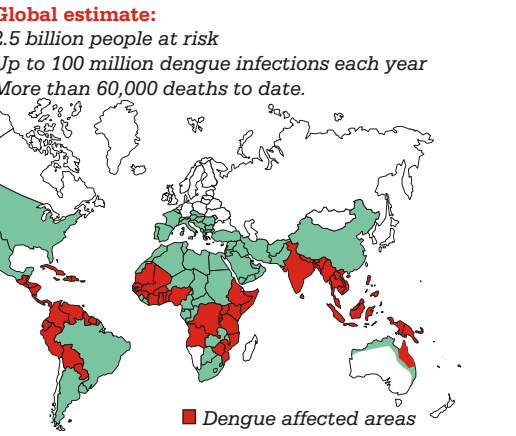
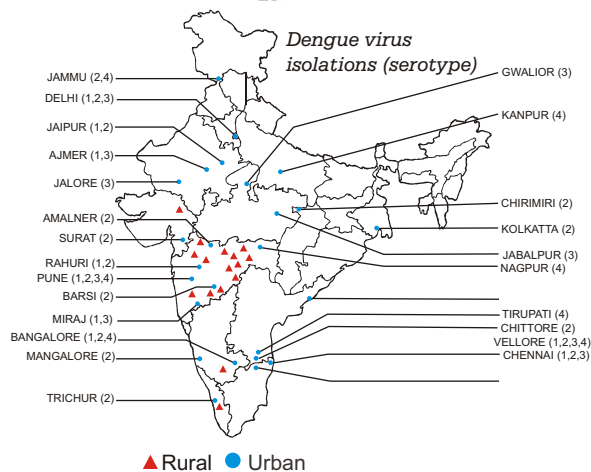


The virus

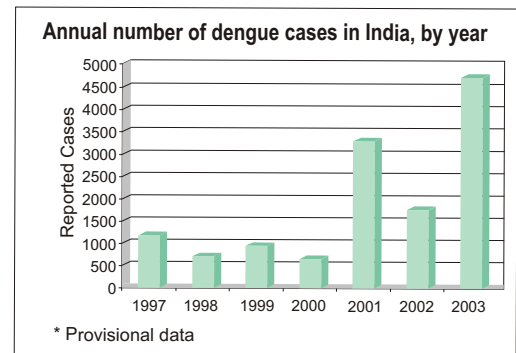
DEN virus belongs to family *Flaviviridae*. It is spherical, 40-60 nm in diameter and contains electron dense core ~30 nm in diameter. Genome is single stranded RNA, about 11 kb in size. Three viral proteins are associated with virion, the envelope, membrane and capsid.

Serotypes

Dengue virus has four serotypes, viz., DEN-1, DEN-2, DEN-3, DEN-4. All four serotypes are reported from India. Often more than one serotype is involved in an outbreak. Some patients also have co-infection with multiple serotypes. It is also postulated that sequential infection with two different serotypes is related to the severity of the disease, leading to DHF.



In India, the fever has been documented from early part of the last century. The virus was first isolated in Calcutta in 1945. Several outbreaks from different parts of the country have been reported during last 50 years. For very long, dengue was recognized exclusively as an urban disease, but now it is also considered an important disease of rural areas.



The number of cases, though grossly under-reported, has shown a rising trend.

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

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/melenae, other overt bleeding.
3. Low platelet count ($100,000/\text{mm}^3$ or less).
4. Objective evidence of "leaky capillaries:"
 - Elevated hematocrit (20% or more over baseline).
 - Low albumen.
 - Pleural or other effusions.

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)

Diagnosis

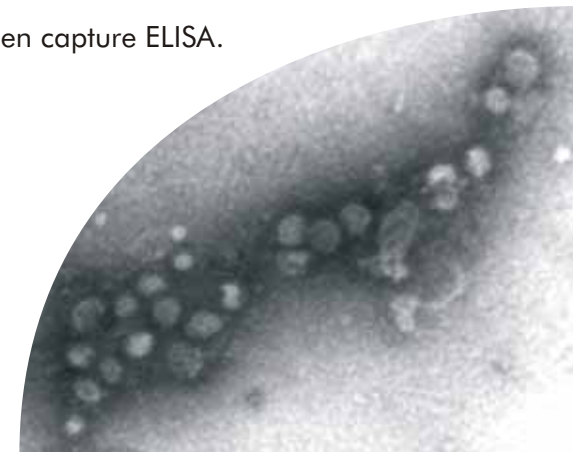
The following diagnostic systems are developed and standardized for regular use at the institute.

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.

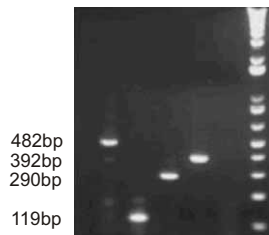


Indigenous MAC - ELISA Kit



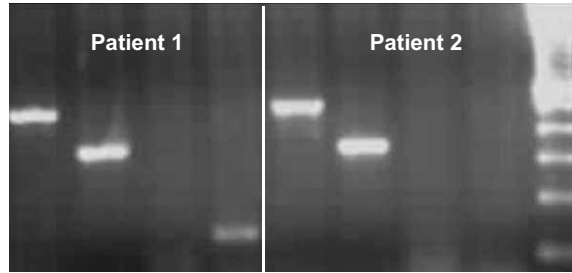
Places	DEN serotypes	Clinical Manifestation
Maharashtra		
Solapur	1, 2, 4	DHF cases
Pune	2, 3, 4	DHF cases
Beed	2	Dengue Fever
Kolhapur	2	DHF
Nagpur	2	DF
Gujarat		
Surat	1, 2, 4	Dengue Fever
Kerala		
	1, 2	DHF

DEN → 1 2 3 4



Detection of viral RNA

- Directly from the clinical samples by RT-PCR.
- Serotyping by RT-PCR, using one primer pair at a time.
- Multiplex RT-PCR wherein all 4 serotypes can be detected in a single reaction.



