Chapter – 1.
Introduction and review of literature.
Chapter - 1.

1.0 Introduction and review of literature.

The term 'dengue' is applied to an acute infectious disease of man characterised by fever, aches and pains in various parts of the body which may range from mild to severe state, generalised rash, lymphadenopathy and leukopenia (Gubler and Kuno, 1998). Its effects may be debilitating to the point of prostration, but uncomplicated classical dengue is rarely fatal. The more serious forms of dengue virus infection are dengue haemorrhagic fever (DHF), or its more severe forms the dengue shock syndrome (DSS). The virus is transmitted to man by mosquitoes of the genus Aedes (Stegomyia) (Gubler, 1988).

The origin of the name 'dengue' is obscure. According to the 'Oxford English Dictionary' the word is derived from the Swahili word 'denga' in the phrase "Ki denga pepo" which refers to a sudden cramp like seizure affecting those involved in a Zanzibar epidemic (Gubler, 1996). Encyclopaedia Britannica considers the name to have been derived from the Spanish name 'dengue', which refers to the stiff dandified gait of those people unfortunate enough to have the illness. An appropriate English name is 'breakbone' fever since the disease is characterised by an extreme pain and stiffness of the joints. The Brazilians called it, 'polka fever'. The name 'Seven day fever' was also applied to it. 'Giraffe fever' and 'Bouquet fever' are French synonyms of this disease. It has been the assumption that 'dengue' spread with the slave trade from East Africa to the Caribbean, where in 1827-28 an extensive outbreak occurred in the West Indies. It was here that the word 'dengue' was first used may be as a Spanish adaptation of the imported word. The Royal College of Physicians of London accepted the term for general use in 1869 (Gubler and Kuno, 1998).

The early history of 'dengue' goes back to epidemics in Java and in Egypt in 1779. Siler, et al, (1926) quote extensively from old documents to support their hypothesis that dengue had occurred in the Western Hemisphere as early as mid 17th century and had its origin in tropical America. Gubler, et al, (1982) on the other hand summarised the evidence, which led him to believe that the disease is originated in South East Asia. Carey (1971) analysed critically the historical accounts of 'dengue' outbreaks during the 18th and 19th century. He furnished arguments in favour of the belief that the earliest recorded epidemics outside the Western Hemisphere (including those in Java,
Egypt and India) were probably due to Chikungunya (CHIK) virus (an alphavirus) rather than dengue viruses.

Benjamin Rush in 1789 (quoted in Siler, et al, 1926; Carey, 1971) was the first to give an accurate clinical description of true dengue fever as it occurred during the Philadelphia epidemic of 1780. It fits, in many details, with the features of the disease seen in well-documented contemporary outbreaks. During the 19th and 20th centuries, extensive outbreaks were reported from tropical and subtropical areas on all continents and from many subcontinents and islands in the South Pacific and in the Caribbean. This extensive geographic spread has continued to the present day (Gubler and Kuno, 1998).

Each of these epidemics affected thousands or millions of people with disease rates in some areas attaining 80% of the population. Such massive outbreaks, even those occurring at a time when methods for virus isolation or serological diagnosis were not available, have aided greatly in the delineation of clinical and epidemiological features of the disease. This was particularly true for the outbreaks in South Africa, the United States, Greece and Japan because these areas have experienced no major subsequent occurrence. It has been possible, through retrospective serological survey, to support the view that these epidemics were indeed caused by dengue viruses (Hammon, et al, 1966; Ehrenkranz, et al, 1971; Hotta, 1965; Hotta, et al, 1968). However, the term 'dengue' is now reserved for the disease caused by at least four antigenically distinct viruses, which belong to the genus Flavivirus within the family Togaviridae.

1.1 Dengue viruses

Dengue is by far the most important arbovirus infection in Southeast Asia. According to WHO dengue has been reported in over hundred countries worldwide and possess threat to approximately 2 billion people. Dengue viruses belong to the family Flaviviridae and possess plus-sense ssRNA as viral genome (Gubler, 1988). Currently arboviruses are defined as follows: "Arboviruses are viruses which are maintained in nature, principally or to an important extent, through biological transmission between susceptible vertebrate hosts by haematophagus arthropods or through transovarial and possibly venereal transmission in arthropods; they multiply and produce viraemia in the vertebrates, multiply in the tissues of arthropods and are passed on to new vertebrates by the bites of arthropods after a period of extrinsic incubation" (WHO, 1985). This definition was modified after the demonstration of vertical transmission of several

Dengue viruses are serologically classified into four antigenically distinct serotypes (DEN-1, 2, 3 and 4). Infection with any of the serotypes generally leads to a mild, self-limiting febrile illness. However, in some cases, vascular and homeostatic abnormalities occur, which may progress to haemorrhage and shock.

1.2 Dengue in India

Occurrence of dengue fevers in India is known for over 200 years. Virologically confirmed cases were recognised subsequent to World War II. Unto the turn of 1970s epidemics were known only from large cities. This was attributed to the urban distribution of their principal vector mosquito, *Ae. aegypti*. Prior to 1952 very little was known about the prevalence of arbovirus infections in India. The existence of sandfly fever and dengue was apparent from their clinical manifestations. “Viral encephalitis” was also a frequently encountered diagnosis, without however any indication as to the aetiological agent involved. Today the prevalence of numerous arboviruses in India has been demonstrated both by the isolation of the viruses as well as by serological diagnostic methods. At present, thirty-one arboviruses are known to exist in India. At present only eleven of these viruses are known to cause disease in man in India, i.e. Chikungunya, dengue types 1, 2, 3 and 4, JE, WN, KFD, Chandipura, Ganjam and the Sandfly Fever group virus. During 1980s a shift in the epidemiological pattern of dengue fever in India was noted. Outbreaks were reported from several villages from different states in India, viz. Kerala, Maharashtra and Gujarat etc.

