International Journal of Veterinary Sciences and Animal Husbandry 2020; 5(6): 01-10

ISSN: 2456-2912
VET 2020; 5(6): 01-10

www.veterinarypaper.com
Received: 01-09-2020
Accepted: 15-10-2020

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Epidemiological surveillance of Dengue fever: An overview

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Abstract
Dengue fever is the most common of all arthropod-borne viral diseases and has emerged as a major public health problem in recent years. Though the disease has been referred in ancient Chinese medical encyclopedia, the first case was reported in 1789 during the epidemics in Asia, Africa and North America. It was then when Benjamin Rush coined the term 'break bone fever' because of the symptoms of myalgia and arthralgia. However, the term dengue fever came into general use only after 1828 (Anonymous, 2010) [6].

Dengue viruses (DENVs) belong to family *Flaviviridae*, which are transmitted through mosquito; *Aedes aegypti* and also by *Ae. albopictus.* There are four sero types of the virus referred to as DENV-1, DENV-2, DENV-3 and DENV-4, actually originated in monkeys and independently jumped to humans in Africa or Southeast Asia between 800 and 100 years ago (Brett *et al.* 2007*)* [19]. Each dengue virus is an encapsulated RNA virus and is composed of three structural protein genes, which encode the nucleocapsid or core (C) protein, a membrane-associated protein, an enveloped (E) glycoprotein and seven non-structural (NS) proteins (Chaturvedi *et al.* 2005) [23, 24, 25, 26, 27, 28, 45, 94]. All four strains are capable of causing three spectra of disease – Dengue fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) (David *et al.* 2010; Gupta *et al.*2012*)* [8, 34, 39, 44, 45].
Today, about 2.5 billion people live in areas where, there is a risk of dengue transmission. It is endemic in over 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. The World Health Organization (WHO) estimates that 50 to 100 million infections occur each year, including 500,000 DHF cases and 22,000 deaths, mostly affecting children (Brett *et al.* 2007*)* [19].
In India, the first confirmed case reports of dengue dates back in the year 1940. Since then many cases have been reported from various states (Gupta *et al.*2012*)* [8, 34, 44, 45]. The first major wide spread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi and Lucknow and then it spreaded to all over the country (Anonymous, 2010) [6].

Keywords: dengue, fever, mosquito, hemorrhagic, infection.

1. Introduction
Dengue viruses (DV) belong to family *Flaviviridae* and have four serotypes (1 to 4). They are transmitted mainly by the *Aedes aegypti* mosquito and also by *Aedes albopictus*. Biologically, DV are highly adapted to the mosquito and are maintained by vertical transmission. DV produces from a subclinical infection to a mild self-limiting disease, the dengue fever (DF) and a severe disease that may be fatal, the dengue hemorrhagic fever/ dengue shock syndrome (DHF/DSS).
The mosquito vectors are present in tropical and subtropical regions of the earth that determines the prevalence of DV in a region. Prior to 1970, only 9 countries had experienced cases of DHF; since then the number has increased more than 4-fold and continues to rise (figure 1a).
The WHO published a global map of the distribution of the dengue epidemic activity during the year 2006 that shows whole India in red colour (figure 1b). Similar maps prepared in earlier years shows that the activity of both the vector and the virus has spread to newer areas, acquiring global public health importance. An estimated 2.5 billion people in more than 100 countries are at risk of acquiring dengue viral infection with more than 50 million new infections being projected annually, 500000 cases of DHF that must be hospitalized and

20000–25000 deaths, mainly in children (Kumar *et al.* 2010; Halstead 2002; Halstead 2007) [21, 35, 67, 68]. Dengue has been an urban disease but now has spread to rural areas of India as well (Chaturvedi *et al.* 2004; Mehendle *et al.* 1991; Kumar *et al.* 2001; Arunachalam *et al.* 2004) [7, 21, 23, 24, 25, 26, 27, 28, 35, 45, 67, 68, 94]. The factors considered responsible for Global resurgence of DF/DHF are unprecedented population growth, unplanned and uncontrolled urbanization, increased Air travel, absence of an effective mosquito control programme and deterioration of Public Health infrastructure. The risk factors for infection with DV are the increased density of the mosquito vector, reinfestation with *Ae. aegypti* of a new geographical area, warm and humid climate, increased population density, water storage pattern in houses, storage of junk in open spaces, including tyres, coconut shells etc that trap rain water and introduction of new serotype of the virus, etc. Vaccines or antiviral drugs are not available for dengue viruses; the only effective way to prevent epidemic DF/DHF is to control the mosquito vector, *Ae. Aegypti* and prevent its bite.