DEN 1,2,4 DEN 1,2
Multiple DEN serotype infections as detected by PCR

Laboratory criteria for confirmation of dengue infection

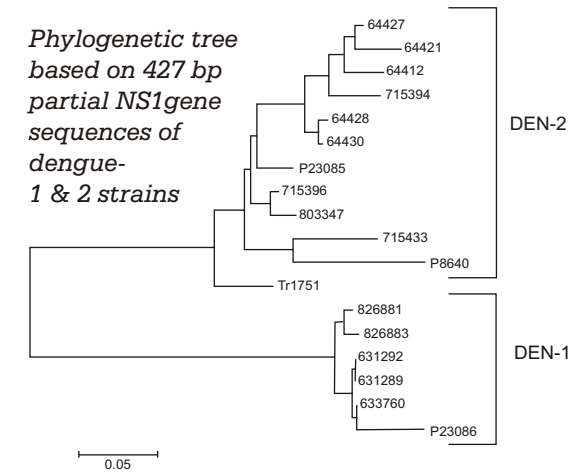
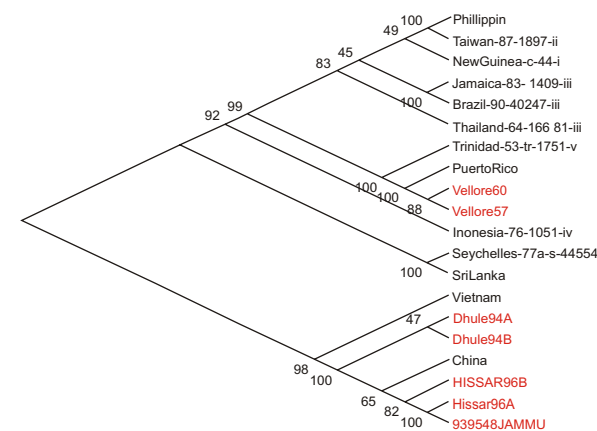
- Isolation of virus from serum, plasma, leucocytes and autopsy samples.
- Presence of IgM antibodies or demonstration of 4-fold or greater increase in IgG titres in convalescent serum samples.
- Detection of antigen by Immunohistochemistry or Immunofluorescence or antigen capture ELISA.
- Detection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

Dengue Serotype Isolated / Detected (Humans)					
Period	DEN-1	DEN-2	DEN-3	DEN-4	Total
1956-1960	2	5	0	4	11
1961-1965	62	33	1	12	108
1966-1970	27	42	95	26	190
1976-1980	7	4	0	0	11
1981-1985	23	4	12	1	40
1986-1990	7	57	5	0	69
1991-1995	4	49	0	0	53
1996-2003	8	26	2	1	37
Total	152	227	115	46	540

Dengue Serotype Isolated (Mosquitoes)					
Period	DEN-1	DEN-2	DEN-3	DEN-4	Total
					0
1961-1965	4	0	0	12	16
1966-1970	24	40	5	6	75
1971-1975	2	2	1	4	9
1976-1980	0	4	0	0	4
1981-1985	0	5	0	1	6
1986-1990	0	24	1	0	25
1991-1995	0	2	0	0	2
1996-2003		5			5
Total	30	79	12	21	142

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.

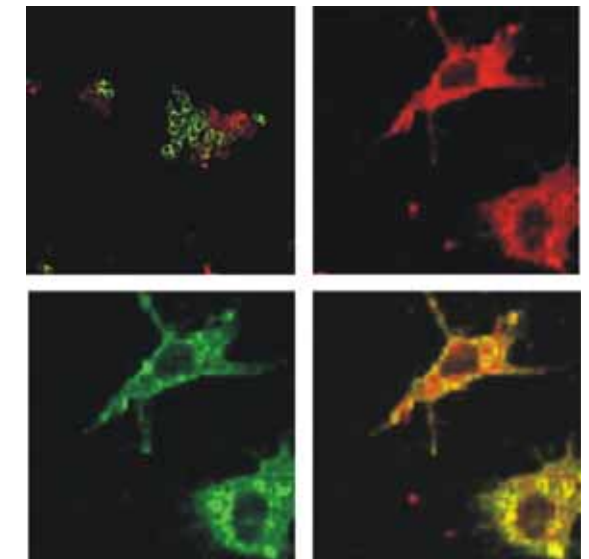


Localization of proteins

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.

Dengue virus proteins in cytoplasm

Golgi bodies (red)



DEN virus antigens (green)

DEN virus antigens co-localized with golgi bodies (yellow)

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.

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.



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.

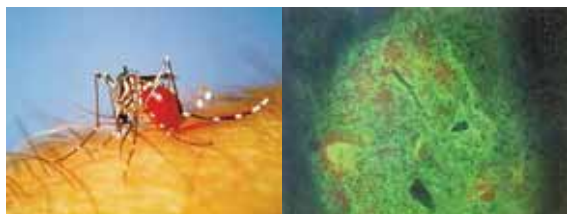
Studies at NIV demonstrated that treatment of potential breeding containers with Abate at a concentration of about 1ppm stops breeding for a period of 8-12 weeks. Government of Maharashtra has included this in their control program strategies.

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.



Aedes aegypti:
The principal vector

Immunofluorescence in
mosquito head squash