The first recognised outbreak of DHF occurred in Calcutta in 1963 and it is known that dengue has continued to circulate in the city (Biswas, *et al*, 1993). In mid-July 1983, an outbreak of a febrile illness was reported from different parts of the city involving mostly children and young adults. As most infected persons did not report to hospitals, no information was available on the actual incidence. Gangopadhyaya and Mukherjee, (1997) who investigated the outbreak estimated that there were several thousand cases. No haemorrhagic manifestations or shock were observed. Virus was isolated from four acute phase sera and identified as dengue-3, which was the first isolation of these serotypes from the city, and from eastern India. Dengue-1, 2 and 4
Clear evidence of the continuing spread of dengue in India was provided by an epidemic which took place in north-west India in Jalore city, Rajasthan in April-May 1985 which would be the first reported dengue outbreak in Western Rajasthan. Earlier epidemics of dengue in north India have usually occurred between August and November, but this epidemic occurred in the summer. The authors (Chouhan, et al, 1990), believe that the shortage of water during the summer led to increased storing of water in houses and hence, to increased breeding of Ae. aegypti. Virus isolates were obtained from serum samples of six patients during the outbreak. The result showed that dengue-3 was the main etiological agent.

Dengue stroked Delhi in an outbreak in September-October 1988, this time clearly associated with haemorrhages and shock. About 41.7% had haemorrhagic manifestations and 12.5% presented with encephalitis. Paired sera from the cases showed the presence of high HI antibody to dengue and JE and to WN. Seroconversion was observed more often for dengue-2 but was also seen for JE, dengue-1, 3 and 4 (Thakre, et al, 1996).

During early nineties, only one episode of epidemic of dengue was investigated in an urban area in Maharashtra state viz. Miraj city. The fever cases reported in March-April 1985 were confirmed to be due to dengue-3 virus. Peculiarity of the epidemic was that all the fever cases occurred in a hospital campus. Later two strains of dengue-2 were isolated from wild caught Ae. aegypti in Pune urban agglomeration between 1983-1985. These were the only evidences of dengue activity in Pune since 1975 episode. During July-August 1986 extensive outbreaks of dengue were reported from the villages covered by Dhaba and Risode primary health centres (PHC) of Akola district and Methikheda PHC of Yavatmal district. While the disease outbreaks continued to occur in different rural areas of these districts during subsequent years, the dengue activity was noted in adjacent districts of Amaravati in 1987.

Another change in the nature of dengue transmission in India (1988) was apparent from the outbreaks of dengue from the rural area of Maharashtra State. Outbreak was reported from the Parbhani district in 1989 and 15 virus isolates were obtained, 12 of which were identified as dengue-2 and one as dengue-1. Overall, 15.09% of the surveyed populations of the villages were affected during the outbreak and the investigators noted a higher prevalence of dengue fever among larger families and in
families that had two or more member's ill (Mehendale, *et al*, 1991; Risbud, *et al*, 1991). The authors rightfully expressed their concern at the evidence that dengue was spreading to rural areas as well as the fact that many of the cases were in villages depending on newly installed tube wells. It was felt that the ease of obtaining water from these tube wells lead to the more frequent storage of water (as the supply of tap water was intermittent) and, consequently favoured increased breeding of *Ae. aegypti*. Ilkal, *et al*, (1991) also reported that the outbreak of dengue fever in villages in Maharashtra State resulted due to the change of practice of storing water which created conditions suitable for the breeding of *Ae. aegypti*.

One of the most extensive data sets in India on dengue virus and its vector *Ae. aegypti* was collected over a 10-year period in Vellore, Tamil Nadu, in the 60s. All 4 serotypes of dengue were isolated from human sera and mosquitoes, and in the last year of the study all 4 were isolated in the same season. During the 60s rural dengue and *Ae. aegypti* were absent in rural villages. Socio-economic progress has led to the proliferation of breeding places suitable for colonisation by *Ae. aegypti*. Recent entomological surveys have shown that the species is breeding in villages of Thanjavur District. Seroepidemiological studies have showed that mild non-epidemic forms of dengue virus is active in these villages Reuben (1994).

Dengue began to appear in rural areas in the south of India. Reuben, (1994) described an outbreak in a single village in Tamil Nadu State in the region of Vellore in 1990. The village population was 2,386 people with 536 cases of fever giving an overall attack rate of 22.5%. *Ae. aegypti*, *Ae. albopictus* and to a lesser extent *Ae. vittatus* were abundant. The serological studies indicated that both dengue-1 and dengue-2 were present in 4 of the 5 paired sera tested. Serious outbreaks of dengue occurred in Delhi in 1988 and in Madras in 1989 dengue and DHF manifestations were reported in 30% of the reported cases.

The salient feature of these reports is that dengue is clearly established in rural areas and that outbreaks continue to occur in endemic villages. The National Institute of Virology, Pune, India, has also investigated and verified outbreaks of dengue in the rural areas of Kerala and Gujarat States. The study shows that the virus has established itself in these regions probably in part through human migration of virus infected persons seeking work in the cities and returning to their home villages. Following the reports of epidemics of febrile illness from several rural and urban areas of Gujarat State in 1988, virological and epidemiological investigations were carried out. Evidence of dengue
virus activity was demonstrated in large cities like Surat and Rajkot as well as several small villages in Sabarkantha district. From human sera 4 strains of dengue-2 virus were isolated of which two were from Surat City and other two were from a village Boria Becharjee in Sabarkantha district.

Entomological investigations showed a widespread distribution of Ae. aegypti in urban and rural areas investigated. Six strains of dengue virus were isolated from Ae. aegypti collected at Chotasan village, Sabarkantha district, four of these were found to be of dengue-2. In the household conditions this mosquito was found to breed predominantly in containers holding non-potable water. The transportation of cement tanks manufactured in cities seemed to play an important role in the dispersal of the species to the villages (Mahadev, et al, 1993).