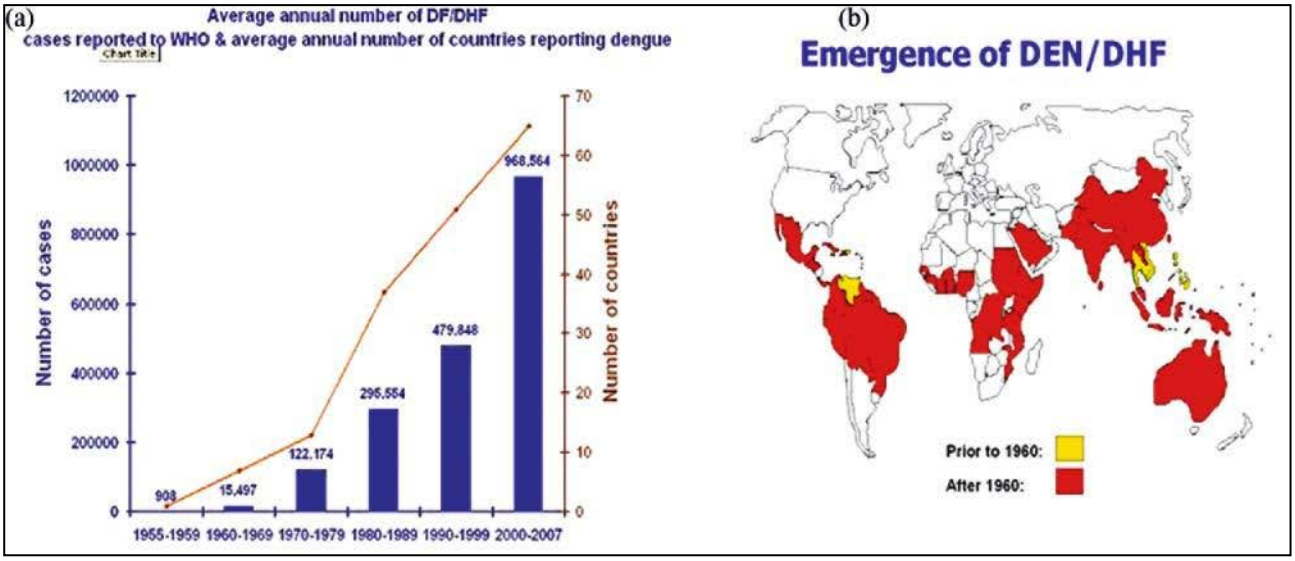


Fig 1: Global prevalence of dengue fever (DF) and dengue hemorrhagic fever (DHF) as shown by the WHO. (a) Average number of cases and the number of countries affected since 1955. (b) World map showing the prevalence of dengue virus before 1960 and during the year 2006.

2. History

Dengue virus was isolated in Japan in 1943 by inoculation of serum of patients in suckling mice (Tewari *et al.* 2004) [52, 81, 97] and at Calcutta (now Kolkata) in 1944 from serum samples of US soldiers (Whitehorn *et al.* 2010) [102]. The first epidemic of clinical dengue-like illness was recorded in Madras (now Chennai) in 1780 and the first virologically proved epidemic of DF in India occurred in Calcutta and Eastern Coast of India in 1963-1964 (WHO 2009; Kimura *et al.* 1944; Sabin *et al.* 1945) [62, 90]. The first major epidemic of the DHF occurred in 1953-1954 in Philippines followed by a quick global spread of epidemics of DF/DHF (Sarkar *et al.*1964) [22, 91, 92]. DHF was occurring in the adjoining countries but it was absent in India for unknown reasons as all the risk factors were present. The DHF started simmering in various parts of India since 1988 (Kabra *et al.* 1992; Bhattacharjee *et al.* 1990; Cherin *et al.* 1994) [16, 20, 53, 54]. The first major wide spread epidemics of DHF/DSS occurred in India in 1996 involving areas around Delhi (Dal *et al.* 1999) and Lucknow (Agraval *et al.* 1999) and then it spread to all over the country (Shah *et al.* 2004) [93].