During 1992 and 1993 small outbreaks of fever, headache and bodyache with varying degree of neurological involvement in few of them were reported from Eastern India. Sera were collected covering three districts of Assam and one of Nagaland. Of these 24.2% sera showed antibodies against dengue-2, 11% had antibodies against JE and dengue-2 but the titres of dengue were four fold higher than JE (Baruah, et.al, 1994).

An epidemic of DHF and DSS occurred in Utter Pradesh for the first time at Shahjahanpur in 1992 with a high case fatality rate. From these cases dengue virus was isolated. Subsequently in 1993 few cases of DHF/DSS were observed at Lucknow also. (Mathur, et al, 1994).

During the course of a serological survey of the human population of the Andaman and Nicobar group of islands 7.67% sera had antibodies to dengue (Padbidri, et.al, 1994). Similarly in 1993 studies on the DHF/DSS cases in Jammu showed 36% of patients had haemorrhagic manifestations in the form of hematemesis, epistaxis and GI tract bleeding. Recent dengue virus infection was recorded in 14 out of these 20 patients who exhibited haemorrhagic manifestations (Padbidri, et al, 1996).

Clinically diagnosed DHF/DSS cases were reported in 1993 from the children aged 7 months to 10 years. These cases were from Mysore, Mandya and Bangalore. Serological diagnosis by MAC-ELISA and HI test indicated dengue-3 and dengue-1 (Prasanna, et al, 1994).

Following an outbreak of fever with suspected dengue viral aetiology, virological, serological and entomological investigations were carried out in August 1992 at Southeast Colliery Ltd., Chirimiri. Recent infection to Flavivirus as revealed in MAC ELISA was recorded in 37% of samples. Adult Aedes species mosquitoes were
also collected from these areas for dengue virus isolation. Dengue-2 virus was also isolated from *Ae. aegypti* mosquitoes (Mahadev, *et al.*, 1997).

Dengue virus isolated from human cases and mosquitoes by National Institute of Virology during 1956 to 1996 has been mentioned in Table 1 and 2.

**Table-1 : Dengue isolates (human) from 1956 to 1996.**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>DEN-1</th>
<th>DEN-2</th>
<th>DEN-3</th>
<th>DEN-4</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956-1960</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>1961-1965</td>
<td>62</td>
<td>33</td>
<td>1</td>
<td>12</td>
<td>108</td>
</tr>
<tr>
<td>1966-1970</td>
<td>27</td>
<td>42</td>
<td>95</td>
<td>26</td>
<td>190</td>
</tr>
<tr>
<td>1971-1975</td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>1976-1980</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>1981-1985</td>
<td>23</td>
<td>4</td>
<td>12</td>
<td>1</td>
<td>40</td>
</tr>
<tr>
<td>1986-1990</td>
<td>7</td>
<td>57</td>
<td>5</td>
<td>0</td>
<td>69</td>
</tr>
<tr>
<td>1991-1996</td>
<td>4</td>
<td>49</td>
<td>0</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>TOTAL</td>
<td>144</td>
<td>201</td>
<td>113</td>
<td>45</td>
<td>503</td>
</tr>
</tbody>
</table>

**Table-2 : Dengue isolates (mosquito) from 1956 to 1996.**

<table>
<thead>
<tr>
<th>YEAR</th>
<th>DEN-1</th>
<th>DEN-2</th>
<th>DEN-3</th>
<th>DEN-4</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1956-1960</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1961-1965</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>1966-1970</td>
<td>24</td>
<td>40</td>
<td>5</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td>1971-1975</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>1976-1980</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>1981-1985</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>1986-1990</td>
<td>0</td>
<td>24</td>
<td>1</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>1991-1996</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>30</td>
<td>74</td>
<td>12</td>
<td>21</td>
<td>137</td>
</tr>
</tbody>
</table>
1.3 Natural hosts and vectors

Many early workers had suspected that dengue was transmitted by the bite of mosquitoes but Graham (1903) first demonstrated the actual transmission. Bancroft (1906) demonstrated that *Ae. aegypti*, when allowed to feed on the blood of a person during the acute phase of illness, was able to transmit the agent to another person after an incubation period of 10 days. Subsequent studies in the Philippines, Indonesia and the island countries in the Pacific showed that *Ae. albopictus* and *Ae. polyensiensis* were also efficient vectors of these viruses (Siler, *et al*., 1926; Gubler and Rosen, 1977 and Snijders, *et al*., 1931). Over 20 species of mosquitoes, all belonging to the genus *Aedes* have either been proved or suspected as vectors of dengue viruses by isolating the virus in nature and/or by experimental studies.

In India, *Ae. aegypti* is known to be the principal vector of dengue and CHIK viruses. Evidence suggests that *Ae. albopictus* may also be a potential vector, at least for dengue viruses. It has been suggested that *Ae. aegypti* may have been introduced into the country through sea communication from the eastern coast of Africa and the Middle East countries. The initial in-land spread probably took place through the river navigation and human settlements along the river villages. In the recent times, however, the intricate network of road transport has been mainly responsible for its spread far and wide, wherever human habitations exist and climatic conditions are suitable.

Besides road transport and increased urbanisation, introduction of tapped water supply is another important factor for creating congenial environment for the establishment of *Ae. aegypti* in India. Piped water supply being generally irregular and intermittent compels people to store water for domestic needs. Such water storage containers offer ideal breeding places for the mosquito throughout the year, particularly during the drier months when water scarcity becomes severe. Thus *Ae. aegypti* which, until recently was thought to be confined to larger towns and cities, has now made inroads even in the small villages, thus increasing the risk of the spread of *Ae. aegypti* borne diseases Dhanda, (1994).

*Ae. aegypti* is mainly an urban mosquito. It has a fairly wide distribution. It breeds in man made containers such as rain water barrels, cisterns, wells, water storage drums, pots, discarded bottles, tin cans, motor vehicle tyres, etc (Christophers, 1960). Similarly, natural habitats such as rain filled tree holes have sometimes been observed harbouring immature *Ae. aegypti* (Rao, *et al*, 1970; Shetty and Geervarghese, 1977).
However, such a situation usually arises due to a spill over from the normal breeding habitats, especially when the population of mosquito reaches a high level (Rao, et al, 1970).

Rao, (1964) in a review on vectors of dengue and CHIK viruses considered *Ae. albopictus* as a suspected vector for dengue. Two other species, which he considered potential vectors, are *Ae. vittatus* and *Ae. w-albus*. The former has been responsible for a large-scale outbreak of yellow fever in Sudan (Satti and Haseeb, 1966). On one occasion dengue-4 virus has been isolated from *Ae. albopictus* at Asansol in West Bengal (NIV, unpublished data). *Ae. albopictus* is mainly a peridomestic mosquito. It is abundant in shady areas with plenty of trees, such as gardens and in the periphery of towns and cities. It breeds mainly in the rainwater accumulated in tree holes but may occasionally be found in outdoor water storage containers and other wooden containers and is found in greatest numbers during and after the monsoon. It is mainly exophilic and exophagic and bites during the day and at dusk.

*Ae. vittatus* and *Ae. w-albus*, like *Ae. albopictus* are peridomestic mosquitoes. They rest and bite outdoors. The former breeds in rock-pools found in various situations, including those formed on the edges of rocky rivers and in stone containers around human habitats, while the latter breeds mainly in tree-holes and other peridomestic containers (Barraud, 1934).

1.3.1 Experimental studies on vectors

Susceptibility of *Ae. aegypti* and *Ae. albopictus* to oral infection with dengue viruses has been studied by Jumali, et al, (1979) and Rosen, et al, (1985). They reported that *Ae. albopictus* is more susceptible and more efficient host for dengue viruses than *Ae. aegypti* mosquitoes. When multiplication of dengue viruses was studied in the parentally infected *Ae. aegypti* and *Ae. albopictus*, it was found that the growth curves for all 4 dengue viruses were similar in both the mosquito species (Tan, et al, 1981). Recently Mitchell, et al, (1987) studied the vector competence of *Ae. albopictus* for dengue 1 to 4 viruses and found that *Ae. albopictus* is competent for the transmission of dengue viruses Frier and Grimstad (1983) and Rosen, (1983) have shown experimentally that other species such as *Ae. polyiensisis, Ae. mediovittatus* and *Ae. triseriatus* have a higher susceptibility to oral infection with dengue viruses than *Ae. aegypti*. Tang, et al, (1987) reported that *Ae. flavopictus* is susceptible to oral infection but not more susceptible than *Ae. aegypti*. Genera of the mosquitoes other than *Aedes*
have been generally found to be poor hosts for dengue viruses. According to Siler, et al, (1926), Simmons, et al, (1931) and Rosen (1985) Culex species are refractory to dengue virus infection and do not play a role in transmission, although a report from China incriminates Cx.quinquefasciatus as a vector (Xue-dong and Gui-Fang, 1985).

In India, experimental studies to determine the vector competence of mosquitoes are conspicuous by their inadequacy, even though dengue fever has been known to occur in the country for almost two centuries. Ae. aegypti has been incriminated as the chief vector of dengue viruses, mainly on epidemiological grounds. It is therefore ironical that the first serious effort to study the susceptibility of Ae. aegypti and Ae. albopictus was as late as 1982 (D'Lima, 1982). He demonstrated that when infected by artificial feeding through a membrane both the species were able to transmit the virus. The rate of infectivity, however, differed significantly in different replicates, a phenomenon that could not be explained satisfactorily. He further showed that two successive infective blood meals at an interval of approximately six days increased the rate of infection in Ae. aegypti as compared to a single infective meal. He also showed that a normal blood meal after 6 days of infective blood meal boosted the virus titre in infected mosquitoes.

In an another study, the Delhi population of Ae. aegypti collected during a dengue epidemic in 1982 was found more susceptible than the laboratory strain from Pune, which had been maintained in laboratory colony for several years (Dhanda, et al, 1983). Experimental studies have been carried out to find out the susceptibility of Ae. aegypti collected from various parts of the world with all the 4 types of dengue viruses by membrane feeding. The study showed variations in the susceptibility of different populations of Ae. aegypti. In a case of laboratory-acquired dengue infection at this Institute, as many as 50% of Ae. aegypti picked up the virus when fed on the patient (Ilkal, et al, 1984). Experimental studies on vector competence of Ae.vittatus for dengue viruses have showed that this species is a poor vector (Mavale, et al, 1992). However, it has been shown to be an efficient vector of CHIK virus (Mourya, et al, 1987).

1.4 Transovarial transmission
The role of transovarial transmission (TOT) in the maintenance cycle of dengue viruses has been reinforced in the recent years. According to Rosen, et al, (1983) French workers in West Africa obtained the first evidence of vertical transmission in
nature. Dengue-2 virus was isolated from a pool of male *Ae. furcifer-taylori* collected in the forest of Ivory Coast. They also isolated dengue-2 virus from another pool of male *Ae. taylori* collected in Senegal. In Burma, Khin and Than (1983) reported isolation of dengue-2 virus from 5 of 199 pools of *Ae. aegypti* larvae (13,930 mosquitoes) which were collected from natural breeding containers in Rangoon. Two of the isolates were from pools of male mosquitoes that had been reared to the adult stage and the sexes separated before testing. In Trinidad, Hull, et al., (1984) succeeded in isolating dengue-4 virus from 1 of 158 pools (10,957 mosquitoes) of adult *Ae. aegypti* collected as eggs. However, by contrast, Watts, et al., (1985) has not succeeded in isolating dengue viruses from 5,766 *Ae. aegypti* larvae collected from houses in Bangkok, Thailand, in which one or more persons had recent dengue virus infection. Recently, TOT of dengue viruses studied in *Ae. mediovittatus* and *Ae. scutellaris* (Gr.) by Freier and Rosen (1987, 1988) have shown that these species transmit all 4 dengue serotypes vertically at much higher rate than other flaviviruses in mosquitoes.