3. Epidemiology and geographical distribution

The epidemiology of dengue fevers in the Indian subcontinent has been very complex and has substantially changed over almost past six decades in terms of prevalent strains, affected geographical locations and severity of disease. The very first report of existence of dengue fevers in India was way back in 1946 (Karamchandani, 1946) [55]. Thereafter, for the next 18 years, there was no significant dengue activity reported anywhere in the country. In 1963-1964, an initial epidemic of dengue fever was reported on the Eastern Coast of India7, (Ramkrishanan *et al.* 1964; Sarkar *et al.* 1964 [91, 92]; Chaudhuri *et al.* 1965; Krishnamurthy *et al.* 1965; Paul *et al.* 1965) [9, 22, 65, 85], it spread northwards and reached Delhi in 1967 (Balaya *et al.* 1969) [9] and Kanpur in 1968 (Chaturvedi *et al.*1968; Chaturvedi *et al.*1970) [23, 24, 25, 26, 27, 28, 45, 94]. Simultaneously it also involved the southern part of the country (Myers *et al.* 1968; Ghosh *et al.* 1968) [42, 43, 74, 75, 76, 77, 78] and gradually the whole country was involved with wide spread epidemics followed by endemic/hyperendemic prevalence of all the four serotypes of DV. The epidemiology of dengue virus and its prevalent serotypes has been ever changing. The epidemic at Kanpur during 1968 was due to DV-4 (Chaturvedi *et al.* 1968) [23, 24, 25, 26, 27, 28, 45, 94] and during 1969 epidemic, both DV-2 and DV-4 were isolated (Chaturvedi *et al.* 1972) [23, 24, 25, 26, 27, 28, 45, 94]. It was completely replaced by DV-2 during 1970 epidemic in the adjoining city of Hardoi (Chaturvedi *et al.* 1974) [23, 24, 25, 26, 27, 28, 45, 94]. Myers *et al* (Myers *et al.* 1968; Myers *et al.* 1970) [74, 75, 76, 77, 78] had reported the presence of DV-3 in patients and *Ae. aegypti* at Vellore during the epidemic of 1966 while during the epidemic of 1968, all the four types of DV were isolated from patients and mosquitoes (Myers *et al.* 1970) [74, 75, 76, 77, 78]. In another study Myers & Varkey (Myers *et al.* 1971) [74, 75, 76, 77, 78] reported an instance of a third attack of DV in one individual. DV-2 was isolated during the epidemics of dengue in urban and rural areas of Gujarat State during 1988 and 1989 (Mahadev *et al.* 1993) [43, 70]. Outbreaks of dengue occurred in Rajasthan by DV-1 and DV-3 (Ghosh *et al.* 1974) [42, 43], DV-3 (Chouhan *et al.* 1990) [32], Madhya Pradesh by DV-3 (Rodrigues *et al.* 1973) [32, 89], Gujarat by DV-2 (Mahadev *et al.* 1993) [43, 70] and in Haryana by DV-2 (Kumar *et al.* 2001) [21, 35, 67, 68]. DV-2 was the predominant serotype circulating in northern India, including Delhi, Lucknow and Gwalior (Dar *et al.* 19799; Agrawal *et al.* 1999;

Parida *et al.* 2002) [15, 20, 33, 34, 35, 38, 44, 82, 98, 99, 100] while DV-1 was isolated during the 1997 epidemic at Delhi (Kurukumbi *et al.* 2001) [69]. The phylogenetic analysis by the Molecular Evolutionary Genetics Analysis programme suggests that the 1996 Delhi isolates of DV-2 were genotype IV. The 1967 isolate was similar to a 1957 isolate of DV-2, from India, and was classified as genotype V. This study indicates that earlier DV-2 strains of genotype V have been replaced by genotype IV (Singh *et al.* 1999) [43, 95, 96]. The Gwalior DV-2 viruses were classified into genotype-IV, and were most closely related to Delhi 1996 DV-2 viruses and FJ 10/11 strains prevalent in the Fujian State of China. However, two earlier Indian isolates of DV-2 were classified into genotype-V. Genotype V of DV-2 has been replaced by genotype IV during the past decade, which continues to circulate silently in north India, and has the potential to re-emerge and cause major epidemics of DF and DHF (Dash *et al.* 2004) [35, 36, 37, 38, 82]. DV-2 has also been reported from southern India - in Kerala alongwith DV-3 (Anoop *et al.* 2010) [1].

DV-3 has been isolated during the epidemics at Vellore in 1966 (Myers *et al.* 1968; Myers *et al.* 1969) [74, 75, 76, 77, 78], at Calcutta in 1983 (Mukherjee *et al.* 1987) [16, 73] and in 1990 (Bhattacharjee *et al.* 1993) [16], at Jalore city, Rajasthan in 1985 (Chouhan *et al.* 1990) [32] at Gwalior in 2003 and 2004 (Dash *et al.* 2005; Paramasivan *et al.* 2010) [35, 36, 37, 38, 81, 82] and at Tirupur, Tamil Nadu in 2010 (Paramasivan *et al.* 2010). [81] Phylogenetic analysis showed that the Madurai isolates were closely related to Gwalior and Delhi isolates. The emergence of DV-4 has been reported in Andhra Pradesh (Dash *et al.* 2011) [35, 36, 37, 38, 82] and Pune, Maharashtra (Dayaraj *et al.* 2011) [40], which was also implicated in increased severity of disease. At Delhi, till 2003, the predominant serotype was DV-2 (genotype IV) but in 2003 for the first time all four dengue virus subtypes were found to co-circulate in Delhi thus changing it to a hyperendemic state (Dar *et al.* 2006) [15, 20, 33, 34, 37, 38, 44, 100] followed by complete predominance of DV serotype 3 in 2005 (Gupta *et al.* 2006) [8, 34, 44, 45]. During the 2004 epidemic of DHF/DSS in northern India a sudden shift and dominance of the DV serotype-3 (subtype III) occurred replacing the earlier circulating serotype-2 (subtype IV) (Dash *et al.* 2005) [35, 36, 37, 38, 82]. Co-circulation of DV serotypes in Delhi in 2003-2004 has also been reported (Dash *et al.* 2005) [35, 36, 37, 38, 82], which may have implications for increased DHF/DSS. Emergence of a distinct lineage of DV-1, having similarity with the Comoros/Singapore 1993 and Delhi 1982 strains, but quite different from the Delhi 2005 lineage and microevolution of the pre-circulating DV-3 has been reported (Kukreti *et al.* 2008). Co-circulation of several serotypes of dengue viruses has resulted in concurrent infection in some patients with multiple serotypes of DV (Bharaj *et al.* 2008) [15, 20]. Further, replacement of DV-2 and 3 with DV-1 as the predominant serotype in Delhi over a period of three years (2007-2009) has been reported (Ryes-Aldasoro, 2017). Concurrent infection by Chikungunya and DV-2 was reported from Vellore (Chakravarti *et al.* 2008) [16, 20, 22, 73] and Delhi (Myers *et al.* 1967) [74, 75, 76, 77, 78] (Table 1).