There have been comparatively few reports of TOT of dengue virus in *Ae. aegypti* mosquitoes. Recently, the occurrence of this phenomenon has been reported from India (Joshi, et al., 1996). However, earlier reports, suggest that, the minimum infection rates (MIR) of the *Ae. aegypti* infected progeny with dengue virus is very low (Rosen, 1983). The low level of TOT of dengue in its main vector *Ae. aegypti* raises an important question whether it has any epidemiological significance? There are several regions in our country where the density of this vector species is almost zeroing during the inter-epidemic season. On several occasions, even during the dengue epidemic the density of *Ae. aegypti* mosquito has been found to be very low.

Earlier studies conducted have shown that there is no animal reservoir involved in dengue virus cycle (Gubler and Kuno, 1998). It is only the “man-mosquito-man” cycle. Once a person gets infected by an infective mosquito bite, presence of virus in the peripheral blood has been seen during 3-6 days of post-infection. Therefore, it is unlikely that during non-mosquitogenic season of very low *Ae. aegypti* density active transmission goes on at a detectable level. The virus is an intracellular obligatory parasite, which cannot survive without the host. This indicates that the probable mechanism of survival of virus during non-epidemic season is the persistence of virus in mosquitoes. Since there are no records of over wintering and diapause phenomenon does not exist in this species of mosquitoes in India, the virus survives in this mosquito species through the TOT in absence of man-mosquito cycle. It is a well-known fact that
once the *Ae. aegypti* eggs get conditioned properly; they can remain viable for many months. When the outside conditions becomes favourable once again they hatch. There is a possibility that dengue virus infected eggs carry the virus horizontally throughout the larval development into the adult stage (Khin and Than, 1983; Hull, *et al*, 1984).

These observations of the vertical transmission of dengue viruses are suggestive that TOT of dengue is an important factor for virus survival especially during inter-epidemic season when the virus remains latent in the egg stage. The earlier published work suggests that the methods used for the detection of virus passing through the gonotrophic cycle were not sensitive enough. In most of the studies pools of large number of mosquitoes were processed in mosquitoes, cell lines and mice. Perhaps these methods were not sensitive enough to detect the virus at very low quantum.

Non-human vertebrate hosts: Experimentally several species of lower primates, *viz.* chimpanzees, gibbons, rhesus, macaques and *Erythrocebus patas* monkey, become infected and develop viraemia high enough to infect mosquitoes (Rosen, 1954; Halsteaed, 1990; Cornet, *et al*, 1984). However, it has been shown that though the lower primates produce viraemia, they do not show obvious signs of illness and appear to be particularly well adapted to the viruses (Gubler, *et al*, 1981). Dengue viruses do not readily infect other vertebrate animals. Baby mice, used for isolation and assay of many other arboviruses, may not show signs of illness after intracerebral inoculation, particularly with unpassaged strains of dengue viruses. Immuno-compromised nude mice do not show signs of illness or viraemia when infected by the oral route (Mourya, *et al*, 1997). No non-human vertebrate host of dengue viruses has so far been found in India.

### 1.5 Vector competence

An important aspect of dengue ecology involves genetic heterogeneity in the mosquito vector population (Degallier, *et al*, 1988). Geographic strains of both *Ae. albopictus* and *Ae. aegypti* vary considerably in their susceptibility to oral infection with dengue viruses, and thus in their ability to transmit the infection. These studies also showed that susceptibility to oral infection was dose related, therefore, a mosquito strain could become infected by increasing the amount of virus ingested, regardless of how resistant it was to dengue virus infection. In both mosquito species, the factors controlling susceptibility to oral infection were the same for all four dengue serotypes;
susceptibility was controlled by a midgut or Mesenteronal escape barrier, and there was no evidence of dissemination or salivary gland barriers to dengue infection. Finally, susceptibility to oral infection with dengue viruses was genetically controlled in both species. Thus, environmental or other selective pressures may cause changes in susceptibility of the mosquito population to dengue viruses, suggesting that vector competence may be an important risk factor for epidemic dengue transmission.

Investigators in several laboratories are studying natural genetic variation among strains of *Ae. aegypti* by isoenzyme analysis using gel electrophoresis. Attempts have been made to characterise strains of *Ae. aegypti* with high and low dengue susceptibility by this technique in an effort to identify genetic markers for susceptibility and refractoriness. Preliminary results have failed to show any conclusive relationship between dengue susceptibility and enzyme banding patterns among either geographic strains or different morphotypes or *Ae. aegypti*. The studies have shown, however, that this species can be separated into geographic groups based on genetic relationships, an observation that has obvious importance in determining the origin of mosquito strains that have reinfested an area after eradication. Whether this type of genetic analysis of mosquito populations will increase our understanding of mosquito vector competence and its role in the distribution and spread of epidemic dengue must be the subject of further study.

Laboratory experiments have demonstrated a wide variation in oral infection among geographic strains of *Ae. aegypti* and *Ae. albopictus*. Further, the rate of mosquito infection varied among strains of virus or serotypes for a given vector strain. Early laboratory experiments established that *Ae. aegypti* is a rather inefficient vector, since oral feeding requires an artificial blood meal containing as much as 10 or more of infectious units per millilitre. However, in most experiments, tests were conducted by using artificial blood meals. As reported for other flaviviruses (West Nile and St Louis encephalitis viruses), the threshold of infection by pledges feeding are often more than 3 dex higher than that by feeding on viraemic mammals with similar virus titre. These observations may be explained by the fact that many factors regulating oral feeding that lead to infection of mosquitoes under natural conditions are still poorly understood.

1.5.1 Intrinsic incubation period and viraemia

The classical experiments on human volunteers established that: (i) the intrinsic incubation period averaged between 4.5 and 7 days, with a small number of
cases exceeding 10 days and (ii) viraemia may exist for 6 to 18 h before onset of symptoms (Siler, et al, 1926). The symptomatic viraemia period is 4 to 5 days, but may be as long as 12 days. The importance of pre-symptomatic viraemia should not be underestimated because of normal human activities and hence contact with vectors in other locations, in contrast to that of symptomatic persons, who are less mobile or bedridden.

During a dengue-2 outbreak investigation in Tonga, despite the application of the most sensitive virus isolation technique (mosquito inoculation) on blood specimens collected daily for as long as 6 days and attempts to isolate virus by permitting uninfected mosquitoes to feed on patients, few virus strains were isolated. It was speculated that the low virus isolation rate reflected a short viraemic period of low magnitude, corresponding with the abortive nature of the outbreak. This in turn, suggested that virus strains with low virulence were most likely the determining factor. A similar observation was documented in the South Pacific with dengue-1 (Gubler, et al, 1978), in Indonesia with dengue-3, and in Taiwan with an unidentified stereotype (Gubler, et al, 1981).

1.5.2 Infection of vectors

Humans infected with dengue vaccine strains may produce low and transient viraemia. Mosquitoes that feed on such viraemic patients may acquire the virus, but they generally fail to transmit the same. The threshold of viraemia in humans for mosquito infection has not been determined. In natural infections, virus titres in humans rise to as high as $10^{8.3}$ MID$_{50}$ per ml. In macaque monkeys infected by mosquito bite, viraemia never exceeded $10^{-6}$ MID$_{50}$ per ml, and Ae. albopictus mosquitoes allowed to feed on those monkeys had infection rates of 10 to 15 percent when viraemia titres were less than $10^{-4}$ MID$_{50}$ per ml. In a highly unusual study in China, it was reported that the virus titres in peripheral blood obtained by finger pricking were approximately $10^{-12}$ TCID$_{50}$ (50 percent tissue culture infectious dose) per ml higher than that in intravenous blood.

1.5.3 Urbanisation and critical human population size for endemic transmission

Enormous population growth in major cities and urbanisation of rural areas in the tropics in the past few decades have definitely contributed to more frequent and
larger dengue epidemics. Thus, reports of a positive correlation between human density and the magnitude of dengue transmission are not surprising.

The minimum number of susceptible persons in a population for maintenance of disease transmission depends on many factors. In locations with smaller populations, after a period of intense transmission, recognisable cases typically disappear. On the other hand, in areas with much larger populations, an endemic situation is often established. Exact estimation of the critical population size that permits endemic transmission in tropical locations is difficult because of the high potential for virus reintroduction, changes in demographics, herd immunity and the effectiveness of vector control.

1.6 Control of urban dengue vectors

Since a vaccine is not available transmission can only be reduced by measures against the mosquito. Thus surveillance of vector species is considered very important. Many countries support dengue control programs, but with few exceptions they are far less successful. Explosive growth of urban areas, limited government resources, poor management, inadequate training of field personnel, excessive reliance on insecticides, incorrect application of insecticides, insecticide resistance, overemphasis on government intervention and insufficient education of the public are responsible for poor surveillance of dengue (Gubler and Clark, 1995).

1.6.1 Indices

Surveys of larva-infested containers are widely used in *Ae. aegypti* control campaigns, whereas the following three indices are commonly cited:

**House Index:** percent of houses positive for containers with *Ae. aegypti* larvae or pupae.

**Breteau Index:** number of containers positive for *Ae. aegypti* larvae or pupae per 100 houses.

**Container Index:** percent of water-holding containers positive for *Ae. aegypti* larvae or pupae.

The House Index is the most widely quoted. Houses are counted as 'positive' regardless of the number of infested containers they harbour (Chan, 1985). In practice, the distribution of such containers is often aggregated. The Breteau Index is a simple
device to counter this non-random distribution. The Container Index gives no information on the prevalence of containers. Attempts have been made to demonstrate mathematical relationship between the indices, but such relationships vary with local conditions (Focks, et al, 1995).

1.6.2 Collection of adults

It is not easy to estimate the adult *Ae. aegypti* population. Adult captures on human bait, indoor sweeps with hand nets, and other manual methods have been used, but are labour intensive, tedious, subject to complex operator and location influences, and in many other ways unsuited for routine surveillance. Electric traps have been devised, but results are inconsistent and their cost and tendency to be stolen for their components preclude routine use. An effective device is the battery-powered backpack aspirator developed by Clark and Rangel (1998). Collections are made from resting sites, principally bedroom closets and other dark indoor sites. A pair of operators can sample 15 to 20 houses in a morning, the period when the mosquito is active. The method has proven very effective in population studies and in the evaluation of control measures.

1.6.3 Adult female mosquito density

Although densities (indoor and outdoor) of female mosquitoes have been determined in dengue-endemic countries, interpretation of results has been difficult because the data were highly variable and the mosquito collection techniques were not standardised. For example, in one study in Thailand, on average, 20 females were found per room, while in another study in Puerto Rico 5 to 10 females were found per house. However, larger numbers of female mosquitoes per residence are not always associated with epidemic transmission. In a localised school outbreak in Malaysia, only three *Ae. aegypti* females were collected in one hostel, where 20 students were infected. Similarly, in a study in India, in one ward where more than 43 percent of the residents suffered from DEN, only three female mosquitoes were found in a survey of 74 houses. Further, in a study conducted during an outbreak in Taiwan, female density was as low as 0.07 per house. Even though indoor mosquito density results may be biased by the variation in efficacy of the mosquito-collecting technique used, the data strongly suggest that an outbreak can occur when mean adult female density per house is much less than 1.