Table 1: Epidemiological studies where dengue virus was identified

Year	Region where study was conducted	
1964	Vellore, Tamil Nadu	DV-2
NA	South India	DV-3
1966	Vellore, Tamil Nadu	DV-3
1968	Vellore, Tamil Nadu	DV- 1,2,3 & 4
1968	Kanpur, Uttar Pradesh	DV-4
1969	Kanpur, Uttar Pradesh	DV-4 and DV-2
1970	Hardoi, Uttar Pradesh	DV-2
NA	NA	DV- 1,2,3 & 4
1983	Kolkata, West Bengal	DV-3
1985	Jalore town, South-West Rajasthan	DV-3
NA	Chikalthana, Pimpalgaon and Waloor villages in Parbhani	DV-1 & 2
	district of Maharashtra.	
1988	Delhi	DV-2
1990	Calcutta, West Bengal	DV-3
1988	Rural and urban areas of Gujarat	DV-2
1993	Mangalore, Karnataka	DV-2
NA	Assam and Nagaland	DV-2
1996	Ludhiana, Punjab	DV- 1,2,3 & 4
1996	Lucknow	DV-2
1996	Delhi	DV-2
1996	Delhi	DV-2
1997	Delhi	DV-1
1996	Delhi	DV-2 (Genotype IV)
NA	Ahmedabad, Gujarat	DV-2
1997	Delhi	DV-1
NA	Delhi	
1996	Rural areas of Haryana	DV-2
2001	Dharmapuri district, Tamil Nadu	DV-2
NA	Andaman and Nicobar Islands	
2001	Gwalior, Madhya Pradesh	DV-2
NA	Northern India	
2001	Chennai, Tamil Nadu	DV-3
2003	Northern India (Delhi & Gwalior)	DV-3
2005	Kolkata, West Bengal	DV-3
2003	Kanyakumari district, Tamil Nadu	DV-3

2003-04	Delhi	DV-3 (subtype III)
2003-05	Delhi	2003 - DV - 1,2,3 & 4 2005 - D - 3
2006	Delhi	DV-3
2006	Delhi	DV-1 & 3
2001-07	North India (Delhi and Gwalior region)	DV-1 (Genotype III)
2006	Delhi	DV-1,3 & 4
2008	Delhi region	DV-1,2 & 3
1956-2005	Entire country	DV-2
2002-06	Delhi	DV-1, 2, 3 & 4
2003	Delhi	DV-3 (Genotype III)
2008	Ernakulam, Kerala	DV-2 & 3
2007	Rural areas of Madurai, Tamil Nadu	DV-3 (Genotype III)
2007	Andhra Pradesh	DV-1 & 4 (Genotype I)
2003-08	Different parts of the country	DV-3 (Genotype III)
2007-09	Delhi	DV 1, 2, 3 & 4
2009-10	Pune, Maharashtra	DV-4 (Genotype I)

4. Causative agent

Dengue virus (DENV) belongs to the family *Flaviviridae*, genus *Flavivirus*, and is transmitted to humans by *Aedes* mosquitoes, mainly *Aedes aegypti*. Flaviviruses have a (+) sense RNA genome and replicate in the cytoplasm of the host cells. The genome mimics the cellular mRNA molecule in all aspects except for the absence of the poly-adenylated (poly-A) tail. This feature allows the virus to exploit cellular apparatus to synthesise both structural and non-structural proteins, during replication. The cellular ribosome is crucial to the replication of the flavivirus, as it translates the RNA, in a similar fashion to cellular mRNA, resulting in the synthesis of a single polyprotein. In general, the genome encodes 3 structural proteins (Capsid, prM, and Envelope) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) (Chahar *et al.* 2009) [15, 20]. The genomic RNA is modified at the 5′ end of positive-strand genomic RNA with a cap-1 structure (me^7-GpppA-me^2).

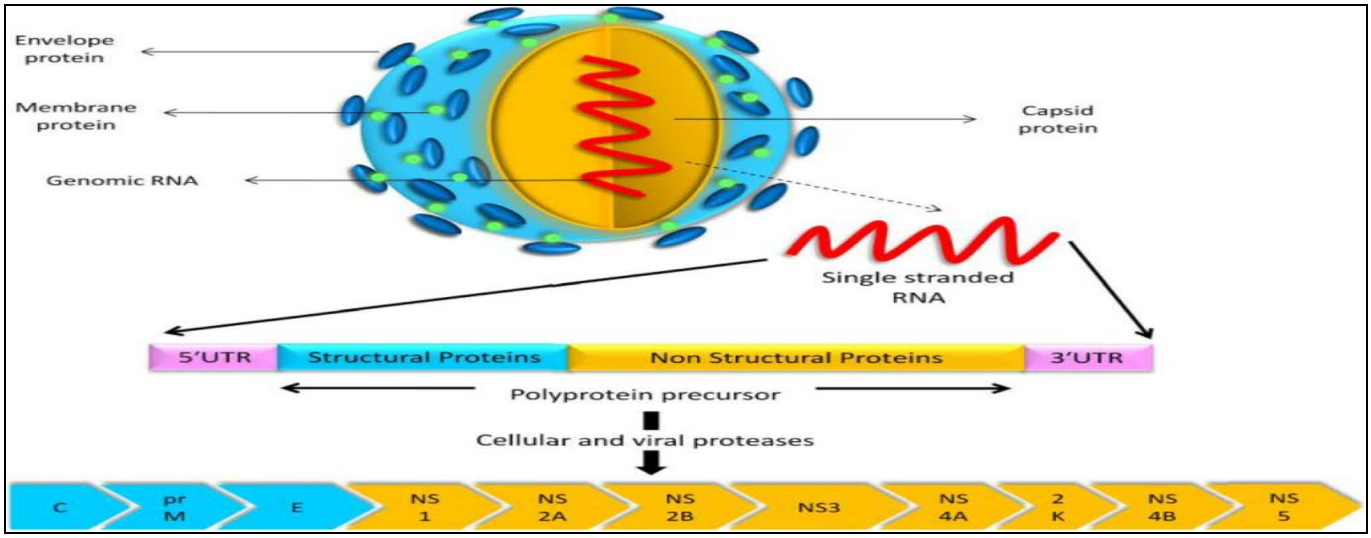
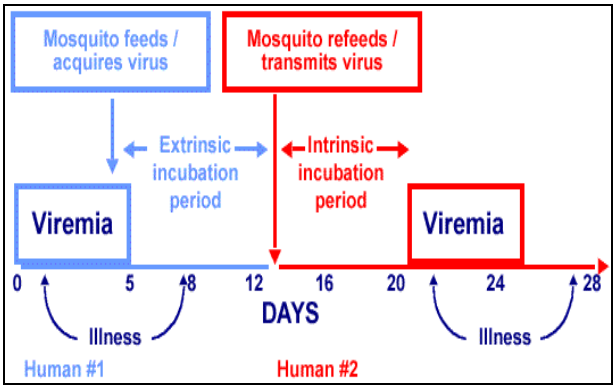


Fig 2: The Detailed structure of Dengue virus

5. Dengue virus and its serotypes

DV-1 was isolated in 1956 at Vellore. All the Indian DV-1 isolates belong to the American African (AMAF) genotype. The Indian DV-1 isolates are distributed into four lineages, India I, II, III and the Africa lineage. Of these, India III is the oldest and extinct lineage; the Afro-India is a transient lineage while India I is imported from Singapore and India II, evolving in situ, are the circulating lineages (Mehendale *et al.* 1991) [71, 72]. The American genotype of DV-2 which circulated predominantly in India during the pre-1971 period, was subsequently replaced by the Cosmopolitan genotype. Post-1971 Indian isolates formed a separate subclade within the Cosmopolitan genotype. DV-2 strains were isolated in India over a time span of more than 50 years (1956-2011). The re-emergence of an epidemic strain of DV type-3 in Delhi in 2003 and its persistence in subsequent years marked a changing trend in DV circulation in this part of India (Dash *et al.* 2004) [35, 36, 37, 38, 82]. Occasional reports of circulation of DV-4 are also seen, though it is not the predominant type in India (Padbidri *et al.* 1995; Barua *et al.* 1996) [10, 68, 79, 80].