Another important factor influencing transmission is the proportion of infective females. Unfortunately, there is a paucity of such data, since virus has been
isolated from pooled, rather than individual, mosquitoes. The minimum infection ratio for *Ae. aegypti* and *Ae. albopictus* in Singapore in four sentinel areas was, on average, 0.51 and 0.59 per 1000 mosquitoes, respectively, while the corresponding figures in another study in Thailand were 61 and 5 per 1000, respectively. While the minimum infection ratio may be a useful index for arboviral diseases in which contact between infectious vector and human occurs outdoors, for dengue transmission, the number of infected female mosquitoes per house or premise appears more useful because of the close association between house and vector. In one of the small number of such investigations, the numbers of virus-infected females/total number of female's captured/number of houses visited in three villages in India during an epidemic were 1/15/20, 1/18/25, and 1/18/32, respectively. Thus, the number of infected females found per residence during dengue transmission was small. In a computer-simulated dengue epidemic in a 40-ha community with an initial population of 10,000 the projected estimate of infected female mosquitoes was determined to be roughly 1 per 100 persons (or about 2 percent of the approximately 5000 females) (Focks, *et al*, 1993, 1995).

1.6.4 Feeding behaviour

Many investigators have recognised that female mosquitoes engage in multiple feeding before completing a gonotropic cycle. The classic experiments by Siler, *et al*, (1926) demonstrated that female mosquitoes, once infected remain infective throughout the adult stage even after repeated bites on humans.

Multiple feeding probably contributes to the explosive nature of dengue outbreaks and may explain that why relatively small numbers of infected females have been found in the residences in endemic foci. Multiple feeding, however, may not always accelerate dengue transmission, depending on the spatial distribution of immune individuals. According to one study, virus acquired by mosquitoes in the second meal from a viraemic person may be neutralised by the first meal. If the first human biting had neutralising antibody it was assumed that after the second viraemic meal infected females would bite more frequently than uninfected mosquitoes. A recent study, however, did not support the assumption (Hardy, 1997).

1.6.5 Extrinsic incubation period

For transmission to continue, the longevity of female mosquitoes is an important parameter. It has been reported that the EIP ranges between 10 and 14 days.
However, the EIP depends on ambient temperature, humidity, viraemia level in the human host, and the virus strain. Although temperature and humidity cannot be separated, generally higher temperature results in a shorter EIP, while lower temperature increases the EIP. A lower temperature, in turn, decreases transmission. Further, the lowest daily temperature, rather than average temperature, is thought to be a more important determinant of dengue transmission seasonality in Bangkok.

Regarding the influence of the virus, the effects of the viraemia level of the human host on virus transmission to the mosquito are described in earlier section on intrinsic incubation period and viraemia. As for the impact of strain variation, a considerable degree of variation in the EIP has been observed. The EIP was 14 days with an unadapted strain as determined by mosquitoes feeding, while with the low-mouse-passage level strains, it was 22 days. As expected, longer EIP were often observed with fully attenuated strains because mosquitoes failed to transmit virus, even though they were infected.

### 1.6.6 Daily survival rate, longevity and gonotrophic cycle

A small change in daily survival rate has a considerable impact on transmission. For example, in a mathematical model of Chikungunya transmitted by *Ae. aegypti*, a change in the daily survival rate from 0.89 to 0.94 altered the course of the disease from a relatively brief, self-limiting epidemic to a stable endemic state. Most, if not all, investigators working on mathematical models of arthropod-borne infections have accepted the assumption of Macdonald that the probability of mosquito survival is constant throughout a mosquito's life. This idea was supported by a more recent publication. According to Smith (1975), however, that is unlikely and suggests further studies under field conditions.

Consensus among entomologists has not been reached regarding the daily survival rate of *Ae. aegypti* adults. Longevity depends on many factors, such as temperature, humidity and nutrition etc. Again, the paucity of data obtained in the field is a major obstacle. Limited data from field studies on female *Ae. aegypti* showed longevity ranging from 8.5 days to 42 days. A recent study suggests it to be at least 9 days under simulation; rates ranging from 0.895 to 0.910 with an average of 0.908 were used. Under laboratory conditions, however, at 27 ± 1°C, females that are given both a 10 percent sucrose solution and blood meals survive, on average, for 55.6 days with a maximum of 100 days. Females that are fed 5 percent sucrose solution and kept at ambient
temperature in the tropics transmit dengue 75 days after feeding on a viraemic human. *Ae. albopictus* females are known to survive for up to 122 days in the laboratory, but there are no reliable records in the field data where daily mortality varies from 7.9 to 15 percent, depending on month.

Temperature affects not only longevity but also length of gonotrophic cycle, which was recognised to be a factor associated with seasonality of dengue in Bangkok. A computer-simulated study by Focks, *et al.*, (1993, 1995) identified it as a significant factor in dengue transmission.

1.7 *Aedes aegypti* control at aquatic stages

1.7.1 Source reduction

Trained inspectors under expert supervision can conduct classic source reduction. Careful records are kept to provide surveillance data for feedback on efficacy. Most larval containers are easily identifiable and can be eliminated. Those considered 'useful' by the householder could usually be inverted or stored in a dry place. Water storage containers can be protected with a cover. Ornamental 'water plants' can be replanted in solid substrate. Roof gutters can be cleaned and graded to ensure drainage. Natural cavities, such as tree holes, can be filled with cement or sand. Animal watering bowls and ornamental fountains can be rinsed and cleaned.

**Larvicides:** Larval sites that cannot be eliminated can be treated to kill the *Ae. aegypti* larvae.

**Oils:** Kerosene, diesel oil and similar products kill larvae and pupae by penetrating the tracheal system and preventing respiration. Spreading agents can improve their effectiveness. Oils fell out of favour when insecticides became available.

**Insecticides:** Perifocal treatments with organochlorine insecticides during the eradication campaign have already been mentioned. The aim was to kill adult mosquitoes that made contact with the sites and make potential containers inhospitable to larvae. When resistance became a major problem, organophosphorus insecticides (fenthion, fenitrothion and malathion) and several pyrethroids were adopted.