6. Transmission of dengue virus



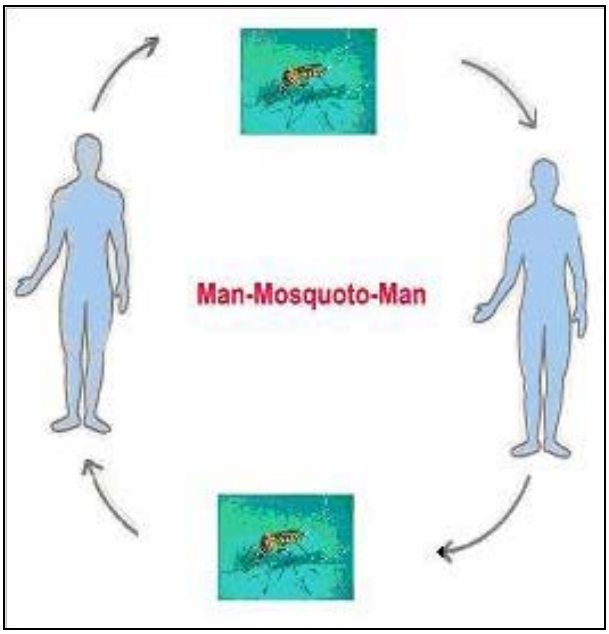


Fig. 3: Transmission cycle of Dengue virus

Dengue virus is primarily transmitted by *Aedes* mosquitoes, particularly *A. aegypti*. These mosquitoes usually live between the latitudes of 35° North and 35° South below an elevation of 1,000 metres (3,300 ft). They typically bite during the early morning and in the evening, but they may bite and thus spread infection at any time of day. Other *Aedes* species that transmit the disease include *A. albopictus*, *A. polynesiensis* and *A. scutellaris* (Kaur *et al.* 1997) [56]. Humans are the primary host of the virus, but it also circulates in nonhuman primates. An infection can be acquired via a single bite. A female mosquito that takes a blood meal from a person infected with dengue fever, during the initial 2- to 10-day febrile period, becomes itself infected with the virus in the cells lining its gut. About 8–10 days later, the virus spreads to other tissues including the mosquito's salivary glandsand is subsequently released into its saliva (Agrawal *et al.* 1998). The virus seems to have no detrimental effect on the mosquito, which remains infected for life. *Aedes aegypti* is particularly involved, as it prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed on people rather than other vertebrates (Kabra *et al.* 1999) [20, 53, 54].

Dengue can also be transmitted via infected blood products and through organ donation. In countries such as Singapore, where dengue is endemic, the risk is estimated to be between 1.6 and 6 per 10,000 transfusions. Vertical transmission (from mother to child) during pregnancy or at birth has been reported. Other person-to-person modes of transmission have also been reported, but are very unusual. The genetic variation in dengue viruses is region specific, suggestive that establishment into new territories is relatively infrequent, despite dengue emerging in new regions in recent decades (Chahar *et al.* 2009; Vajpayee *et al.* 1999) [15, 20, 100].

6.1 Predisposition

Severe disease is more common in babies and young children, and in contrast to many other infections, it is more common in children who are relatively well nourished. Other risk factors for severe disease include female sex, high body mass index, and viral load. While each serotype can cause the full spectrum of disease, virus strain is a risk factor (Joshi *et al.* 2000) [51, 79, 80]. Infection with one serotype is thought to produce lifelong immunity to that type, but only short-term protection against the other three. The risk of severe disease from secondary infection increases if someone previously exposed to serotype DENV-1 contracts serotype DENV-2 or DENV-3, or if someone previously exposed to DENV-3 acquires DENV-2. Dengue can be life-threatening in people with chronic diseases such as diabetes and asthma (Singh *et al.* 2001) [43, 95, 96].

7. Clinical features and pathogenesis

The patients initially develop an abrupt onset of high fever (39–40°C) with headache, retro-orbital pain, malaise, nausea, vomiting, and myalgia. The acute febrile stage lasts 2–7 days and may be followed by recovery but patients feel weakness. During defervescence some patients develop hemorrhagic manifestation that may be mild petechial haemorrhage, and bleeding at the nose, gastrointestinal tract and gums, which may be severe. Menorrhagia has been more prevalent due to the increasing number of affected adolescents, but haematuria is rare. Hepatomegaly is common with soft and tender liver. Thrombocytopoenia and rising haematocrit due to plasma leakage are usually detectable before the onset of the subsequent stage of shock (DSS) with an abrupt fall to normal or subnormal levels of temperature, varying degrees of circulatory disturbances lasting for 24–48 h. In recent publications a number of atypical manifestations of dengue, such as encephalitis/encephalopathy (Victor *et al.* 2002) [101] and myocarditis, hepatitis and cholecystitis etc. have been reported (Padbidri *et al.* 2002) [68, 79, 80]. The majority of patients have rapid uneventful recovery without sequelae in the convalescent stage. The sequence of events in a patient after bite of infected mosquito is summarized in figure 2. The clinical diagnosis of DHF is based on four main characteristic manifestations (Sarkar *et al.* 1964) [22, 91, 92]: (i) continuous high fever lasting 2–7 days; (ii) hemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis; (iii) thrombocytopoenia (platelet count less than 100×10^9/l); and (iv) evidence of plasma leakage manifested by hemoconcentration (an increase in haematocrit 20% above average for age, sex and population), pleural effusion and ascites etc. But some patients with bleeding or anemia may not have a rising haematocrit. Therefore, close observation, serial haematocrit and daily platelet count monitoring are suggested to fulfill the clinical diagnostic criteria. The severity of DHF is categorized into four grades (Sarkar *et al.* 1964) [22, 91, 92]: grade I, being the mildest and grade IV being most severe, with circulatory failure manifested by a rapid and weak pulse with narrowing of pulse pressure (20 mmHg) or hypotension, with the presence of cold clammy skin and restlessness. There may be profound shock in which pulse and blood pressure are not detectable (DSS). In such patients the mortality rate is high.

8. Diagnosis

Diagnosis of dengue virus infection is confirmed in the laboratory. During the stage of fever there is viraemia (figure 2) with presence of NS1 antigens in blood. The presence of virus in blood is detected either by isolation of the virus using infant mice or in tissue culture or by RT-PCR and the NS1 is detected by ELISA. During the post-febrile stage lasting a few weeks, IgM and IgG antibodies are present and are detected by Capture- ELISA (Figure 4). During primary infection, viraemia and fever coincides, but during a secondary infection (second time infection with DV), the viraemia is present for 2

to 3 days, and NS1 antigens in blood lasts little longer. With a newer approach, artificial NS1 receptors have been implanted on a reuseable microchip that can capture and identify NS1 instantly and may be used for bedside diagnosis of dengue virus infection (Kabilan *et al.* 2004) [7, 52].

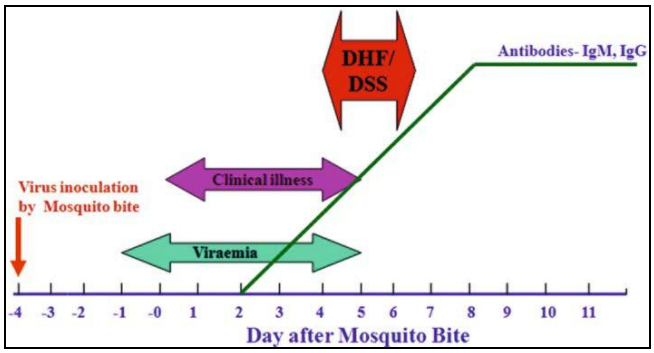


Fig. 4: Sequence of events during dengue virus infection following the bite of infected mosquito.

9. Treatment

The management of dengue virus infection is essentially supportive and symptomatic. No specific treatment is available. However, there are Indian studies which have contributed in terms of better management of DHF/DSS. A rapid response to platelet and fresh frozen plasma (FFP) transfusion is reported in a study. Anti-D has been used in children with DHF and severe refractory thrombocytopenia. In experimental study pre-feeding mice with trivalent chromium picolinate (CrP) in drinking water could abolish the adverse effects of DV infection on most of the haematological parameters. *Hippophae rhamnoides* (Seabuckthorn, SBT) leaf extract has been shown to have a significant anti-dengue activity.

9.1 Vaccine for dengue virus

Dengue vaccines have been under development since the 1940s, but a tetravalent vaccine which simultaneously provides long-term protection against all DV serotypes is round the corner 205. A tetravalent antigen was designed by splicing the EDIIIs of DV-1, DV-2, DV-3 and DV-4 using flexible pentaglycyl linkers. A synthetic gene encoding this tetravalent antigen was expressed in *Pichia pastoris* and purified to near homogeneity. This tetravalent antigen when injected into inbred BALB/c mice, elicited neutralizing antibodies specific to each of the four DVs in plaque reduction neutralization tests206. Efforts are underway to present the tetravalent antigen on a chimeric VLP platform. Some promising dengue antigens have been developed using different systems (Table 2).