Larvicides are used to treat drinking water in drums and cisterns, as well as cemetery vases and other non-disposable containers. For the past 30 years, temephos (at a dosage of 1 ppm active ingredient) has been the compound of choice, mainly because of its relatively low mammalian toxicity (oral LD$_{50}$ for rats: 8600 mg/kg). Alternatives to
temephos are methoprene, a synthetic compound that interferes with insect metamorphosis and mosquito-specific toxin derived from the bacterium *Bacillus thuringiensis* serotype H-14. Toxin is available in floating slow-release that are useful for treating deep water, where particulate insecticides tend to settle out of reach of the larvae. All three treatments are effective for 1 to 2 months, but many householders discard the treated water to remove the toxicant, a response that has probably grown with public awareness of the hazards of insecticides and other chemicals.

The concept of using live predators to kill *Ae. aegypti* is attractive, but although many organisms have been studied, few are in routine use. Larvivorous fish are effective in cisterns and other large containers, provided that householders do not object to their presence. Adult *Toxorhynchites* mosquito oviposite in *Ae. aegypti* larval habitats and their larvae feed voraciously on *Ae. aegypti* larvae. Unfortunately, *Toxorhynchites* is difficult and expensive to rear in large numbers and field trials have given variable results. The copepod *Mesocyclops*, a micro-crustacean predator, feeds on newly hatched *Ae. aegypti* larvae. It is easy to rear at minimal cost and can be applied by hand sprayer. Several species have given encouraging results in pilot trials. Dragonflies, beetle larvae and even a small aquatic turtle have also given good results in some studies.

**Protection of water storage containers:** Tightly fitting covers can prevent oviposition in water storage vessels.

**Water supply:** A reliable piped water supply should eliminate the need to store water, but in many communities storage is a cultural habit, reinforced by failures of the piped supply. Intermittent use of stored water will enhance mosquito production if vessels accumulate leaves and other litter as sources of larval nutrition.

**Solid waste:** The proliferation of disposable containers is a contemporary factor that has contributed to an increase in *Ae. aegypti* populations. Regular garbage collections can diminish the problems, but availability does not ensure effective use. Moreover, collection services often reject important items, such as used tires, abandoned car bodies, old kitchen appliances and discarded toilet bowls.

**Tyre management:** Discarded rubber tyres are of critical importance in dengue control because they are favoured oviposition sites.
1.7.2 *Aedes aegypti* control at adult stages

**Residual insecticides:** Insecticides that are chemically stable can be applied as a residue to surfaces onto which mosquitoes alight. Transient contact with these residues can be sufficient to kill susceptible insects. Ideally the treatment should last for several months. Indoor treatments have been extensively used in anti-malaria campaigns, and contributed significantly to the elimination of *Ae. aegypti* from the Mediterranean and other regions after World War II.

**Indoor space sprays:** Low volume and ULV aerosols are more effective when applied as indoor space sprays because adult *Ae. aegypti* are highly endophilic. A number of portable devices are available for such treatments. Field trials of fenitrothion (estimated at 800-ml active ingredient ha-1) gave effective control for 6 to 7 months, and mosquito densities were substantially reduced for up to a year. Presumably, a residual effect was involved. Excellent results were also obtained with thermal fogs of malathion.

1.7.3 Personal protection

**Screens:** Mosquito-proof screens on doors and windows can greatly reduce the number of mosquitoes inside a building, provided that doors are kept closed and there are no larval sites within the screened area. A study in Puerto Rico indicated that screens reduced the risk of dengue infection.

**Bed nets:** *Ae. aegypti* is a day-biting mosquito, so bed nets are usually considered irrelevant. However, in many dengue-endemic countries people rest in the afternoon, during the period of maximum biting activity. In addition, some biting occurs in the hours after dawn and even in the dark. Bed nets give protection from bites to persons resting at these times, and throughout the day to infants, nightshift workers, and the bed-ridden. Little attention has been paid to the isolation of dengue patients to prevent the infection of mosquitoes, but here again, bed-nets can be an effective barrier, particularly if impregnated with a residual insecticide.

**Mosquito repellents:** Repellents containing N,N-Diethyl-m-toluamide (DEET) can be applied to exposed skin to prevent mosquito bites, and are recommended for use by travellers visiting dengue endemic areas. At tropical temperatures this can mean frequent application because of perspiration. Repellents containing DEET at concentrations above 30% should be avoided. Care must be taken when treating small
children and no material should be applied to their hands or faces. Formulations containing active ingredients other than DEET is available, but is not as effective. Clothing can be treated with DEET or permethrin to give a repellent effect.

1.7.4 Aedes aegypti and insecticide resistance

All insect populations exhibit genetic variation in their susceptibility to insecticides. The use of insecticides exerts a selection pressure that favours the survival of strains with increased resistance. A variety of biochemical mechanisms are involved. In many cases, these confer cross-resistance to compounds of the same chemical group, even if they have not been involved in the selection process. In some cases, cross-resistance extends to chemically unrelated groups.

Since the late 1940s, resistance to a range of insecticides has been documented in Ae. aegypti from many parts of the world. Adult Ae. aegypti in Puerto Rico have a high 'knock down resistance' (kdr) to organochlorine insecticides, presumably a legacy of the malaria and Ae. aegypti eradication campaign during the DDT era. The mechanism of kdr confers cross-resistance to pyrethroids as well. In the Americas, significant resistance to temephos has been demonstrated in Ae. aegypti larvae from many countries. However, despite widespread use of ULV malathion, resistance to this insecticide has not been observed. This is not surprising in view of the lack of efficiency and, thus, the low selection pressure exerted by the ULV method against this species.

Insecticide susceptibility should be routinely monitored in any campaign that uses insecticides. Enhanced ovitraps are useful for obtaining large numbers of Ae. aegypti eggs from the field, ensuring an optimum sample size for testing.

It would be misleading to write with confidence and authority on the control of dengue vectors. Successful control has rarely been achieved and hardly ever sustained. We cannot assume that methods used in the past will be effective if reapplied today. We no longer live in the heady days of DDT optimism and the Ae. aegypti eradication campaign. Heavy reliance on ULV aerosols is clearly not the answer. Therefore, if we assume that it is possible to succeed, we must explore new approaches, with attention to cost versus effect, and an open mind to the roles of government, industry and the community at large.