Table 2: Dengue antigens developed with potential for vaccine purposes

Expression	Antigen	Antigen design/ salient findings	Ref.
Escherichia	DV 4 envelope	Overexpressed in the form of insoluble inclusion bodies	86
coli	domain III		
	DV 4 envelope	Molecular interaction with heparan sulphate, refolded and purified to homogeneity	87
	domain III		
	rDen 4 EDIII	Highly immunogenic with compatible adjuvants	88
	r-D2EIII	Purified from inclusion bodies; protected cells against DV-2 challenge	89
	r-DME-G	Multiepitope antigen containing IgG-specific epitopes	90
	r-DME-M	Multiepitope antigen containing IgM-specific epitopes; used to develop a rapid	91
		strip assay	
	r-HD	Domain II of *M. tuberculosis* Hsp70 fused to r-DME-G; enhanced immunogenicity	92
		of r-DME-G did not elicit DENV neutralizing antibodies	
	r-EDIII-4/2	Fusion of envelope domain IIIs of DENV-4 and DENV-2; elicit neutralizing	93
		antibodies to DENV-4 and DENV-2	
	r-EDIII-T	Envelope domain IIIs of the four types linked in a tandem array; detects anti-DV	94, 95
		IgM & IgG antibodies, sensitivity is enhanced by coating biotinylated r-EDIII-T	
		on streptavidin plates	
	b-EDIII-T	*In vivo* biotinylated version of r-EDIII-T antigen	96
Pichia	Den2E-HBsAg	A hybrid antigen containing the ectodomain of DV-2 E (aa 1-395) fused to	97
pastoris		hepatitis B surface antigen	
	Den2E-HBsAg	Exist as virus like particles and acts as a bivalent immunogen	98
	EDIII-2	Antigen corresponding to DV-2 envelope domain III; expressed in methanol-	99
		induced *Pichia* cells; elicit DV-2-specific neutralizing antibodies	
	sEDIII-2	Secrets recombinant DV-2 envelope domain III	100
	r-EDIII-T	A tetravalent envelope antigen domain IIIs linked in a tandem array; unlike its	101
		E. coli-expressed counterpart, the *Pichia*-expressed tetravalent antigen elicited	
		neutralizing antibodies specific to all four DENV serotypes	
Adenovirus	DENV-2 E	Last 31 aa of DV-2 prM + the first 395 aa of E encoded by an adenovirus vector;	102
		elicit DV-2 specific neutralizing antibodies	
	DENV-2 EDIII	Monovalent DV-2 EDIII gene expressed using plasmid and adenoviral vectors;	103
		elicit DV-2-specific neutralizing antibodies and T cell responses	
	EDIII-4/2	Fusion of envelope domain IIIs of DV-4 and DV-2, expressed using plasmid and	104

		adenoviral vectors elicit neutralizing and T cell responses DV-2 and DV-4	
	EDIII-T	The EDIII-based tetravalent antigen expressed using plasmid and adenoviral vectors	105
		elicit neutralizing antibodies and T cell responses specific to four DV serotypes	

9.2 Recombinant dengue virus antigens

Several studies have contributed in terms of developing new reagents or technology for diagnostic purposes (Table 2). A recombinant DV3 envelope domain III (rDen 3 EDIII) protein has been produced in *Escherichia coli* for potential use in diagnosis. A biotinylated chimeric dengue antigen to exploit the high affinity of biotin-streptavidin interaction to detect anti-dengue antibodies has been developed which incorporates the envelope domain III of all four DV serotypes. Immunosensor has been established for label free and real time assay for the serological diagnosis of DV infection. Scope for development of biosensors for diagnosis was demonstrated. The recombinant dengue multiepitope (rDME-M) protein specific to IgM in *E. coli* was produced in a 5-L fermentor for use in diagnostic purpose.

10. Prevention and control

Aedes aegypti is the commonest vector of DV in India, followed by *Ae. albopictus*. Larval indices indicate that *Ae. aegypti* is well established in peri-urban areas and is beginning to displace *Ae. albopictus*. Water-holding containers, *viz.* plastic, metal drums and cement tanks facilitate breeding of *Ae. aegypti*. Expansion in the risk area of diseases borne by it in the context of urbanization, transport development and changing habitats is a major concern.

10.1 Vector control

Vector control is known to be a good method for prevention of vector borne diseases. There are several reports from India which have demonstrated resistance of mosquito vector with anti larval substances like DDT and dieldrin but susceptibility to malathione is reported. Temephos is relatively more effective in controlling *Ae. aegypti*, followed by fenthion, malathion and DDT. Peridomestic thermal fogging reduced the resting and biting for the 3 days after treatment,
whereas indoor fogging suppressed adult populations for 5 days.

Plant based repellent against mosquito borne diseases have also been described. Flavonoid compounds derived from *Poncirus trifoliata* compounds have various activities against different life stages of *Ae. aegypti*. Larvicidal and ovicidal activities of benzene, hexane, ethyl acetate, methanol and chloroform leaf extract of *Eclipta alba* have shown potential for controlling *Ae. aegypti* mosquito. Hydrophobic nanosilica at 112.5 ppm is effective against mosquito species.

11. Public health importance

Dengue is one of the major public health problems which can be controlled with active participation of the community.
Need is to organize health education programmes about dengue disease to increase community knowledge and
sensitize the community to participate in integrated vector control programmes.

12. Conclusions

Dengue disease continues to involve newer areas, newer populations and is increasing in magnitude, epidemic after epidemic. Every aspect of dengue viral infection continues to be a challenge; the pathogenesis of severe dengue disease is not known, no vaccine is yet available for protection and the vector control measures are inadequate. Dengue virus was isolated in India in 1944, but the scientific studies addressing various problems of dengue disease have been carried out at limited number of centres. Though clinical studies have reported on dengue disease in India, but these are largely based on diagnosis made by kits of doubtful specificity and sensitivity. A lot more remains to be achieved for creating an impact.

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